Synthesis and Antitumor Activity of 9-Substituted Nitrogen Mustard Derivatives of 6-Alkylthiopurines[1,2]

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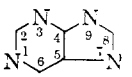
The preparation of 6-alkylthio-9-(β-chloroethyl)purines and 6-alkylthio-9-[bis(β-chloroethyl)aminoethyl]-purines was reported. Direct alkylation of 6-(alkylthio)purines with ethylene bromohydrin or 2-bromoethyl chloride in dimethyl sulfoxide gave 6-(alkylthio)-9-(β-hydroxyethyl)purines or 6-(alkylthio)-9-(β-chloroethyl)-purines, respectively, in good yield. These compounds were identical with those prepared by alternate and unambiguous synthetic routes. Although cyclization of some 4-(β-chloroethylamino)pyrimidines to dihydroimidazolo[2,3-*c*]pyrimidines has been reported, the closely related 2-amino-6-alkylthio-9-(β-chloroethyl)purines did not undergo similar type of cyclization. Several 6-alkylthio-9-(β-hydroxyethyl)- and -9-(β-chloroethyl)-purines possess antitumor activity against Adenocarcinoma 755 system. In addition, significant activities in Friend virus leukemia (solid) and in tissue culture studies have also been observed in some 6-(aralkylthio)-9-(β-chloroethyl)purines.

Since the discovery of the antitumor activity of 6-mercaptopurine in 1954,[4] various structural modifications have been explored by many investigators. Screening results indicated[5,6] that none of the purine derivatives substituted at positions 1, 3, and 7 exhibited



any significant activity. Substitution at position 8 gave varied results, and an amino group at position 2 of the purine ring often is the only substituent at this position which does not result in the loss of carcinostatic effect. A number of 6-substituted purines, especially 6-S-substituted, have been shown to possess significant activity. Substitution at the 9-position has rewarded cancer chemotherapeutists with many encouraging results. For instance, although 2,6-diamino-9-(*p*-tolyl)purine[7] is the only active compound among a number of 9-aryl-substituted purines, a wide variety of 9-alkyl-substituted purines were found to have antitumor activity equal to, or greater than, their parent compound. This information clearly suggested that, with regard to antitumor activity, future investigation involving the purine ring system might well be centered about the 9-alkyl-6-alkylthiopurines (and the corresponding derivatives of 6-thioguanine).

Nitrogen mustard and its derivatives have been used widely as carcinostatic agents.[8] A recent publication reported that combination therapy with 6-thioguanine and uracil nitrogen mustard produced a synergistic response in Sarcoma 180 ascites tumor system.[9] This indicated that a nitrogen mustard moiety attached directly or indirectly to some 6-S-substituted purines might be of particular interest. Synthesis of various 6-substituted purines with a nitrogen mustard moiety at the 9-position has been reported by other investigators: 9-(β-chloroethyl)adenine, as indicated by Timmis and co-workers,[10] was found to inhibit the growth of the C_{1300} experimental tumor. Lin and Price[11] reported that 9-[bis(β-chloroethyl)aminopropyl]hypoxanthine is active against Ehrlich ascites 6C3HED and several other experimental tumors in mice.[12] Burckhalter and Bariana noticed that N^6-(α-[bis(β-chloroethyl)amino]-4-ethoxy-*m*-tolyl)adenine possessed activity against L1210 in mice and Dunning leukemia (solid) in rats.[13] Levin, Sugiura, and Brown[14] have recently prepared a nitrogen mustard derivative of 6-thiopurine wherein the bis(β-chloroethyl)aminoethyl moiety was attached to the 6-S-position.

In the present work the preparation of 9-substituted nitrogen mustard derivatives of 6-(alkylthio)purines has been accomplished in our laboratories.

Treatment of 6-chloro-9-(β-hydroxyethyl)purine[15] (I) with thiourea in refluxing ethanol yielded 9-(β-hydroxyethyl)-6-purinethiol (II). Compound II was smoothly alkylated with alkyl halides or aralkyl halides, according to the procedure of Noell and Robins,[16] to give IV. These 9-(β-hydroxyethyl) derivatives (IV)

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(2) Presented in part before the Division of Medicinal Chemistry, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964.

(3) Taken in part from the Master's Thesis of James D. Westover submitted to the Department of Chemistry, Arizona State University, Tempe, Ariz., 1961.

(4) G. H. Hitchings and C. P. Rhodes, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

(5) For a detailed discussion of the antitumor activity and structural relationships of purine derivatives, see R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964), and references cited therein.

(6) (a) Ciba Foundation Symposium on the Chemistry and Biology of Purines, Little, Brown and Co., Boston, Mass., 1957; (b) H. E. Skipper and L. L. Bennett, Jr., *Ann. Rev. Biochem.*, **27**, 137 (1958); (c) J. A. Montgomery, *Cancer Res.*, **19**, 447 (1959).

(7) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Am. Chem. Soc.* **81**, 3046 (19591.

(8) (a) A collection of 240 references and data on 2255 nitrogen mustard derivatives (one- and two-armed) has been published in a recent survey; *cf.*, R. P. Bratzel, R. B. Ross, T. H. Goodridge, W. T. Huntress, M. T. Flather, and D. E. Johnson, *Cancer Chemotherapy Rept.*, **No. 26**, 1 (1963); (b) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co., London, 1962; (c) S. S. Brown, *Advan. Pharmacol.*, **2**, 243 (1963), and references cited therein; (d) E. Hirschberg, *Cancer Res.* (Supplement), **23**, 521 (1963).

(9) B. A. Booth and A. C. Sartorelli, *Science*, **138**, 518 (1962).

(10) (a) J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, 327 (1960); (b) S. S. Epstein and G. M. Timmis, *Biochem. Pharmacol.*, **11**, 743 (1962).

(11) H. H. Lin and C. C. Price, *J. Org. Chem.*, **26**, 266 (1961).

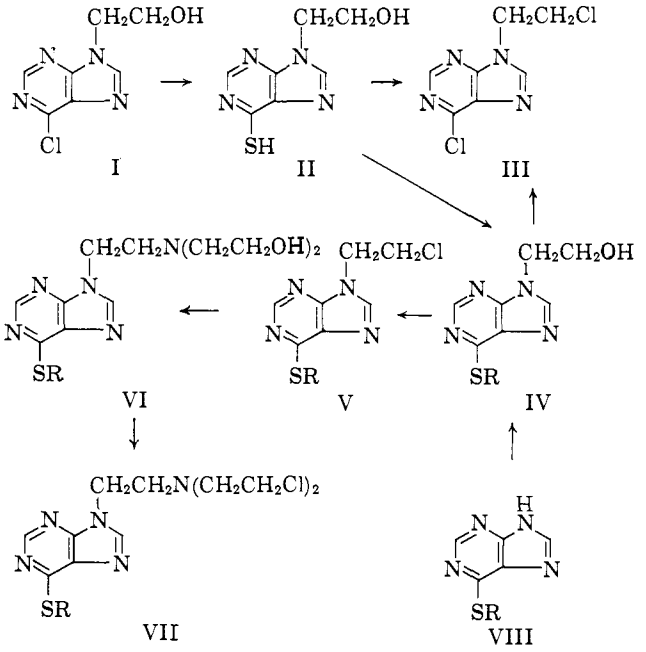
(12) R. J. Rutman, F. S. Lewis, S. Buckner, C. C. Price, F. Llewellyn, and E. Owens, *Cancer Res.* (Supplement), **22**, 559 (1962).

(13) J. H. Burckhalter and D. S. Bariana, unpublished work.

(14) G. Levin, K. Sugiura, and G. B. Brown, *J. Med. Chem.*, **7**, 357 (1961).

(15) (a) M. Ikehara and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **9**, 27 (1961); (b) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

(16) C. W. Noell and R. K. Robins, *ibid.*, **81**, 5997 (1959).

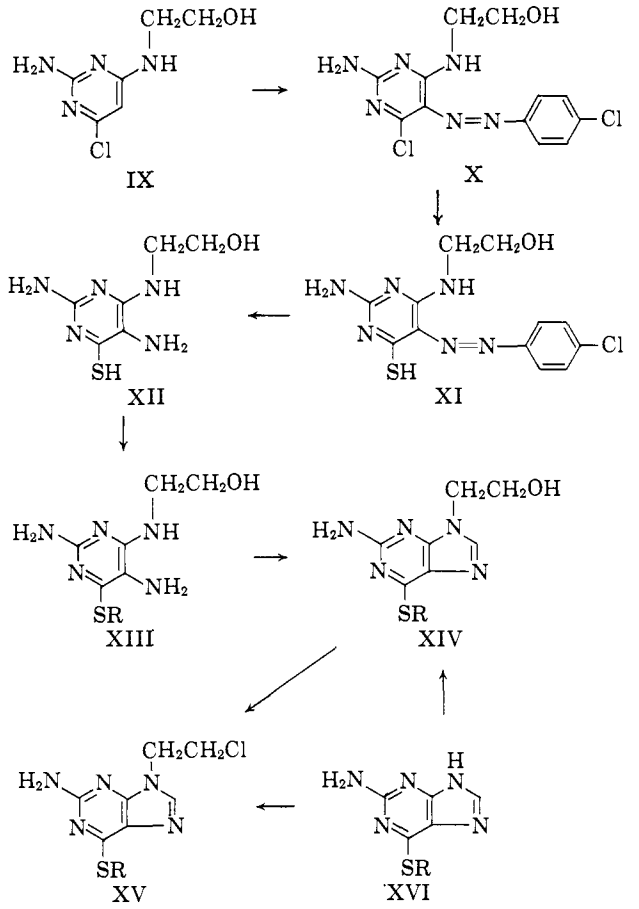


could also be prepared by direct alkylation of 6-(alkylthio)purines (VIII) with ethylene bromohydrin in dimethyl sulfoxide. The procedure used was similar to that described by Montgomery and Temple[15b] for the alkylation of various chloropurines. Chlorination of IV with thionyl chloride afforded the 6-alkylthio-9-(β-chloroethyl)purines (V) in good yield. Attempted preparation of 9-(β-chloroethyl)-6-purinethiol by the chlorination of II with thionyl chloride, however, yielded 9-(β-chloroethyl)-6-chloropurine (III). This is the first example of a mercapto group being changed to a chloro group by means of thionyl chloride.

The reaction between V and diethanolamine has been found to proceed quite readily in 2-ethoxyethanol. In this manner the 6-alkylthio-9-[bis(β-hydroxyethyl)-aminoethyl]purines (VI) were obtained. Thionyl chloride then successfully converted VI to the desired 6-alkylthio-9-[bis(β-chloroethyl)aminoethyl]purines (VII), which were isolated and purified as hydrochloride salts.

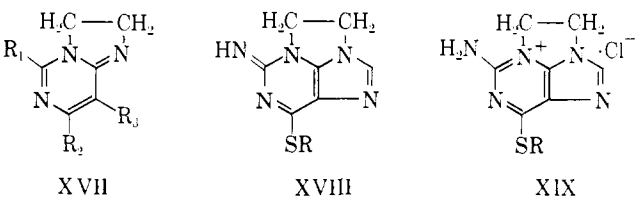
The corresponding thioguanine derivatives were prepared as follows. 2-Amino-4,6-dichloropyrimidine[17] reacted with 2-aminoethanol to give a monosubstituted (β-hydroxyethyl)amino derivative (IX). Utilizing the procedure of Boon,[18] IX was coupled with *p*-chlorobenzenediazonium chloride to yield a 5-(*p*-chlorophenylazo) derivative (X). Treatment of X with sodium hydrosulfide in refluxing ethanol readily replaced the chlorine atom by the thio group to form XI. A sodium hydrosulfite reduction of XI yielded 2,5-diamino-4-[(β-hydroxyethyl)amino]-6-pyrimidinethiol (XII). When XII was treated with benzyl chloride, the corresponding 6-(benzylthio)pyrimidine (XIII, R = $C_6H_5CH_2$) was obtained in good yield.

Cyclization of XIII (R = $C_6H_5CH_2$) to 2-amino-6-benzylthio-9-(β-hydroxyethyl)purine (XIV, R = $C_6H_5CH_2$) was readily accomplished in a mixture of triethyl orthoformate and acetic anhydride. Compounds XIV can be prepared readily by direct alkylation of 6-substituted thioguanine (XVI) with ethylene bromohydrin, which provided the desired products in better yield and higher purity.



The conversion of XIV to the 9-substituted one-armed mustard derivative of 6-(alkylthio)guanines (XV) was performed by procedures similar to those used for the conversion of 6-alkylthio-9-(β-hydroxyethyl)purines (IV) to the corresponding 6-alkylthio-9-(β-chloroethyl) derivatives (V). Compounds XV could also be prepared by direct alkylation of 2-amino-6-(alkylthio)purine (XVI) with 2-bromoethyl chloride in 40–50% yield.

Ramage and co-workers[19] noted that some 4-(β-chloroethylamino)pyrimidines having either a 5-amino group or a potentially tautomeric group at position 2 readily cyclized to a dihydroimidazolo[2,3-*c*]pyrimidine (XVII). Since compounds XV contain a 2-amino group as well as a β-chloroethylamino moiety [the latter corresponds to a 4-(β-chloroethylamino) moiety substituted to a pyrimidine ring system], the possibilities of XV to possess structures XVIII or XIX were not overlooked. Elementary analyses of



these compounds rule out structure XVIII, and negative tests for ionizable halide eliminated the proposed alternate structure XIX for these chlorinated products. These results clearly indicated that internal cyclization[19] was not noted with compounds XV and hence

(17) H. Büttner, *Ber.*, **36**, 2227 (1903).
(18) W. R. Boon, *J. Chem. Soc.*, 2146 (1957).
(19) (a) G. R. Ramage and G. Trappe, *ibid.*, 4410 (1952); (b) J. Clark and G. R. Ramage, *ibid.*, 2821 (1958).

the structural assignment for XV as 2-amino-6-alkylthio-9-(β-chloroethyl)purines is correct.

Preliminary testing results (see Table I) indicated that with the exception of 6-methylthio-9-(β-hydroxyethyl)purine all the 6-S-substituted 9-(β-hydroxyethyl)- or 9-(β-chloroethyl)purines possess antitumor activity in Adenocarcinoma 755 system in mice. In particular, both 6-benzylthio-9-(β-chloroethyl)purine and 6-(*o*-chlorobenzylthio)-9-(β-chloroethyl)purine are active against Solid Friend virus leukemia and in the cell culture testing system. The corresponding 9-[bis(β-hydroxyethyl)aminoethyl] and 9-[bis(β-chloroethyl)aminoethyl] derivatives are much less active than their parent 9-β-hydroxyethyl or 9-β-chloroethyl compounds.

Experimental[20]

9-(β-Hydroxyethyl)-6-purinethiol (II).—A mixture of 6-chloro-9-(β-hydroxyethyl)purine (19.9 g., 0.1 mole) and thiourea (22.8 g., 0.3 mole) in 1 l. of absolute ethanol was refluxed with stirring for 3 hr. The resulting precipitate was separated by filtration, washed with cold ethanol, and dried at 80°. The product was recrystallized from ethanol.

9-(β-Chloroethyl)-6-chloropurine (III).—A solution of 9-(β-hydroxyethyl)-6-purinethiol (II) (3.92 g., 0.02 mole) in thionyl chloride (100 ml.) was heated at reflux for 1 hr. At the end of this time, the solution was evaporated to dryness at reduced pressure. The residual sirup was covered with absolute ethanol (50 ml.), heated to reflux, and once again evaporated to dryness. The resulting yellow solid was recrystallized twice from heptane to give 2.82 g. (65% yield) of analytically pure product, m.p. 106–108°, λ_{max}^{EtOH} 263 mμ (ε 10,100). The product was found to be identical with that prepared by Montgomery and Temple.[15b]

6-Alkylthio-9-(β-hydroxyethyl)purines (IV). General Procedure. A.—A solution of II (19.6 g., 0.1 mole) in 350 ml. of 1 *N* NaOH was warmed on a water bath at 45°. An equivalent amount of alkyl halide in 50 ml. of absolute ethanol was added dropwise to the warm solution with gentle stirring. After the addition was complete the mixture was stirred at 45–50° for 2 hr. and then allowed to cool slowly to room temperature. The precipitated product was separated by filtration, washed with water, ice-cold ethanol, and ether, and then dried at 80°. The products were recrystallized from water.

B. Preparation of 6-Benzylthio-9-(β-hydroxyethyl)purine (IV, R = $C_6H_5CH_2$).—To a solution of 6-benzylthiopurine[21] (VIII, R = $C_6H_5CH_2$) (24.2 g., 0.1 mole) in 250 ml. of dimethyl sulfoxide was added anhydrous K_2CO_3 (27.6 g., 0.2 formula wt.) and ethylene bromohydrin (25.0 g., 0.2 mole). The mixture was stirred at room temperature for 3 hr. after which time the resulting solid was separated by filtration, washed with ice-cold water, and finally recrystallized from water to give 16.3 g. (57% yield) of the desired product, m.p. 139–140°. Analytical and ultraviolet absorption data indicated that the product was identical with that prepared by method A.

6-Alkylthio-9-(β-chloroethyl)purines (V). General Procedure.—Five grams of IV was added to 100 ml. of thionyl chloride at room temperature. The mixture was refluxed on the steam bath for 1 hr., and the resulting solutions were evaporated to dryness under reduced pressure. The residue was recrystallized twice from petroleum ether (b.p. 60–100°) to afford a product of analytical purity.

6-Alkylthio-9-[bis(β-hydroxyethyl)aminoethyl]purines (VI). General Procedure.—A solution of 0.1 mole of V and 21.0 g. (0.2 mole) of diethanolamine in 250 ml. of 2-ethoxyethanol was refluxed for 14 hr., then evaporated under reduced pressure. The residual brown oil was dissolved in 200 ml. of water, and the resulting solution was extracted with three 150-ml. portions of chloroform. The chloroform extract was treated with decolorizing charcoal, dried (Na_2SO_4), and evaporated to dryness. The resulting residue was recrystallized from a mixture of ethyl acetate and heptane to yield VI of analytical purity.

6-Alkylthio-9-[bis(β-chloroethyl)aminoethyl]purines (VII). General Procedure.—To a well-stirred solution of 0.025 mole of VI in anhydrous chloroform maintained at 5° was added dropwise a solution of 50 ml. of thionyl chloride in 50 ml. of chloroform. During the addition a resinous material separated but gradually redissolved giving a complete solution at the end of addition. The solution was then stirred and heated gently at reflux for 1 hr. after which time it was evaporated under reduced pressure to yield a yellow sirup. The residual thionyl chloride was removed by covering the sirup with methanol, heating at reflux for 10 min., and evaporating again under reduced pressure. The resulting glass-like product was dissolved in 200 ml. of absolute ethanol, cooled to 0°, and saturated with anhydrous HCl. The clear solution was evaporated to dryness at reduced pressure to yield a colorless glass, which gradually crystallized after triturating with anhydrous ether. Although repeated attempts to crystallize the product had failed, a product of analytical purity could be obtained by dissolving the material in anhydrous chloroform and precipitating it with anhydrous ether. These products were quite hygroscopic, but good analytical data could be obtained by drying the samples overnight at room temperature and 0.25 mm. pressure.

2-Amino-4-chloro-6-[(β-hydroxyethyl)amino]pyrimidine (IX). A suspension of 2-amino-4,6-chloropyrimidine[15] (24.6 g., 0.15 mole) and 2-aminoethanol (18.3 g., 0.3 mole) in 250 ml. of absolute ethanol was stirred and heated at reflux for 2 hr. The solution was treated with decolorizing charcoal and filtered. The yellow filtrate was evaporated to dryness under reduced pressure to yield a semisolid, which was triturated with 200 ml. of water and chilled, and the crude product was separated by filtration. The product was then purified by *recrystallizing* from water to give 20.6 g. (73% yield) of pure product: m.p. 146–148°; $\lambda_{max}^{pH\ 1}$ 231 mμ (ε 11,900), 298 mμ (ε 3800); $\lambda_{max}^{pH\ 7}$ 273 mμ (ε 7700); $\lambda_{max}^{pH\ 13}$ 237 mμ (ε 10,400), 285 mμ (ε 9300).

Anal. Calcd. for $C_6H_9ClN_4O$: C, 38.2; H, 4.7; N, 29.7. Found: C, 38.0; H, 4.8; N, 29.2.

2-Amino-4-chloro-5-(*p*-chlorophenylazo)-6-[(β-hydroxyethyl)amino]pyrimidine (X).—A solution of 18.9 g. (0.1 mole) of IX and 100 g. of sodium acetate in 500 ml. of 50% acetic acid was stirred at room temperature. To this soution was added a solution of *p*-chlorobenzenediazonium chloride [prepared by the usual procedure from 12.6 g. (0.1 mole) of *p*-chloroaniline in 200 ml. of 2 *N* HCl]. The resulting mixture was stirred at room temperature for 20 hr. The solid product was collected by filtration, washed with water and ethanol, and air dried. Recrystallization of the product from a mixture of dimethylformamide and water gave 23.0 g. (71% yield) of analytically pure product: m.p. 229–231°; $\lambda_{max}^{pH\ 1}$ 237 mμ (ε 17,000), 367 mμ (ε 27,100); $\lambda_{max}^{pH\ 10}$ 304 mμ (ε 5900), 396 mμ (ε 14,400).

Anal. Calcd. for $C_{12}H_{10}Cl_2N_6O$: C, 44.0; H, 3.7; N, 25.6. Found: C, 43.7; H, 3.9; N, 25.1.

2-Amino-5-(*p*-chlorophenylazo)-6-(β-hydroxyethyl)amino-4-pyrimidinethiol (XI).—A suspension of X (32.7 g., 0.1 mole) and sodium hydrosulfide (32.7 g.) in 500 ml. of absolute ethanol was refluxed for 20 hr. with gentle stirring. The precipitated product was separated by filtration, washed with water and ethanol, and air dried. The product was purified by recrystallizing from a mixture of dimethylformamide and water to give 25.1 g. (78% yield) of analytically pure product: m.p. 265–267° dec.; $\lambda_{max}^{pH\ 1}$ 292 mμ (ε 17,200), 326 mμ (ε 8100), 450 mμ (ε 21,000); $\lambda_{max}^{pH\ 10}$ 292 mμ (ε 20,400), 415 mμ (ε 17,500).

Anal. Calcd. for $C_{12}H_{13}ClN_6OS$: C, 44.3; H, 4.0; N, 25.8. Found: C, 44.6; H, 4.1; N, 26.0.

2,5-Diamino-6-(β-hydroxyethyl)amino-4-pyrimidinethiol (XII).—To a stirred suspension of XI (10.0 g., 0.031 mole) in 100 ml. of boiling water was added, in small portions, 20 g. of sodium hydrosulfite. During this addition the yellowish color gradually faded yielding a colorless solution.

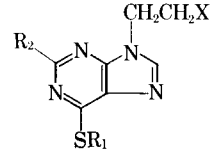
After the addition was complete, the solution was boiled for 20 min., treated with decolorizing charcoal, and filtered. The filtrate was allowed to cool, and the resulting white crystals were collected by filtration. Recrystallization of the product from a minimum amount of water gave 3.2 g. (52%) of analytically pure product: m.p. 227–229°; $\lambda_{max}^{pH\ 1}$ 233 mμ (ε 18,500), 310 mμ (ε 24,000); $\lambda_{max}^{pH\ 7}$ 317 mμ (ε 13,300).

Anal. Calcd. for $C_6H_{11}N_5OS$: C, 35.8; H, 5.5; N, 34.8. Found: C, 35.6; H, 5.8; N, 34.6.

(20) All melting points (corrected) were taken on a Thomas-Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2. See Table II for analytical data.

(21) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

Table I: Antitumor Activities of 9-Substituted Nitrogen Mustard Derivatives of 6-S-Substituted Purines and Related Compounds[a]

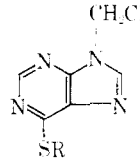


			Dose,[b]	Test/Control									KB cell culture	
R_1	R_2	X	mg./kg.	Ca755	S180	LE-L1210	WA 256	HE 129	FV	LL	DL	DA	Slope[c]	ED_{50},[d] γ/ml.
CH_3	H	OH	25	1.19										
$C_6H_5CH_2$	H	OH	100	0.01										
			50	0.03										
			25	0.17										
o-$ClC_6H_4CH_2$	H	OH	300	0.00										
			150	0.05										
			75	0.07										
CH_3	H	Cl	100				0.53						...	$>1.0 \times 10^2$
			75	0.00										
			25	0.00			0.93				1.00			
			12.5	0.04										
			6.2	0.34										
$C_6H_5CH_2$	H	Cl	240							0.46	1.20			
			180								1.08	1.36	−0.21	9.3×10^{-1}
			120	0.00			1.28	0.85	0.35	0.77		1.25		
			25	0.01			0.92					1.25		
			18						0.85					
			12.5	0.05								1.09		
			6.2	0.17										
			3.1	0.63										
o-$ClC_6H_4CH_2$	H	Cl	240	0.00						0.64			−2.2	8.1×10^{-1}
			200						0.25					
			100				1.15		0.43	0.58	1.08	1.14		
			60	0.00						0.75		1.14		
			50	0.00			1.06		0.43			1.07		
			30	0.03	1.14						1.08			
			25	0.03			0.77		0.60			1.07		
			12.5	0.08					0.40					
			6.2	0.30										
CH_3	NH_2	OH											...	$>1.0 \times 10^2$
$C_6H_5CH_2$	NH_2	OH											−1.10	2.7×10^1
$C_6H_5CH_2$	H	$N(CH_2CH_2OH)_2$	500		1.29								−0.81	1.6×10^1
			400	0.68		1.03								
o-$ClC_6H_4CH_2$	H	$N(CH_2CH_2OH)_2$											−1.10	3.2×10^1
CH_3	H	$N(CH_2CH_2Cl)_2$	20		0.32								−0.73	5.6×10^1
			5		0.69									
			4	0.52		1.05								
$C_6H_5CH_2$	H	$N(CH_2CH_2Cl)_2$											...	$>1.0 \times 10^2$
o-$ClC_6H_4CH_2$	H	$N(CH_2CH_2Cl)_2$											−0.80	3.6×10^1

[a] The biological testing was performed by the screening contractors of the Cancer Chemotherapy National Service Center. Ca755 = Adenocarcinoma 755, S180 = Sarcoma 180, LE-L1210 = lymphoid leukemia L1210, WA 256 = Walker 256 (subcutaneous), HE 129 = Hepatoma 129, FV = Friend virus leukemia (solid), LL = Lewis lung carcinoma, DL = Dunning leukemia (solid), DA = Dunning ascites leukemia. [b] Below toxicity level. [c] Slope: change of response for each one-log change of dose. [d] ED_{50}: the dose that inhibits growth to 50% of control growth.

TABLE II

6-ALKYLTHIO-9-(β-SUBSTITUTED)ETHYLPURINES



R	R′	Yield, %	M.p., °C.	% calcd. C	% calcd. H	% calcd. N	% found C	% found H	% found N	Ultraviolet absorption, pH 1: λmax, mμ	pH 1: ε × 10⁻³	pH 11: λmax, mμ	pH 11: ε × 10⁻³
H	OH	75	283–284	42.8	4.1	28.6	43.1	4.3	28.8	224	9.6	232	15.1
										320	21.8	308	23.0
CH_3	OH	90	197–198	45.7	4.7	26.7	45.5	4.4	26.4	293	17.7	289	20.0
$CH_2C_6H_5$	OH	87	139–140	58.8	4.9	19.6	59.1	5.0	19.5	293	20.1	289	20.9
m-$CH_2C_6H_4Cl$	OH	85	130–131	52.4	4.1	17.5	52.5	4.1	17.3	293	18.2	289	20.2
CH_3	Cl	66	152–153	42.0	3.9	24.5	42.3	4.0	24.7	283[a]	17.8		
$CH_2C_6H_5$	Cl	76	93–94	55.2	4.3	18.4	55.2	4.4	18.5	285[a]	20.2		
m-$CH_2C_6H_4Cl$	Cl	94	91–92	49.5	3.5	16.5	49.2	3.7	16.1	285[a]	20.6		
CH_3	$N(CH_2CH_2OH)_2$	46	86–87	48.4	6.8	23.5	48.3	6.7	23.3	229	12.2	228	8.9
										306	19.0	286	19.0
$CH_2C_6H_5$	$N(CH_2CH_2OH)_2$	70	89–91	57.9	6.2	18.7	57.8	6.6	18.4	291	19.4	292	18.7
m-$CH_2C_6H_4Cl$	$N(CH_2CH_2OH)_2$	73	100–101	53.0	5.4	17.2	53.0	5.5	17.3	291	19.2	289	19.2
CH_3	$N(CH_2CH_2Cl)_2$	71	190–191 dec.	38.9[b]	4.8[b]	18.8[b]	38.7	4.9	18.4	224	10.7	230	6.1
										293	16.6	290	17.3
$CH_2C_6H_5$	$N(CH_2CH_2Cl)_2$	55	104–106 dec.	46.5[c]	5.2[c]	15.1[c]	46.2	4.9	14.9	293	19.2	289	20.8
m-$CH_2C_6H_4Cl$	$N(CH_2CH_2Cl)_2$	72	60–62 dec.	43.3[c]	4.5[c]	14.0[c]	42.9	4.6	13.6	292	17.4	297	20.0

[a] Determined in ethanol. [b] Isolated and analyzed as a hydrochloride salt. [c] Isolated and analyzed as a hydrochloride monohydrate.

2,5-Diamino-4-benzylthio-6-[(β-hydroxyethyl)amino]pyrimidine (XIII, R = $C_6H_5CH_2$).—A solution of benzyl chloride (6.9 g., 0.055 mole) in 25 ml. of absolute ethanol was added dropwise to a stirred solution of XII (10.1 g., 0.05 mole) in 200 ml. of 5% KOH at room temperature. The product gradually precipitated during the addition. After the addition was complete, the mixture was stirred at room temperature for 2 hr., and the precipitate was collected by filtration. Recrystallization from benzene gave 12.3 g. (85%) of analytically pure product: m.p. 128–129°; $\lambda_{max}^{pH\ 1}$ 238 mμ (ε 15,400), 318 mμ (ε 9000); $\lambda_{max}^{pH\ 11}$ 225 mμ (ε 18,000), 316 mμ (ε 10,200).

Anal. Calcd. for $C_{13}H_{17}N_5OS$: C, 53.7; H, 5.9; N, 24.0. Found: C, 53.5; H, 6.0; N, 23.5.

2-Amino-6-benzylthio-9-(β-hydroxyethyl)purine (XIV, R = $C_6H_5CH_2$). A.—A solution of 10 g. (0.034 mole) of XIII (R = $C_6H_5CH_2$) in 125 ml. of triethyl orthoformate and 125 ml. of acetic anhydride was refluxed for 4 hr. The solution was then evaporated under reduced pressure. The residual glass was covered with 100 ml. of 1 *N* NaOH and this mixture refluxed for 5 min. The crude tan solid product was filtered, washed with water, and air dried. Recrystallization from ethyl acetate gave 5.2 g. (51% yield) of analytically pure product: m.p. 181–182°; $\lambda_{max}^{pH\ 1}$ 247 mμ (ε 9600), 319 mμ (ε 12,000); $\lambda_{max}^{pH\ 11}$ 245 mμ (ε 11,400), 310 mμ (ε 12,300).

Anal. Calcd. for $C_{14}H_{15}N_5OS$: C, 55.8; H, 5.0; N, 23.2. Found: C, 55.6; H, 4.9; N, 22.9.

B.—To a solution of 25.7 g. (0.1 mole) of 2-amino-6-(benzylthio)purine[22] (XVI, R = $C_6H_5CH_2$) in 180 ml. of dimethyl sulfoxide stirred at room temperature was added anhydrous K_2CO_3 (27.6 g., 0.2 formula wt.) followed by ethylene bromohydrin (25.0 g., 0.2 mole). The resulting mixture was stirred at room temperature for 3 hr. and then poured onto 500 g. of flaked ice. The ice mixture was allowed to stand for 15 hr., and the precipitated product was separated by filtration. Recrystallization from ethyl acetate gave 16.3 g. (54% yield) of analytically pure product. Melting point, elementary analysis, ultraviolet absorption, and paper chromatography of the product indicated that it was identical with that prepared by method A.

2-Amino-6-methylthio-9-(β-hydroxyethyl)purine (XIV, R = CH_3).—To a stirred solution of 18.1 g. (0.1 mole) of 2-amino-6-(methylthio)purine[23] (XVI, R = CH_3) in 100 ml. of dimethyl sulfoxide at room temperature was added anhydrous K_2CO_3 (27.6 g., 0.2 formula wt.) and ethylene bromohydrin (25.0 g., 0.2 mole). After the addition was complete, the resulting mixture was stirred at room temperature for 3 hr. and then evaporated below 80° under reduced pressure. The solid residue was extracted with 500 ml. of boiling isopropyl alcohol and the extract was evaporated. Recrystallization of the residue from a small amount of isopropyl alcohol gave 10.1 g. (45%) of pure product: m.p. 164–166°; $\lambda_{max}^{pH\ 1}$ 247 mμ (ε 10,800), 319 mμ (ε 11,900); $\lambda_{max}^{pH\ 11}$ 244 mμ (ε 12,800), 308 mμ (ε 12,400).

Anal. Calcd. for $C_8H_{11}N_5OS$: C, 42.6; H, 4.9; N, 31.1. Found: C, 42.8; H, 5.2; N, 30.6.

2-Amino-6-benzylthio-9-(β-chloroethyl)purine (XV, R = $C_6H_5CH_2$). A.—A mixture of 3.0 g. (0.01 mole) of XIV (R = $C_6H_5CH_2$) and 75 ml. of thionyl chloride was heated to reflux on the steam bath for 1 hr. (A complete solution was formed within 5 min.) The solution was evaporated under reduced pressure to yield a red tarry material. The residual thionyl chloride was removed by covering the material with methanol and heating at reflux for 10 min., and was evaporated once again under reduced pressure. The red residue was then covered with 50 ml. of ethanolic ammonia (anhydrous ethanol saturated with ammonia at 0°). The resulting solution was evaporated under reduced pressure to yield a yellow solid. The semisolid was extracted with three 200-ml. portions of boiling heptane, and the heptane extracts were evaporated to 100 ml., then allowed to cool. White crystals which deposited on cooling were collected by filtration and air dried. The crude product was recrystallized twice from heptane and once from a minimum amount of absolute ethanol to give 0.75 g. (23.5% yield) of XV (R = $C_6H_5CH_2$), which failed to yield a positive ionizable halide test; m.p. 123–124°; $\lambda_{max}^{ethanol}$ 245 mμ (ε 12,700), 310 mμ (ε 11,200).

Anal. Calcd. for $C_{14}H_{14}ClN_5S$: C, 52.6; H, 4.4; N, 21.9. Found: C, 52.6; H, 4.7; N, 22.1.

B.—Anhydrous potassium carbonate (27.6 g., 0.2 formula wt.) and 2-bromoethyl chloride (15.7 g., 0.11 mole) were added at room temperature to a stirred solution of 2-amino-6-(benzylthio)-purine (XVI, R = CH_3) (25.8 g., 0.1 mole) in 100 ml. of dimethyl sulfoxide. After stirring 3 hr. at room temperature, the reaction mixture was poured onto 500 g. of ice flakes. The mixture was stirred for 30 min. and the precipitated product was separated by filtration. The product was washed with water, air dried, and recrystallized from a mixture of benzene and heptane (1:10) to give 14.1 g. (44%), m.p. 123–124°. The product was

(22) F. O. Leonard, C. G. Skinner, E. M. Lansford, Jr., and W. Shive, *J. Am. Chem. Soc.*, **81**, 907 (1959).

(23) J. A. Montgomery and L. B. Holum, *ibid.*, **79**, 2185 (1957).

found to be identical with that prepared by the preceding procedure.

2-Amino-6-methylthio-9-(β-chloroethyl)purine (XV, R = CH_3).—A solution of 18.1 g. (0.1 mole) of 2-amino-6-(methylthio)purine (XVI, R = CH_3) in 100 ml. of dimethyl sulfoxide was stirred at room temperature. To this solution was added anhydrous K_2CO_3 (27.6 g., 0.2 formula wt.) and 2-bromoethyl chloride (15.7 g., 0.11 mole). After stirring for 3 hr. at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The yellow residue was extracted with two 75-ml. portions of boiling benzene and the crude product was obtained by evaporation of the benzene extract. Recrystallization from a 1:10 mixture of benzene–heptane gave 11.2 g. (46%) of pure product which gave a negative ionizable halide test; m.p. 141–142°; $\lambda_{max}^{ethanol}$ 243 mμ (ε 14,800), 307 mμ (ε 11,500).

Anal. Calcd. for $C_8H_{10}ClN_5S$: C, 39.4; H, 4.1; N, 28.7. Found: C, 39.4; H, 4.4; N, 28.5.

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Nitrogen Mustards Derived from 3,4-Dihydro-2,4-dioxo-1(2H)-pyrimidinepropionic and -butyric Acids[1]

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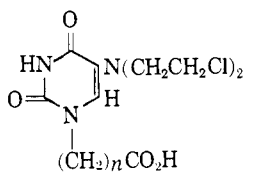
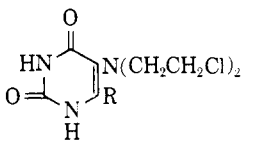
The two uracil nitrogen mustards, ω-[5-bis(2-chloroethyl)amino-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidine]-propionic and -butyric acids (I and II) have been synthesized. Reaction of β-ethoxyacryloyl isocyanate with the appropriate ω-aminoalkanoic acid ester and cyclization in base afforded the uracilalkanoic acid. Nitration gave the nitrouracilalkanoic acid which was esterified, reduced to the amine, and hydroxyethylated. Chlorination with phosphoryl chloride proceeded smoothly to afford the mustards I and II. These demonstrated low toxicity and borderline activity against Walker 256 in the rat.

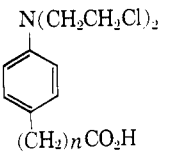
Uracil mustard (III) and its 6-methyl derivative, Dopan (IV), are useful clinically in the treatment of various malignancies in man.[2] Another nitrogen mustard that has clinical utility is chlorambucil (V, $n = 3$).[3a] Its homologs (V, $n \neq 3$) show less biological activity than V ($n = 3$) but more than the parent aromatic amine, bis(2-chloroethyl)aniline.[3b] The alkanoic acid side chain causes significant changes in chemical and biological properties. Consideration of the activity of both types of alkylating agents III and V suggested the synthesis of the uracil nitrogen mustards I and II. They contain the elements of both and may be considered as uracil analogs of chlorambucil in which alkanoic acid side chains are attached to the heterocyclic amine III. If I and II should possess antitumor activity, conceivably one may show greater activity than the other. The synthesis and antitumor evaluation of I and II are presented in this manuscript.



I, $n=2$
II, $n=3$



III, R=H
IV, R=CH_3



V

The uracilpropionic acid VIIIa was prepared by the general scheme of uracil synthesis of Shaw and his co-workers.[4] For the following steps, the procedures were simplified and the yields increased significantly. Sodium β-ethoxyacrylate was converted to the acid chloride,[4c] then to β-ethoxyacryloyl isocyanate (VI),[4a] and condensed with ethyl β-alanate, all in one step, to afford the acylurea VIIa. This was cyclized to the uracilpropionic acid VIIIa[5] in quantitative yield. The literature method[4b] was modified by heating at 60° for 2.5 hr. and using a medium of aqueous sodium hydroxide and 1,2-dimethoxyethane. Omission of the 1,2-dimethoxyethane caused incomplete solution of VIIa and gave lower yields of product. Reaction for shorter periods at higher temperatures was also less satisfactory.

Nitration of VIIIa proceeded in either sulfuric acid or acetic acid–sulfuric acid to give the nitrouracil IXa. Esterification to Xa, followed by catalytic hydrogenation over palladium on charcoal afforded the aminouracil XIa.

Hydroxyethylation of XIa with 2 moles of ethylene oxide in glacial acetic acid gave a chromatographically

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) H. G. Petering, H. H. Buskirk, E. A. Musser, and J. S. Evans, *Cancer Chemotherapy Rept.*, **27**, 1 (1963), have summarized the biological activities of III and IV.

(3)(a) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953); (b) W. C. J. Ross, *Ann. N. Y. Acad. Sci.*, **68**, 669 (1958).

(4)(a) G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 157 (1958); (b) J. H. Dewar and G. Shaw, *ibid.*, 583 (1962); (c) G. Shaw and R. N. Warrener, *ibid.*, 153 (1958).

(5) After the completion of this work, the preparation of VIIIa by the reaction of uracil with acrylic acid derivatives in liquid ammonia[6] and with acrylonitrile in aqueous alkali[7] was reported.

(6) Y. P. Shvachkin, M. T. Azarova, and I. L. Rapanovich, *Vestn. Mosk. Univ. Ser. II, Khim.*, **18**, 68 (1963); *Chem. Abstr.*, **59**, 15283 (1963).

(7) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1762 (1964).