Synthesis and Biological Activities of Some N^4-Substituted 4-Aminopyrazolo[3,4-*d*]pyrimidines[1]

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Syntheses and biological activities of 26 N^4-substituted 4-aminopyrazolo[3,4-*d*]pyrimidines as analogs of naturally occurring modified nucleic acid bases, *N*-(purin-6-ylcarbamoyl)-L-threonine and N^6-(Δ^2-isopentenyl)adenine, are described. 4-Aminopyrazolo[3,4-*d*]pyrimidine was converted into the desired intermediate, ethyl pyrazolo[3,4-*d*]pyrimidine-4-carbamate (**2**). 4-Ureidopyrazolo[3,4-*d*]pyrimidines (**6–26**) were prepared by displacement of the ethoxy group of the carbamate **2** by amino acids and a variety of amines and by a reaction of 4-aminopyrazolo-[3,4-*d*]pyrimidine (**1**) with isocyanates. N^4-Alkylaminopyrazolo[3,4-*d*]pyrimidines were generally prepared by displacement of the chlorine from 4-chloropyrazolo[3,4-*d*]pyrimidine with various amines. Several analogs exhibited moderate to very good growth inhibitory activities against cultured L1210 leukemia and 6410 human leukemic myeloblasts.

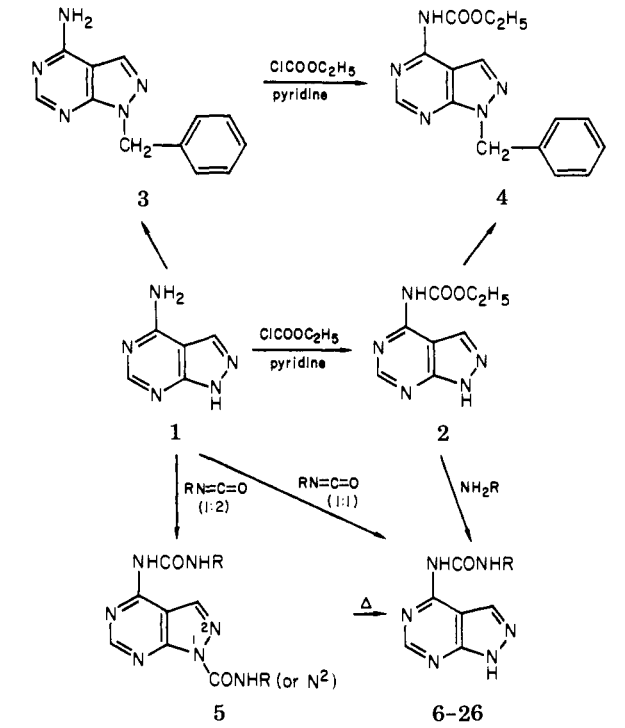
Various structural changes in the naturally occurring purine derivatives have resulted in pharmacologically useful compounds. Since the analogs of the naturally occurring tRNA bases, *N*-(purin-6-ylcarbamoyl)-L-threonine and N^6-(Δ^2-isopentenyl)adenine, have shown biological activities,[2,3] various isomeric analogs derived from the pyrazolo[3,4-*d*]pyrimidine nucleus were prepared. This paper describes the synthesis, chemical properties, and growth inhibitory activities of the 26 4-ureido- and 4-alkylaminopyrazolo[3,4-*d*]pyrimidines.

Chemistry. The 4-ureidopyrazolo[3,4-*d*]pyrimidines (**6–26**) were prepared by the urethane and the isocyanate methods (Scheme I). In the case of the urethane method, the key intermediate, ethyl pyrazolo[3,4-*d*]pyrimidine-4-carbamate (**2**), was prepared in 86% yield by reaction of ethyl chloroformate with 4-aminopyrazolo[3,4-*d*]-pyrimidine[4] (**1**). Unlike the reaction of adenine with ethyl chloroformate,[2a] the acylation occurred at the N^4 position of **1** when equimolar amounts of 4-aminopyrazolo[3,4-*d*]pyrimidine (**1**) and ethyl chloroformate in pyridine were allowed to react. The structure of ethyl pyrazolo[3,4-*d*]pyrimidine-4-carbamate (**2**) was assigned on the basis of chemical analysis, chemical reactions, and spectral data as described below. Ethyl 1-benzylpyrazolo[3,4-*d*]-pyrimidine-4-carbamate (**4**) was synthesized by reaction of 1-benzyl-4-aminopyrazolo[3,4-*d*]pyrimidine (**3**)[5] with ethyl chloroformate in pyridine. Alternatively, compound **4** was also obtained by an alkylation of carbamate **2** with benzyl chloride. These reactions show that the carboethoxy group in **2** cannot be at the N^1 or N^2 position. The NMR spectrum of compound **1** in Me_2SO-d_6 showed the proton absorption peak for the 4-amino group at δ 7.68, while the corresponding peak in compound **2** was absent. It was replaced by an absorption at δ 11.1 for NH in **2**, thus indicating that the 4-amino group was substituted. Similarly, the NMR spectra of ethyl 1-benzylpyrazolo-[3,4-*d*]pyrimidine-4-carbamate (**4**) when compared to those of 1-benzyl-4-aminopyrazolo[3,4-*d*]pyrimidine (**3**) show that the peak for the 4-amino group of **3** at δ 7.72 was replaced by an absorption at δ 11.1 for NH of **4**. The relative stability of compound **2** in alkaline media as compared to the labile nature of known ring N-acylated adenine and pyrazolo[3,4-*d*]pyrimidine derivatives further supports the structure of the urethane **2**.

In the displacement reaction with amino acids and amines the urethane **2** was less reactive than the purine analog, ethyl purine-6-carbamate. Reactions with alkyl and aralkylamines and amino acids required a longer reaction time (16 h) to obtain the 4-ureidopyrazolo[3,4-*d*]pyrimidines (**6–26**) in 50–70% yield. The reaction with arylamines, such as aniline and *o*-fluoroaniline, for 24 h gave very poor yields of the desired product, since in this time period the urethane **2** degraded to 4-aminopyrazolo-[3,4-*d*]pyrimidine (**1**). In such cases the isocyanate method was useful. A reaction with equimolar amounts of 4-aminopyrazolo[3,4-*d*]pyrimidine (**1**) and an alkyl or an aryl isocyanate at room temperature for 24 h gave mostly 4-ureidopyrazolo[3,4-*d*]pyrimidine along with a small amount of the $N^1(N^2)$,N^4-dicarbamoyl derivative **5**. A reaction with an excess of isocyanate for prolonged time periods gave a mixture of the 4-ureido compound and the dicarbamoyl derivative **5**. The analogs belonging to series **5** with aryl or alkyl side chains can be converted to the 4-ureido compound by treatment with 1 N NaOH at 100° for 10 min. Since compounds **6–26** and the dicarbamoyl derivatives such as **5** moved very closely on TLC in various solvent systems, the products were differentiated by their ir spectra. The 4-ureido compounds **6–26** exhibited the C=O absorption at 1680–1690 cm^{-1} while the dicarbamoyl derivatives **5** showed an additional peak at 1720 cm^{-1}. 4-Alkylaminopyrazolo[3,4-*d*]pyrimidines (**27–31**) were prepared in about 70% yield from reaction of 4-chloropyrazolo[3,4-*d*]pyrimidine[4] and the corresponding amines.

In general, these pyrazolo[3,4-*d*]pyrimidine derivatives

Scheme I



were very insoluble in H_2O, thus requiring the preparation of more soluble derivatives such as nucleosides and nucleotides for testing in biological systems. These studies are in progress.

Biological Activity. The compounds were tested for their ability to inhibit growth of the cultured cells derived from the buffy coat of a normal individual (Nc 37) and a patient with myeloblastic leukemia (RPMI 6410) and mouse leukemia cells L1210 (Table I).[2b] In order to relate the magnitude of the antiproliferative activity, the compounds were assigned the rating of >++, ++, +, ±, and NA at 10^{-4} M (Table I) as described in the Experimental Section.

In our earlier work it was noted that 6-ureidopurines in which the side chain was derived from amino acids were usually inactive against the above cell lines.[2b] The L-threonine derivative **6** of pyrazolo[3,4-*d*]pyrimidine, however, showed excellent growth inhibitory activity against RPMI 6410 and L1210. The glycine derivative **7** was marginally active against these cell lines. The 6-ureidopurines with alkyl side chains such as *n*-butyl, *n*-pentyl, and isoamyl were quite active (70% inhibition at 10^{-4} M) against RPMI 6410.[2b] The corresponding pyrazolo[3,4-*d*]pyrimidine derivatives also showed strong to marginal growth inhibitory activity against RPMI 6410 and L1210 cells. Particularly the allyl (**9**), *tert*-butyl (**13**), *n*-pentyl (**14**), neopentyl (**16**), *n*-octyl (**17**), and adamantyl (**19**) derivatives were potent (60–70% inhibition) toward L1210. The propargyl (**10**), *n*-pentyl (**14**), *n*-octyl (**17**), ethoxyethyl (**18**), and adamantyl (**19**) compounds were also active against the leukemic myeloblasts RPMI 6410. In the alkylureido series the most active compounds ranged in the following order: *n*-octyl (**17**) > adamantyl (**19**) > L-threonyl (**6**) > *n*-pentyl (**14**). Among the phenyl-substituted side chains the phenylpropyl (**20**) and phenylbutyl (**21**) derivatives showed marginal activity against RPMI 6410. The phenylbutyl (**21**) compound was quite potent against L1210 cells.

The 6-phenylureidopurines were marginally active against RPMI 6410.[2b] However, corresponding phenyl (**22**), *m*-chlorophenyl (**25**), and *p*-chlorophenyl (**26**) analogs of pyrazolo[3,4-*d*]pyrimidines were very potent toward 6410, while *o*-fluorophenyl (**23**) and *o*-chlorophenyl (**24**) compounds were marginally active. Compounds **25** and **26** also showed strong activity against the growth of L1210 cells. Among the four phenylureidopyrazolo[3,4-*d*]pyrimidines, *m*-chloro (**25**) and *p*-chloro (**26**) derivatives were the most potent compounds. In the case of 4-alkylaminopyrazolo[3,4-*d*]pyrimidines, allyl (**29**), isoamyl (**30**), and benzyl (**31**) derivatives were quite potent toward RPMI 6410 and the latter two compounds were also very active against L1210. The glycine compound (**7**), propargylureido (**10**), phenylureido (**22**), *o*-fluorophenylureido (**23**), and *o*-chlorophenylureido (**24**) derivatives exhibited greater antiproliferative activity toward cells of malignant origin as compared to the cells derived from a normal individual. On the whole, 4-ureidopyrazolo[3,4-*d*]pyrimidines exhibited greater growth inhibitory activity than the 6-ureidopurines.

Experimental Section

Melting points and ir, uv, and mass spectra were recorded as reported previously.[2a,b] NMR spectra were determined in Me_2SO-d_6 on a Varian A-60A and XL-100 spectrometer, using Me_4Si as an internal reference. TLC was carried out on Bakerflex silica gel 1B-F using the following solvent systems: (A) EtOH-EtOAc (1:49); (B) EtOAc-*n*-PrOH-H_2O (4:1:2); (C) EtOAc-2-ethoxyethanol-16% HCOOH (4:1:2). In a two-phase system the upper phase was used for development of a chromatogram. The C, H, and N analyses were carried out by Heterocyclic Chemical Corp., Harrisonville, Mo. 64701.

Ethyl Pyrazolo[3,4-*d*]pyrimidine-4-carbamate (2). To a stirred suspension of 13.5 g (0.1 mol) of 4-aminopyrazolo[3,4-*d*]pyrimidine[4] (**1**) in 350 ml of anhydrous pyridine was added dropwise 35 ml of ethyl chloroformate at −10°. The mixture was then slowly brought to room temperature and stirred further for 3 h. This was then refluxed for 3 h and evaporated to dryness. The residue was triturated with 400 ml of hot H_2O and the light tan solid was collected on a filter and washed with hot H_2O: yield 17.9 g (86.5%); mp >300° dec. The analytical sample was recrystallized from boiling EtOH: mp >300° dec; uv λ max (95% EtOH) 260 nm (ε 12400), 280 (shoulder); uv λ max (0.1 N HCl) 265 nm (ε 10800); uv λ max (0.1 N NaOH) 290 nm (ε 10200); ir max 1755 cm^{-1} (urethane C=O); NMR (Varian XL-100) (Me_2SO-d_6) δ 1.33 (t, 3, J = 8 Hz, CH_3), 4.33 (q, 2, J = 8 Hz, CH_2), 8.46 (s, 1, 3 or 6-H), 8.55 (s, 1, 3 or 6-H), and 11.10 ppm (s, 1, NH) (the NH of **2** at 11.10 and the NH_2 of the 4-aminopyrazolo[3,4-*d*]pyrimidine (**1**) were exchanged when D_2O was added to the Me_2SO solution); mass spectrum *m/e* (rel %) 208 ($M^{\cdot+}$ + 1, 10), 207 ($M^{\cdot+}$, 32), 162 (14), 161 (36), 135 (100), 119 (18), and 108 (36). Anal. ($C_8H_9N_5O_2$·0.5H_2O).

Ethyl 1-Benzylpyrazolo[3,4-*d*]pyrimidine-4-carbamate (4). (a) To a stirred suspension of 118 mg (0.5 mmol) of 4-amino-1-benzylpyrazolo[3,4-*d*]pyrimidine (**3**)[5] in 25 ml of anhydrous pyridine was added 109 mg (1.0 mmol) of ethyl chloroformate at −10°. The mixture was then slowly brought to room temperature and stirred for 2 h. The mixture was evaporated to dryness and the residue was crystallized from hot EtOH: total yield 66 mg (44.5%); mp >300° dec; uv λ max (50% EtOH) 257 nm (ε 10160), 263 (10900), 283 (7560); uv λ max (0.1 N HCl) 266 nm (ε 14170); uv λ max (0.1 N NaOH) 298 nm (ε 23400), 306 nm (shoulder); ir max 1750 cm^{-1} (urethane C=O); NMR (Me_2SO-d_6) (data obtained on Varian XL-100) δ 1.30 (t, 3, J = 7 Hz, CH_3), 4.26 (q, 2, J = 7 Hz, CH_2), 5.61 (s, 2, CH_2), 7.27 (s, 5, C_6H_5), 8.49 (s, 1, 3 or 6-H), 8.61 (s, 1, 3 or 6-H), and 11.10 ppm (s, 1, NH); mass spectrum *m/e* (rel %) 298 ($M^{\cdot+}$ + 1, 5), 297 ($M^{\cdot+}$, 18), 296 (8), 252 (13), 251 (33), 250 (35), 225 (8), 224 (20), 223 (10), 196 (20), 174 (15), and 91 (100). Anal. ($C_{15}H_{15}N_5O_2$) C, H, N.

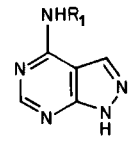
(b) The urethane **2** was allowed to react with benzyl chloride and K_2CO_3 according to the literature procedure.[5] Among the several products present in the reaction mixture, compound **4** was isolated by preparative TLC. Uv spectra and chromatographic mobilities of this material were identical with the material prepared by (a) as described above.

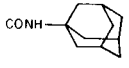
***N*-(Pyrazolo[3,4-*d*]pyrimidine-4-ylcarbamoyl)glycine (7). Method A.** A stirred mixture of 1.03 g (5 mmol) of **2** and 0.75 g (10 mmol) of glycine in 50 ml of anhydrous pyridine was heated in a glass bomb at 120° for 18 h. After cooling to room temperature, the solid was filtered and washed with EtOH and H_2O. The crude product was dissolved in 1 N NaOH and treated with charcoal. After filtering charcoal, the filtrate was adjusted to pH 4 with concentrated HCl. The white solid was filtered and washed with H_2O and EtOH: yield 537 mg (45.6%); mp 240–248° dec; ir max 1700, 1670 (ureido C=O), 1600 and 1540 cm^{-1} (C=C, C=N); NMR (Me_2SO-d_6) δ 4.05 (d, 2, J = 6 Hz, CH_2), 8.56 (s, 2, 3-H and 6-H), and 9.56 ppm (t, 1, J = 5.5 Hz, CONH); mass spectrum *m/e* (rel %) 218 ($M^{\cdot+}$ − 18, 3), 162 (16), 161 (100), 135 (90), 119 (32), and 108 (37).

Table I lists the compounds prepared in an analogous manner. Generally the reaction time period was 18 h. Compound **6** was recrystallized from solvent C after the crude product was obtained from the reaction mixture.

***N*-(Pyrazolo[3,4-*d*]pyrimidine-4-ylcarbamoyl)butylamine (11). Method B.** A stirred mixture of 1.35 g (10 mmol) of 4-aminopyrazolo[3,4-*d*]pyrimidine (**1**) and 0.991 g (10 mmol) of *n*-butyl isocyanate in 20 ml of anhydrous Me_2SO was heated in a glass bomb at 90° for 5 h. After keeping at room temperature overnight, the white solid was filtered and washed with EtOH. The filtrate was evaporated to dryness and the residue was triturated with 200 ml of hot EtOH. Upon cooling at 4° overnight, additional product separated: total yield 1.18 g (50.5%); mp 255–259° (soften), >300° dec; ir max 1690 (ureido C=O) and 1550 cm^{-1} (C=C, C=N); NMR (in Me_2SO-d_6) δ, 0.98 (d, 3, J = 6 Hz, CH_3), 1.67–1.17 (m, 4, CH_2), 3.33 (q, 2, J = 7 Hz, NCH_2), 8.57 (s, 1, 3 or 6-H), 8.60 (s, 1, 3, or 6-H), 9.33 (t, 1, J = 6 Hz, CONH), and 10.48 ppm (s, 1, NHCO); mass spectrum *m/e* (rel %) 234

Table I. Pyrazolo[3,4-*d*]pyrimidines (1)



Compd no.	R_1	Method	Yield, %	Mp,[a] °C	Formula[b]	Uv max, nm (ε × 10^{-3}) 0.1 N HCl	95% EtOH[c]	0.1 N NaOH[d]	Biological activity[e] Nc 37	RPMI 1640	L1210
6	CONHCH(COOH)CH(CH$_3$)(OH)	A	45	173–174	$C_{10}H_{12}N_6O_4 \cdot 2H_2O$	267 (16.3)	265 (16.8)	272 (13.7), 296 (5.4)	++	++	++
7	CONHCH$_2$COOH	A	45	240–248 dec	$C_8H_8N_6O_3$	267 (13.8)	265 (13.9)	271 (11.6), 297 (4.4)	NA	+	±
8	CONHCH(CH$_3$)$_2$	A	55	>300 dec	$C_9H_{12}N_6O$	271 (15.9)	266 (14.9)	273 (11.8), 298 (3.8)	++	±	±
		B	45								
9	CONHCH$_2$CH=CH$_2$	A	53	>300 dec	$C_9H_{10}N_6O$	270 (14.2)	266 (13.5)	273 (10.4), 298 (3.7)	++	NA	>++
10	CONHCH$_2$C≡CH	A	63	>300 dec	$C_9H_8N_6O$	266 (14.6)	264 (15.1)	272 (11.3), 297 (5.0)	NA	++	+
11	CONHCH$_2$CH$_2$CH$_2$CH$_3$	B	50	>300 dec	$C_{10}H_{14}N_6O$	270 (14.6)	264 (14.2)	272 (11.4), 298 (4.2)	±	±	+
		D	61	>300 dec	$C_{10}H_{14}N_6O$						
12	CONHCH$_2$CH(CH$_3$)$_2$	A	67	>300 dec	$C_{10}H_{14}N_6O$	272 (16.1)	267 (13.8)	272 (13.5), 294 (5.7)	+	+	+
13	CONHC(CH$_3$)$_3$	B	57	>300 dec	$C_{10}H_{14}N_6O$	272 (15.6)	266 (14.6)	273 (11.3), 300 (3.7)	+	+	++
14	CONHCH$_2$(CH$_2$)$_3$CH$_3$	A	54	>300 dec	$C_{11}H_{16}N_6O$	272 (14.4)	266 (13.3)	272 (11.7), 295 (5.0)	>++	++	++
15	CONHCH$_2$CH$_2$CH(CH$_3$)$_2$	A	64	>300 dec	$C_{11}H_{16}N_6O$	270 (16.0)	265 (15.0)	272 (12.0), 297 (4.1)	+	+	+
16	CONHCH$_2$C(CH$_3$)$_3$	A	30	>300 dec	$C_{11}H_{16}N_6O$	270 (16.4)	267 (15.6)	272 (12.7), 298 (4.2)	+	+	++
17	CONHCH$_2$(CH$_2$)$_6$CH$_3$	B	41	>300 dec	$C_{14}H_{22}N_6O$	269 (16.8)	265 (16.6)	272 (13.1), 297 (4.3)	>++	>++	>++
18	CONHCH$_2$CH$_2$OCH$_2$CH$_3$	A	69	>300 dec	$C_{10}H_{14}N_6O_2$	271 (16.3)	267 (16.6)	272 (13.5), 295 (6.0)	±	++	±
19		A	11	>300 dec	$C_{16}H_{20}N_6O$	272 (16.6)	267 (15.9)	272 (12.5), 296 (4.1)	++	++	>++
20	CONHCH$_2$CH$_2$CH$_2$-C$_6$H$_5$	A	65	>300 dec	$C_{15}H_{16}N_6O$	270 (16.4)	266 (15.3)	272 (12.1), 299 (4.0)	++	+	+
21	CONHCH$_2$CH$_2$CH$_2$CH$_2$-C$_6$H$_5$	A	74	>300 dec	$C_{16}H_{18}N_6O$	269 (16.0)	265 (15.6)	272 (12.6), 296 (4.4)	+	±	++
22	CONH-C$_6$H$_5$	B	42	>300 dec	$C_{12}H_{10}N_6O$	283 (19.9)	280 (20.9)	279 (21.9), 305 (8.5)	NA	++	+
23	CONH-C$_6$H$_4$-*o*-F	B	40	>300 dec	$C_{12}H_9FN_6O \cdot 1.5H_2O$	281 (21.6)	280 (21.6)	280 (22.0), 307 (10.3)	NA	+	+
24	CONH-C$_6$H$_4$-*o*-Cl	A	2.3	>300 dec	$C_{12}H_9ClN_6O$	278 (19.4)	280 (19.5)	280 (18.2), 307 (7.4)	NA	±	+
		B	40	>300 dec	$C_{12}H_9ClN_6O$						
25	CONH-C$_6$H$_4$-*m*-Cl	C	65	>300 dec	$C_{12}H_9ClN_6O$	281 (23.5)	277 (24.6)	280 (21.9), 310 (12.6)	>++	>++	>++
26	CONH-C$_6$H$_4$-*p*-Cl	B	38	290 s, >300 dec	$C_{12}H_9ClN_6O$	283 (21.6)	279 (23.1)	280 (21.9), 307 (11.2)	++	++	++
27	CH$_2$CH$_3$	D	76	257–259[f]	$C_7H_9N_5$	265 (9.8)	278 (12.5)	268 (9.6)	NA	NA	+
28	CH(CH$_3$)$_2$	D	72	251–253[g]	$C_8H_{11}N_5 \cdot 0.5H_2O$	265 (10.8)	279 (15.4)	269 (12.3)	++	+	NA
29	CH$_2$CH=CH$_2$	D	70	187–191	$C_8H_9N_5 \cdot 0.5H_2O$	265–267 (10.4)	277.5 (14.1)	267–269 (10.3)	+	++	+
30	CH$_2$CH$_2$CH(CH$_3$)$_2$	D	76	178–179	$C_{10}H_{15}N_5$	269 (9.7)	278.5 (14.7)	268 (10.9)	>++	++	>++
31	CH$_2$-C$_6$H$_5$	D	75	210–212[h]	$C_{12}H_{11}N_5$	291.5 (11.1)	278 (15.3)	268 (11.1)	++	++	>++

[a] s = soften; ef = melts with effervescence; dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N. The analytical results were within ± 0.4% of the theoretical values. [c] At neutral pH shoulders at 275 and 285 nm for compounds 6–21. [d] Shoulders at 266–268 nm for compounds 6–21. [e] The notation represents the viable cell number relative to the controls after 72 h of incubation in the tissue cultures (concentration 1 × 10^{-4} M): >++, 0–30%; ++, 30–60%; +, 60–80%; ±, 80–90%; NA, not active 90–110%. [f] Lit.[4] mp 259–260°. [g] Lit.[4] mp 253–254°. [h] Lit.[4] mp 215–217°.

($M^{\cdot+}$, <1), 191 (2), 162 (10), 161 (71), 135 (48), 119 (17), 108 (12), 98 (19), 73 (10), and 48 (100).

The same product was also obtained by stirring the above reaction mixture at room temperature for 24 h.

N-(Pyrazolo[3,4-d]pyrimidin-4-ylcarbamoyl)-o-chloroaniline (24). Method C. A stirred mixture of 1.35 g (10 mmol) of 4-aminopyrazolo[3,4-d]pyrimidine (1), 3.08 g (20 mmol) of o-chlorophenyl isocyanate, and 20 ml of anhydrous Me_2SO was stirred in a glass bomb at room temperature for 24 h. The white solid was filtered and washed with hot EtOH. Additional product was obtained from the filtrate: total yield 1.89 g (65.4%); mp >300° dec; ir max 1700 (ureido C=O), 1590 and 1540 cm^{-1} (C=C, C=N); NMR (in Me_2SO-d_6) δ 7.65–6.97 (m, 4, phenyl ring), 8.60 (s, 1, 3 or 6-H), 8.68 (s, 1, 3 or 6-H), and 11.12 ppm (s, 1, NH); mass spectrum m/e (rel %) 245 ($M^{\cdot+}$, <1), 162 (1), 161 (7), 156 (4), 155 (32), 154 (9), 153 (100), 135 (19), 127 (21), 125 (32), 90 (28), and 63 (14).

These reactions, when carried out with excess alkyl or aryl isocyanates for a prolonged period of time (48 h) at room temperature, resulted in the formation of a mixture of the N^4-carbamoyl and the $N^1(N^2)$,N^4-dicarbamoyl-4-aminopyrazolo-[3,4-d]pyrimidine 5. In these cases the mixture was dissolved in 1 N NaOH and heated on a steam bath for 10 min. After cooling to room temperature and then adjusting to pH 4.0 with concentrated HCl, the precipitated 4-ureidopyrazolo[3,4-d]pyrimidines (4) were collected on a filter and washed with water.

N^4-Allylaminopyrazolo[3,4-d]pyrimidine (29). Method D. A mixture of 1.54 g (10 mmol) of 4-chloropyrazolo[3,4-d]pyrimidine and 1.99 g (23.5 mmol) of allyamine in 10 ml of H_2O was heated at 100° for 4 h. Upon cooling to room temperature the white crystalline product was obtained: yield 1.19 g (70%); mp 187–191°; ir max 1600 cm^{-1} (C=C, C=N); NMR (in Me_2SO-d_6) δ, 4.22 (m, 2, NCH_2), 5.24 (m, 2, CH_2), 5.93 (m, 1, CH), 8.24 (s, 1, 3 or 6-H), and 8.30 ppm (s, 1, 3 or 6-H); mass spectrum m/e (rel %) 175 (M^+ + 1, 26), 174 ($M^{\cdot+}$, 32), 160 (75), 147 (11), 135 (10), 119 (15), 108 (10), 93 (15), 66 (29), and 56 (100).

Growth Inhibition Assays. Cultured cells derived from the buffy coat of a normal individual (Nc 37) and a patient with myeloblastic leukemia (RPMI 6410) and mouse leukemia cells L1210 were used for growth inhibition studies (Table I).[2b] The compounds were dissolved in Me_2SO and allowed to remain at 37° overnight; this achieved sterilization. Sterile growth medium (RPMI No. 1640 + 10% fetal calf serum) was then added to bring the final concentration of Me_2SO to 0.5% and that of a compound to 10^{-4} M. At this Me_2SO concentration, growth of control cultures was unaffected. The assays were performed in test tubes without agitation. The tubes were inoculated with cells from stock cultures in logarithmic growth to a starting density previously determined to be near minimum required for initiation of logarithmic growth of control cultures (between 2–5 × 10^5 cells/ml). At 24-h intervals, 0.2 ml was removed and mixed with trypan blue to a final concentration of 0.05% and the total and viable cells were counted in a hemocytometer. Cells able to exclude the dye were scored as viable. The compounds were rated as shown in Table I to indicate the following viable cell densities relative to controls after 72 h of incubation: >++, 0–30%; ++, 30–60%; +, 60–80%, ± 80–90%; NA, (not active) 90–110%.

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Catechol O-Methyltransferase. 8. Structure–Activity Relationships for Inhibition by 8-Hydroxyquinolines

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A series of 5- and 7-substituted 8-hydroxyquinolines was evaluated as inhibitors of catechol O-methyltransferase (COMT, E.C. 2.1.1.6). The electronic character of the substituents in the 5 position appeared to have only a small effect if any on the inhibitory activity of these compounds. A significant factor which contributes to the inhibitory activity of these compounds appears to be the nature of the 7-substituent. The structure–activity relationship for this series of inhibitors is discussed relative to the nature of the enzymatic binding site.

Catechol O-methyltransferase (COMT,[2] E.C. 2.1.1.6) plays an important role in the inactivation of catecholamines and the detoxification of various xenobiotic catechols. COMT is a soluble enzyme which requires magnesium to catalyze the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the catechol acceptor.[3,4] Of the various different classes of synthetic inhibitors of this transmethylation which have been identified some of the most potent in vitro inhibitors are derivatives of 8-hydroxyquinoline (8-HQ).[5] From kinetic studies carried out in our laboratory, we have shown that 8-HQ's inhibit COMT by binding reversibly to the catechol binding site on this enzyme.[5]

As part of our continuing studies of this enzymatic transmethylation, we have attempted to determine what structural modifications of 8-HQ would afford optimal inhibitory activity. In addition, we felt such comprehensive structure–activity relationship studies could provide useful information concerning the topography surrounding the catechol binding site of this enzyme. From earlier studies in our laboratory,[5] it was demonstrated that the 8-hydroxyl group and the quinoline nitrogen of 8-HQ were absolute