Synthesis and Antiviral Activity of Certain 4-Substituted and 2,4-Disubstituted 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines

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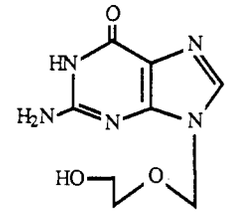
Treatment of the sodium salt of 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**2**) with (2-acetoxyethoxy)methyl bromide (**3**) has provided 4-chloro-2-(methylthio)-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**4**). Ammonolysis of **4** at room temperature gave 4-chloro-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**5**). However, ammonolysis of **5** at 130 °C furnished 4-amino-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (**6**), which on desulfurization with Raney Ni yielded 4-amino-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**7**) (acyclic analogue of tubercidin). The oxidation of **6** with *m*-chloroperbenzoic acid provided the sulfone derivative **8**. A nucleophilic displacement of the 2-methylsulfonyl group from **8** with methoxide anion provided 4-amino-2-methoxy-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**9**). Demethylation of **9** with iodotrimethylsilane gave 4-amino-2-hydroxy-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**10**). Treatment of 2,4-dichloropyrrolo[2,3-*d*]pyrimidine (**11**) with **3** gave the protected acyclic compound **12**, which on deacetylation and ammonolysis under controlled reaction conditions gave 2,4-dichloro-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**13**) and 4-amino-2-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**14**), respectively. The condensation of 2-acetamido-4-chloropyrrolo[2,3-*d*]pyrimidine (**15**) with **3** gave the protected acyclic compound **16**, which on concomitant deacetylation and ammonolysis with methanolic ammonia at an elevated temperature yielded 2,4-diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**17**) in moderate yield. In tests involving human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1), only slight activity and cytotoxicity were observed. The most active compounds (**12** and **13**) were slightly more active against HCMV than acyclovir, but both compounds were inactive against HSV-1. The activity against HCMV, however, was not well separated from cytotoxicity leading to the conclusion that these compounds did not merit further study.

The most important antiviral drug discovered over the past few years is the acyclic analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, **1**). This compound potently and selectively inhibits the in vitro and in vivo replication of herpes simplex viruses[1,2] and is clinically efficacious in the treatment of certain herpesvirus infections.[3-6] The biochemical basis for the antiviral activity of acyclovir involves its specific phosphorylation to the corresponding monophosphate by a herpesvirus-encoded pyrimidine deoxynucleoside kinase.[1,7] The monophosphate is phosphorylated further by cellular kinases[8,9] to acyclovir triphosphate, a potent and selective inhibitor of the virus-encoded DNA polymerase.[1,10-12] The combination of specificity and selectivity for virus-encoded enzymes leads to a paucity of cytotoxic effects by the drug in uninfected cells and assures the usefulness of acyclovir as an antiviral agent.



acyclovir (1)

The success of acyclovir as an antiviral drug has prompted intensive efforts by several groups to prepare and evaluate many structurally related acyclic analogues.[13-16] In our laboratory, the synthesis of pyrrolo[2,3-*d*]pyrimidine nucleosides as potential antitumor agents has been under active investigation for a number of years.[17-19] More recently, we have been investigating this class of nucleosides as potential antiviral drugs. We have found that arabinosyl- and deoxyribosylpyrrolo[2,3-*d*]pyrimidines are active against HCMV and HSV-1.[20] This observation led us to expand the series of compounds

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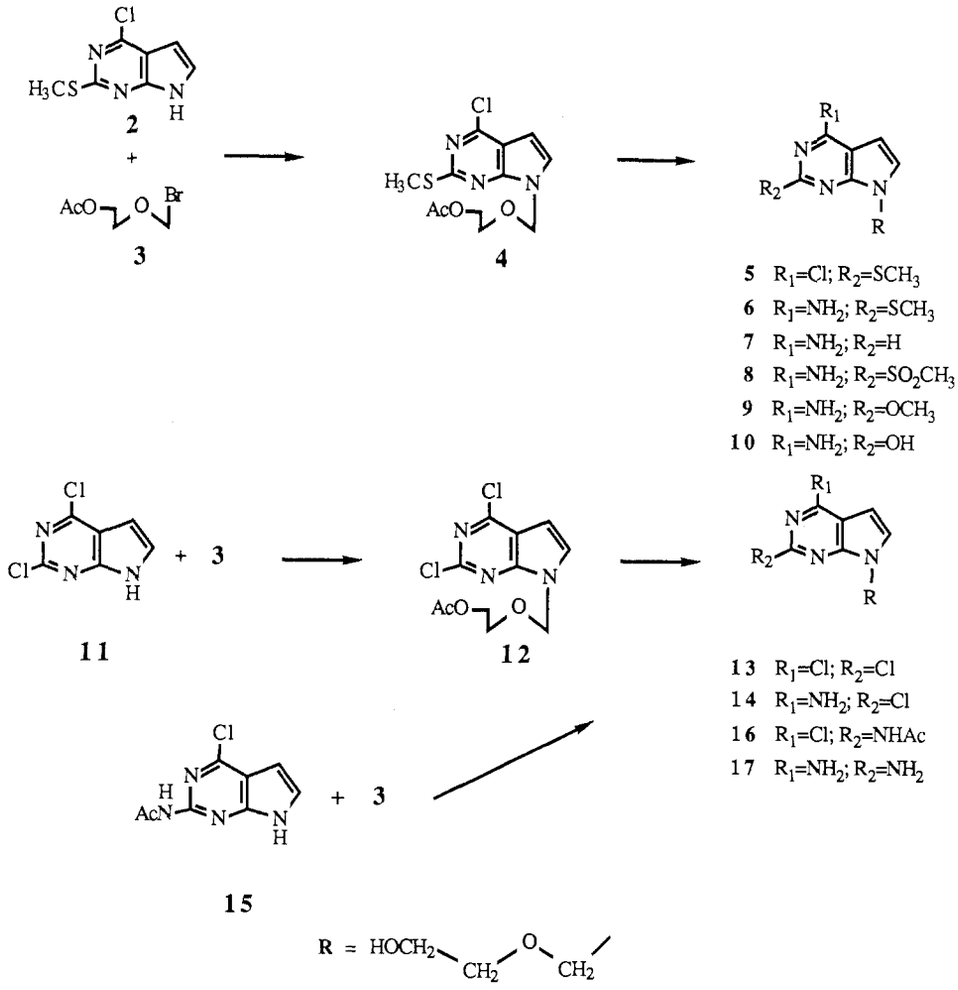
Scheme I



and to initiate the synthesis of acyclovir analogues in which the guanine base was replaced by selected 4-substituted and 2,4-disubstituted pyrrolo[2,3-*d*]pyrimidines. We report herein the first evaluation of such compounds for antiviral activity.

Results and Discussion

Chemistry. The successful alkylations and glycosylations of pyrrolo[2,3-*d*]pyrimidines have been reported by using various conditions, e.g., DMF/NaH,[21] phase-transfer conditions,[22] aqueous sodium hydroxide,[23] trimethylsilylation,[24] acid-catalyzed fusion procedures,[24] etc. We elected to use the procedure recently reported for the stereospecific synthesis of various 2′-deoxynucleosides of pyrrolo[2,3-*d*]pyrimidines.[25,26a] The sodium salt of 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**2**) was generated by the treatment of the heterocycle with sodium hydride in DMF. This sodium salt was condensed with (2-acetoxyethoxy)methyl bromide[26b] (**3**) in DMF to furnish the blocked acyclic compound **4** in 68% yield after column chromatography (Scheme I). That alkylation had occurred at N-7 rather than at N-3 or N-1 was initially assumed by the fact that peaks assigned to the protons at C-5 and C-6 were observed in the ^{1}H NMR spectrum as doublets at δ 6.7 and 7.6 (J = 3.8 Hz). Previous studies in our laboratory have shown[27] that the C-5 and C-6 protons in pyrrolo[2,3-*d*]pyrimidines, without any substituent at N-7, will appear as quartets, due to coupling with the NH proton at N-7. The observance of two sharp doublets, rather than two quartets, in the ^{1}H NMR spectrum of **4** clearly established that the N-7 position had been alkylated. There have been previous reports[19b,28] that glycosylation of 4-chloropyrrolo[2,3-*d*]pyrimidine or other trisubstituted pyrrolo[2,3-*d*]pyrimidines at N-7 results in a small hypsochromic shift in the ultraviolet spectrum when compared to the ultraviolet spectrum of the corresponding aglycon, whereas glycosylation at N-1 produces a definite bathochromic shift relative to that observed for the aglycon. The ultraviolet spectrum for compound **4** showed values very close to those observed for the aglycon and provides additional support for our assignment of N-7 for the site of glycosylation. Treatment of **4** with methanolic ammonia at room temperature gave a good yield of 4-chloro-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (**5**). More vigorous treatment of

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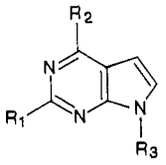
Table I. Antiviral Activity and Cytotoxicity of 4-Substituted and 2,4-Disubstituted 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines



compd	substituent			50% inhibitory concentration, μM				
				plaque reduction assay		cytotoxicity		
	R_1	R_2	R_3	HCMV	HSV-1	HFF[a]	BSC[a]	KB[b]
4	SCH_3	Cl	AEM[c]	>100[d]	>100	100	>100	33
5	SCH_3	Cl	HEM	>100	>100	30	>100	
6	SCH_3	NH_2	HEM	>100	>100	>100	>100	
7	H	NH_2	HEM	>100	>100	>100	>100	>100
8	SO_2CH_3	NH_2	HEM	>100	>100	>100	>100	
9	OCH_3	NH_2	HEM	>100	>100	>100	>100	
10	OH	NH_2	HEM	>100	>100	>100	>100	
12	Cl	Cl	AEM	40	>100	100	>100	
13	Cl	Cl	HEM	37	>100	100	>100	
14	Cl	NH_2	HEM	>100	>100	>100	>100	
16	NHAc	Cl	HEM	>100	>100	>100	>100	112
17	NH_2	NH_2	HEM	>100	>100	>100	>100	
1 (acyclovir)	NH_2	OH	HEM	63[e]	2.6[e]	>100	>100	>100
ganciclovir	NH_2	OH	DHPM	8.8[f]	3.0[f]	>100	>100	1000

[a] Visual cytotoxicity scored on uninfected HFF or BSC-1 cells at time of HCMV or HSV-1 plaque determination. [b] Average percent inhibition of DNA, RNA, and protein synthesis determined in KB cells as described in the text. [c] Abbreviations used; AEM: 7-[(2-acetoxyethoxy)methyl], HEM: 7-[(2-hydroxyethoxy)methyl], DHPM: 7-[(dihydroxypropoxy)methyl. [d] I_{50} concentration not reached at highest concentration tested (100 μM). [e] Average of two and four experiments, respectively, with HCMV and HSV-1. [f] Average of 23 and two experiments, respectively.

compound **5** with $MeOH/NH_3$ at 135 °C effected a displacement of the 4-chloro group to give 4-amino-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**6**) in quantitative yield. Desulfurization of **6** with Raney Ni provided the acyclic analogue of tubercidin, 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**7**), in 68% yield. The same compound (**7**) has been previously reported[28] but in a low yield. An oxidation of the 2-methylthio group of compound **6** to a methylsulfonyl group was accomplished by using *m*-CPBA to afford **8** in 48% yield.[29] A nucleophilic displacement of the methylsulfonyl group was attempted with aqueous sodium hydroxide in an effort to obtain the isoguanine analogue **10**. However, this was unsuccessful and led to the recovery of starting material. This inertness of the methylsulfonyl group toward nucleophilic displacement with alkali (NaOH) might be due to the generation of a sulfone anion after the abstraction of an α-methyl proton thereby inhibiting the attack of hydroxide anion at C-2. A facile displacement of the methylsulfonyl group was observed on treatment of compound **8** with sodium methoxide in methanol to give 4-amino-2-methoxy-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**9**) in fairly good yield. Demethylation of **9** with iodotrimethylsilane in acetonitrile[30] furnished 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidin-2-one (**10**) in 24% yield.

For the synthesis of some additional 2,4-disubstituted pyrrolo[2,3-*d*]pyrimidines, specifically the 2,4-diamino compound, we elected to use 2,4-dichloropyrrolo[2,3-*d*]-pyrimidine (**11**) as our starting material. The synthesis of **11** has been recently reported[26a] but in a very poor yield. We synthesized **11** by an improved method in 27% yield by treating pyrrolo[2,3-*d*]pyrimidin-2,4-dione[31] with pyrophosphoryl chloride. The sodium salt of **11** was condensed with (2-acetoxyethoxy)methyl bromide (**3**) (Scheme I) by using essentially the same reaction conditions as we used for the preparation of compound **4**. 2,4-Dichloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**12**) was obtained, after silica gel column chromatography, as yellow fluorescent crystals in 50% yield. Treatment of compound **12** with $MeOH/NH_3$ at room temperature led to the formation of a mixture of 2,4-dichloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**13**) and 4-amino-2-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**14**) in a 60:40 ratio. The successful deacetylation of **12**, without a concomitant displacement of the 4-chloro group, was achieved by the treatment of compound **12** with $MeOH/NH_3$ at −10 °C to afford compound **13** in a 35% yield, after column chromatography on silica gel. Treatment of compound **13** with $MeOH/NH_3$ in a sealed steel reaction vessel at 135 °C for 10 h furnished compound **14** in 65% yield. Subsequent attempts to obtain a displacement of both chloro groups by ammonia under more stringent conditions, e.g., elevated temperature, etc., only provided mixtures with some decomposition products. This prompted us to initiate an alternate route for the preparation of 2,4-diamino-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**17**). We elected to use the sodium salt of 2-acetamido-4-chloropyrrolo[2,3-*d*]pyrimidine (**15**), which was condensed with **3** to give 2-acetamido-4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine as a viscous oil in 80% yield, after purification by column chromatography on silica gel. This oil was covered with $MeOH/NH_3$ and allowed to stand at 0–5 °C for 5 h to obtain 2-acetamido-4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**16**) in 47% yield. Compound **16** was treated with $MeOH/NH_3$ at 135 °C in a sealed steel reaction vessel for 24 h in order

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to effect a nucleophilic displacement of the 4-chloro group and remove the protecting group from the amino group to give 2,4-diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**17**) in 27% yield.

Biological Evaluations. Compounds were evaluated for activity against HCMV and HSV-1 by using plaque-reduction assays. Cytotoxicity of each compound was examined in normal human diploid cells (human foreskin fibroblasts, HFF cells), monkey kidney cells (BSC-1 cells), and in some cases a human neoplastic cell line (KB cells). Table I shows that nearly all compounds were inactive against HCMV and HSV-1. These compounds also were not cytotoxic at 100 μM. Compounds **12** and **13** showed some activity against HCMV but also induced cytotoxicity at 100 μM in HFF cells. Thus, even though activity was similar to that of acyclovir, it was judged to be insignificant due to toxicity. In contrast, ganciclovir (DHPG) was active against HCMV and HSV-1 at <10 μM and required 1000 μM to produce cytotoxicity. The activity of DHPG against both viruses[32] and acyclovir against HSV-1 but not HCMV[33] is well established.

The lack of activity in this pyrrolo[2,3-*d*]pyrimidine series is consistent with recent work at Burroughs Wellcome. Beauchamp et al.[34] reported that the pyrrolo[2,3-*d*]pyrimidine analogue of acyclovir was inactive against HSV-1. Apparently HSV-1 thymidine kinase did not phosphorylate the compound. Although it is tempting to speculate, we have not explored whether or not our compounds are substrates for viral or cellular kinases.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 270 MHz with a IBM WP 270 SY spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent (DMSO-d_6). Ultraviolet spectra were recorded on a Hewlett-Packard 8450A spectrophotometer, and the infrared spectra were measured on a Perkin-Elmer 281 spectrophotometer. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was on silica gel 60 F-254 plates (Analtech, Inc.). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. Detection of components on TLC was made by UV light (254 nm). Rotary evaporations were carried out under reduced pressure with the bath temperature below 30 °C unless specified otherwise.

4-Chloro-2-(methylthio)-7-[(2-acetoxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (4). Sodium hydride (0.06 g, 60% in mineral oil) was added to a solution of 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine[35] (**2**, 0.25 g) in dry DMF (4 mL) in small portions at 0–5 °C under a nitrogen atmosphere. When all hydrogen evolution had ceased, (2-acetoxyethoxy)methyl bromide (**3**, 0.3 g) was added dropwise to the above mixture, with stirring, under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for an additional hour. The reaction mixture was diluted with ice-cold water (20 mL), and the pH was adjusted immediately with glacial acetic acid acid to pH ~7. The reaction mixture was extracted with chloroform (3 × 20 mL), washed with water, and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo at a reduced temperature to afford a thick syrup. This syrup was then rotary evaporated onto 2 g of silica gel and applied to the top of a column (1.8 × 2.5 cm) wet packed with 15 g of silica gel, and benzene was used as an eluent. Elution with this solvent afforded a colorless syrup, which was crystallized from ethyl alcohol to afford 0.25 g (61.5%) of **4** as colorless needles: mp 91–92 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.6 (d, 1, J = 3.8 Hz, C-6-H), 6.78 (d, 1, J = 3.8 Hz, C-5-H), 5.78 (s, 2, N_7CH_2), 4.3 (m, 2, OCH_2), 2.8 (m, 2, CH_2), 2.75 (s, 3, SMe), 2.15 (s, 3, OAc); IR (KBr) 1730 (OAc) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 206 (1.7), 250 (2.2), 273 (0.8), 310 (0.6); pH 1, 207 (1.7), 251 (2.1), 275 (0.7), 311 (0.5); pH 11, 228 (0.8), 251 (2.1), 275 (0.7), 310 (0.6). Anal. ($C_{12}H_{14}N_3O_3ClS$) C, H, N.

4-Chloro-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (5). Compound **4** (0.2 g) was dissolved in dry MeOH (10 mL), and to this solution was added 20 mL of methanolic ammonia (methanol previously saturated with ammonia at 0 °C). The reaction mixture was then stirred in a pressure bottle at room temperature for 20 h. The solvent was removed under reduced temperature and pressure, and the oily syrup obtained was applied to the top of a column (1 × 40 cm) packed with wet silica gel in chloroform. The column was eluted with 2% MeOH in $CHCl_3$ to yield a colorless syrup, which upon trituration with diethyl ether furnished a crystalline compound. The solid was collected by filtration and then recrystallized from water to obtain 0.12 g (80%) of **5** as needles: mp 90–96 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.7 (d, 1, J = 3.7 Hz, C-6-H), 6.6 (d, 1, J = 3.7 Hz, C-5-H), 5.62 (s, 2, N_7CH_2), 4.66 (m, 1, exchangeable with D_2O, OH), 3.4 (m, 4, CH_2CH_2), 2.5 (s, 3, SMe); IR(KBr) 3400 (OH) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 207 (1.8), 250 (2.6), 274 (0.8), 311 (0.8); pH 1, 207 (2.0), 251 (2.7), 276 (0.8), 311 (0.7); pH 11, 227 (1.1), 251 (2.7), 275 (0.9), 311 (0.8). Anal. ($C_{10}H_{12}N_3O_2SCl$) C, H, N.

4-Amino-2-(methylthio)-7-[(2-hydroxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (6). Compound **5** (0.5 g) was covered with MeOH (20 mL, previously saturated with ammonia at 0 °C), and the reaction mixture was then heated in a steel vessel at 135 °C for 10 h. The vessel was cooled, and the solvent was removed in vacuo to give a colorless syrup. Trituration of this syrup with diethyl ether gave a colorless crystalline compound, which was collected by filtration and then washed with diethyl ether. The solid was crystallized from boiling water to obtain **6** as needles: 0.3 g (78%); mp 175–176 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.11 (d, 1, J = 3.6 Hz, C-6-H), 7.10 (br s, 2, exchangeable with D_2O, NH_2), 6.52 (d, 1, J = 3.6 Hz, C-5-H), 5.47 (s, 2, CH_2), 3.43 (br s, 1, exchangeable with D_2O, OH), 3.3 (m, 4, CH_2CH_2), 2.5 (s, 3, SMe); IR (KBr) 3400 (OH), 3324 (NH_2) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 205 (1.4), 234 (1.8), 280 (1.0); pH 1, 223 (1.5), 281 (0.9); pH 11, 236 (1.5), 282 (0.9). Anal. ($C_{10}H_{14}N_4O_2S$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (7). Raney Ni (2 g) was added to a solution of compound **6** (0.36 g) in ethanol (45 mL) containing 15% aqueous NH_4OH (40 mL). The reaction mixture was heated at reflux for 1.5 h at which time TLC showed no starting material. The solvent was decanted, and the Raney Ni was washed several times with ethanol (3 × 40 mL). The filtrate and all washing fractions were combined, and the solvent was removed in vacuo. The resulting semisolid mass was applied to the top of a column (1 × 40 cm) packed with wet silica gel in chloroform. Elution of the column with MeOH/$CHCl_3$ (5:95 v/v) afforded a colorless crystalline compound. This compound was recrystallized from ethyl acetate to afford 0.2 g (68%) of **7**: mp 124–125 °C (lit.[28] mp 127–129 °C); ^{1}H NMR (Me_2SO-d_6) δ 7.65 (s, 1, C-2-H), 7.12 (d, 1, J = 4.0 Hz, C-6-H), 6.65 (d, 1, J = 4.0 Hz, C-5-H), 5.6 (s, 2, N-7-CH_2), 3.6 (br s, 4, CH_2CH_2), 3.72 (br s, 1, exchangeable with D_2O, OH), 8.3 (br s, 2, exchangeable with D_2O, NH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 215 (1.6), 271 (1.2); pH 1, 226 (1.8), 273 (1.1); pH 11, 226 (0.6), 271 (1.1). Anal. ($C_9H_{12}N_4O_2$) C, H, N.

4-Amino-2-(methylsulfonyl)-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (8). Compound **6** (0.4 g) was dissolved in ethyl alcohol (10 mL), and to this solution was added *m*-chloroperbenzoic acid (*m*-CPBA) (85% technical grade) (0.816 g) in several portions over a period of 4 h. After the final addition of the *m*-CPBA, the reaction mixture was allowed to stir for an additional hour. The reaction mixture was then poured into diethyl ether (100 mL), and the precipitate that separated was collected by filtration. This compound was recrystallized from methanol to afford 0.2 g (48%) of **8**: mp 186–187 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.7 (s, 2, exchangeable with D_2O, NH_2), 7.5 (d, 1,

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J = 3.5 Hz, C-6-H), 6.7 (d, 1, J = 3.5 Hz, C-5-H), 5.57 (s, 2, N-7-CH_2), 4.6 (t, 1, J = 2.58, 3.0 Hz, exchangeable with D_2O, OH), 3.7 (m, 4, CH_2), 3.35 (s, 3, SO_2Me); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 221 (2.5), 270 (1.1), 297 (1.1); pH 1, 213 (3.1), 270 (1.2), 301 (1.1); pH 11, 227 (1.9), 270 (1.1), 301 (1.1). Anal. ($C_{10}H_{14}N_4O_4S$) C, H, N.

4-Amino-2-methoxy-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (9). A solution of sodium methoxide (Na 0.16 g dissolved in 5 mL MeOH) was added to a solution of compound **8** (0.09 g) in dry MeOH (10 mL). This reaction mixture was heated at reflux for 1 h, the pH was adjusted to 7 with glacial actic, and the solvent was then removed in vacuo. The residue was extracted (3 × 50 mL) with ethyl acetate, and the extracts were combined, washed (3 × 100 mL) with water, and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a thick syrup. This syrup was rotary evaporated on to 1 g of silica gel and applied to the top of a column (1 × 30 cm) wet packed with 10 g of silica gel with chloroform/methanol (5:95 v/v). Elution of the column with the same solvent system afforded a crystalline compound, which was recrystallized from ethyl alcohol to obtain 0.3 g (47.3%) of **9**: mp 189–190 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.07 (br s, 2, exchangeable with D_2O, NH_2), 7.03 (d, 1, J = 3.6 Hz, C-6-H), 6.5 (d, 1, J = 3.6 Hz, C-5-H), 5.42 (s, 2, N-7-CH_2), 4.63 (m, 1, exchangeable with D_2O, OH), 3.79 (s, 3, OMe), 3.44 (m, 4, CH_2CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7 217 (2.4), 250 (1.1); pH 1 204 (1.3), 226 (2.5), 260 (0.7), 286 (0.8); pH 11 224 (1.2), 273 (1.0). Anal. ($C_{10}H_{14}N_4O_3$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidin-2-one (10). Iodotrimethylsilane (0.53 mL) was added to a solution of compound **9** (0.15 g) in dry acetonitrile (18 mL) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h. The solvent was then removed in vacuo, and the resulting dark yellow crystalline compound was dissolved in methanol (20 mL) and heated at reflux for 3 h. The solution was concentrated to ~10 mL and then allowed to stand at 5 °C for 18 h. The yellow compound that had precipitated was collected by filtration and recrystallized from methanol to afford 0.06 g (43%) of **10**, mp 224–225 °C dec. An analytical sample was prepared by prep TLC with a solvent system of $MeOH/CHCl_3$ (20:80 v/v): ^{1}H NMR (Me_2SO-d_6) δ 10.45 (br s, 1, exchangeable with D_2O, NH), 7.30 (br s, 2, exchangeable with D_2O, NH_2), 6.78 (d, 1, J = 3.8 Hz, C-6-H), 6.40 (d, 1, J = 3.8 Hz, C-5-H), 5.25 (s, 2, CH_2), 4.66 (br s, 1, exchangeable with D_2O, OH), 3.37 (m, 4, CH_2CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 225 (2.01), 256 (0.6), 308 (0.5); pH 1, 226 (1.6), 295 (0.6); pH 11, 226 (1.3), 256 (0.6), 294 (0.5). Anal. ($C_9H_{12}N_4O_3 \cdot {}^1/_2MeOH$) C, H, N.

2,4-Dichloropyrrolo[2,3-*d*]pyrimidine (11). A mixture of pyrrolo[2,3-*d*]pyrimidin-2,4-dione[31] (5 g, 33 mmol) and pyrophosphoryl chloride (40 mL) was heated in a steel bomb at 165 °C for 19 h. After cooling, the brown solution was decanted from any solid residue, and the excess pyrophosphoryl chloride was removed under reduced temperature and pressure. The resulting thick syrup was poured onto ~200 g of crushed ice. The solid precipitate was collected by filtration, and the filtrate was extracted with diethyl ether (2 × 200 mL). The diethyl ether extracts were combined, washed with water, and dried over anhydrous $MgSO_4$. The ether was removed under reduced pressure to give 1.65 g (26.5%) of **11**. Recrystallization from $CHCl_3$ with a few drops of ethyl acetate gave an analytical sample: mp 247–250 °C (lit.[26a] mp 248–250 °C); ^{1}H NMR (Me_2SO-d_6) δ 6.68 (d, 1, C-5-H), 7.73 (d, 1, C-6-H), 12.72 (br s, 1, NH). Anal. ($C_6H_3N_3Cl_2$) C, H, N.

2,4-Dichloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (12). Sodium hydride (60% oil emulsion, 0.076 g) was added to a solution of compound **11** (0.3 g) in dry DMF (6 mL) in small batches over a period of 30 min. (2-Acetoxyethoxy)methyl bromide (**3**, 0.395 g) was added to this solution at room temperature, and the reaction mixture was allowed to stir for 2 h under a nitrogen atmosphere at room temperature. Water (30 mL) was added, and the product was extracted with ethyl acetate (3 × 30 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a thick syrup, which was rotary evaporated onto 1 g of silica gel. The silica gel was then applied to the top of a column (1 × 30 cm) wet packed with 10 g of silica gel with chloroform as an eluent. Elution of the column with the same solvent afforded a crystalline compound, which was recrystallized from ethyl alcohol to obtain 0.25 g (50%) of **12**: mp 83–85 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.90 (d, 1, J = 3.67 Hz, C-6-H), 6.78 (d, 1, J = 3.6 Hz, C-5-H), 5.6 (s, 2, CH_2), 4.05 (m, 2, CH_2CH_2), 3.55 (m, 2, CH_2CH_2), 1.93 (s, 3, CH_3); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 229 (3.6), 288 (0.5); pH 1, 230 (3.8), 290 (0.6); pH 11, 231 (3.6), 288 (0.6). Anal. ($C_{11}H_{11}N_3O_3Cl_2$) C, H, N.

2,4-Dichloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (13). Compound **12** (0.2 g) was dissolved in dry methanol (5 mL), and to this solution was added MeOH (20 mL, previously saturated with NH_3 at 0 °C) at −10 °C in an ice–salt bath. The reaction mixture was placed in a pressure bottle and stirred at −10 °C for 1 h. The solvent was removed under reduced pressure and temperature, and the colorless syrup was applied to the top of a column (1 × 40 cm) prepacked with wet silica gel in chloroform. Elution of the column with chloroform yielded a colorless syrup, which was crystallized from benzene to afford 0.05 g (35.7%) of **13**: mp 115–116 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.85 (d, 1, J = 3.8 Hz, C-6-H), 6.75 (d, 1, J = 3.8 Hz, C-5-H), 5.63 (s, 2, CH_2), 4.63 (t, 1, J = 5.23 and 4.25 Hz, exchangeable with D_2O, OH), 3.35 (m, 4, CH_2CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 211 (2.2), 231 (3.3), 282 (0.8); pH 1, 205 (1.8), 231 (3.3), 276 (0.6); pH 11, 233 (3.0), 278 (0.6). Anal. ($C_9H_9N_3O_2Cl_2$) C, H, N.

4-Amino-2-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (14). Compound **13** (0.12 g) was covered with 15 mL of methanol (previously saturated with NH_3 at 0 °C) and heated in a sealed steel reaction vessel at 100 °C for 10 h. The solvent was removed in vacuo to give a semisolid mass, which was applied to the top of a column (1 × 30 cm) prepacked with wet silica gel in chloroform. Elution of the column with $MeOH/CHCl_3$ (5:95 v/v) yielded a crystalline compound, which was recrystallized from ethyl acetate to give 0.06 g (63.15%) of **14** as a colorless powder: mp 208–209 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.55 (br s, 2, exchangeable with D_2O, NH_2), 7.25 (d, 1, J = 3.5 Hz, C-6-H), 6.57 (d, 1, J = 3.5 Hz, C-5-H), 5.43 (s, 2, CH_2), 4.63 (t, 1, J = 5.2 and 4.18 Hz, exchangeable with D_2O, OH), 3.55 (m, 4, CH_2CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7 215 (2.8), 274 (1.6); pH 1 228 (2.3), 277 (1.4); pH 11 226 (1.3), 275 (1.5). Anal. ($C_9H_{11}N_4O_2Cl \cdot {}^1/_4H_2O$) C, H, N.

2-Acetamido-4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (16). Sodium hydride (60% oil emulsion, 0.11 g) was added to a solution of 2-acetamido-4-chloropyrrolo[2,3-*d*]pyrimidine[36] (**15**, 0.5 g) in small batches. When the evolution of hydrogen had ceased, (2-acetoxyethoxy)methyl bromide (**3**, 0.55 g) was added in one portion, with stirring, at room temperature. The reaction mixture was then stirred at room temperature under a nitrogen atmosphere for 5 h, cold water (20 mL) was added, and the product was extracted with ethyl acetate (3 × 50 mL). The extracts were combined and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the brown syrup was rotary evaporated onto silica gel (1 g). This silica gel was then applied to the top of a column (1 × 40 cm) prepacked with wet silica gel in chloroform. Elution of the column with $MeOH/CHCl_3$ (2:98, v/v) yielded a viscous oil (0.7 g). This oil was covered with methanolic ammonia (25 mL) (methanol that had been previously saturated with ammonia at 0–5 °C), and the reaction mixture was stirred at 0–5 °C for 5 h. The solvent was removed in vacuo, and the resulting yellow solid was recrystallized from ethyl acetate to afford 0.25 g (42%) of **16** as light yellow crystals: mp 126–127 °C; ^{1}H NMR (Me_2SO-d_6) δ 10.67 (br s, 1, exchangeable with D_2O, NH), 7.67 (d, 1, J = 3.70 Hz, C-6-H), 6.61 (d, 1, J = 3.70 Hz, C-5-H), 5.59 (s, 2, CH_2), 4.63 (br s, 1, exchangeable with D_2O, OH), 3.64–3.41 (m, 4, CH_2CH_2), 2.20 (s, 3, CH_3); UV λ_{max} nm ($\epsilon \times 10^4$) pH 7, 205 (1.6), 242 (3.0), 279 (7.5), 295 (7.5); pH 1, 205 (1.7, 242 (2.7), 282 (7.6); pH 11, 216 (2.4), 240 (1.8), 288 (7.4). Anal. ($C_{11}H_{13}N_4O_3Cl$) C, H, N.

2,4-Diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (17). Compound **16** (0.15 g) was dissolved in dry methanol, and to this solution was added 20 mL of methanol (previously saturated with NH_3 at 0 °C). This reaction mixture was then heated at 135 °C in a sealed steel reaction vessel for 10 h. The solvent was removed in vacuo, and the brown syrup was

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subjected to preparative (precoated silica gel F-254, 0.5 mm) TLC. The compound obtained from the appropriate band was then purified by radial chromatography by using a chromatatron with a solvent system of methanol/chloroform (10:90 v/v). The single UV absorbing band was eluted with the same solvent system and collected in 25-mL fractions (total of 10). The compound was obtained in the first three (25 mL) fractions. The fractions were combined, and the solvent was removed in vacuo to give a light green syrup. Trituration of this syrup with diethyl ether gave a crystalline solid, which was recrystallized from methanol to obtain 0.03 g (27.27%) of **17**: mp 150–151 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.83 (d, 1, J = 3.6 Hz, C-6-H), 6.74 (br s, 2, exchangeable with D_2O, C-4-NH_2), 6.40 (d, 1, J = 3.5 Hz, C-5-H), 5.32 (s, 2, CH_2), 4.6 (br s, 1, exchangeable with D_2O, OH), 3.4 (m, 4, CH_2CH_2); UV λ_{max} nm ($\epsilon \times 10^4$) pH 1, 206 (2.0), 232 (3.0), 262 (1.4), 299 (1.0); pH 11, 226 (2.8), 262 (1.6), 282 (1.2). Anal. ($C_9H_{13}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

Biological Evaluations. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hanks salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and diploid cell cultures of human foreskin fibroblast (HFF) cells were grown in MEM with Earle salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[37] The plaque-purified isolate, P_0, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[20]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 plaque reduction experiments were performed with use of monolayer cultures of BSC-1 cells. The assay was performed exactly as referenced above for HSV-1 titration assays except that the 0.2 mL of virus suspension contained approximately 100 PFU of HSV-1 and the compounds to be tested were dissolved in the overlay medium.

(c) Cell Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF and BSC-1 cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Cytopathology was estimated at 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of radioactive precursors into DNA, RNA and protein as detailed elsewhere.[20]

(d) Data Analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceeding sections against log drug concentrations; 50% inhibitory (I_{50}) concentrations were calculated from the regression lines. The three I_{50} concentrations for inhibition of DNA, RNA, and protein synthesis were averaged to give the values reported in Table I for KB cell cytotoxicity. Samples containing positive controls (acyclovir, ganciclovir, or vidarabine) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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Synthesis and Thromboxane A_2 Antagonist Activity of *N*-Benzyltrimetoquinol Analogues

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It is currently believed that the platelet thromboxane A_2 (TXA_2/PGH_2) receptor is different from the vascular TXA_2/PGH_2 receptor. While the majority of TXA_2 receptor antagonists are structurally related to the prostaglandins, trimetoquinol (TMQ) represents a unique nonprostanoid antagonist. TMQ also possesses β-adrenergic activity; however, an *N*-benzyl substituent on TMQ has been shown to impart some selectivity for platelet antiaggregatory activity versus β-adrenergic activity. In this study, we examined the synthesis and TXA_2 antagonist activity of a series of substituted *N*-benzyl analogues of TMQ. While these analogues showed an apparent direct correlation between platelet antiaggregatory activity and electron-donating ability of the *N*-benzyl substituents, no such correlation could be demonstrated for the inhibition of contractile responses. Thus, nonprostanoid TXA_2 antagonists can be used to demonstrate differences between platelet and vascular TXA_2/PGH_2 responses.

Thromboxane A_2 (TXA_2) is a potent vasoconstrictor and platelet aggregatory agent that mediates its effects by receptor-stimulated breakdown of polyphosphoinositides.[1-3] Inhibition of TXA_2 at the receptor level is thus a useful approach to the treatment of various ischemic and thrombotic disorders.[4] The TXA_2 receptor is usually

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