Synthesis and Antiherpes Simplex Virus Activity of 9-[(1,3-Dihydroxy-2-propylthio)methyl]guanine[1]

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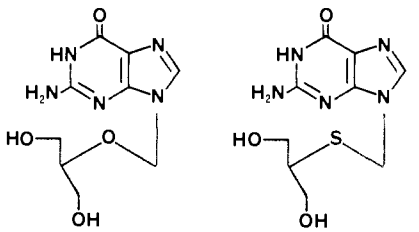
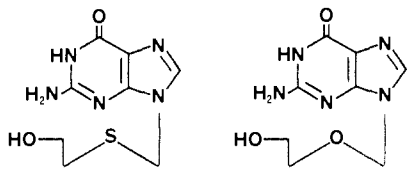
The synthesis of the thio analogue (thio-DHPG, **2**) of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, **1**) is described. The synthesis of **2** proceeded via the condensation of acetoxymethyl sulfide **9** with diacetylguanine **10** to give the protected nucleoside analogue **11**. Although catalytic hydrogenolysis failed, the benzyl ether functionalities of **11** were successfully cleaved by an acetolysis reaction to furnish **14**. Ammonolysis of **14** gave **2**, which was also transformed to sulfoxide **15** and sulfone **16**. Preliminary in vitro screening indicated that **2** exhibited comparable activity to DHPG against herpes simplex virus type 1 (HSV-1) but was less active against the type 2 virus (HSV-2) and human cytomegalovirus (HCMV). In a mouse encephalitis model (HSV-2), subcutaneous treatment with **2** led to a 53% reduction in mortality at a dose of 100 mg/kg per day.

The synthesis and antiviral activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, **1**) an acyclic



1 **2**

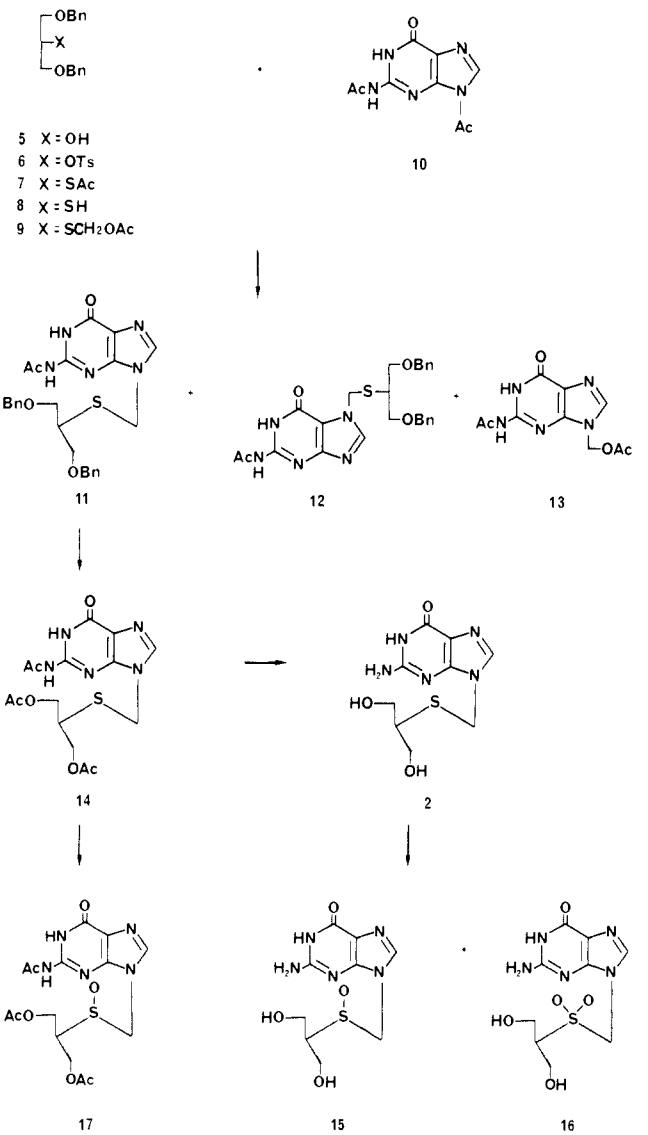


3 **4**

analogue of 2′-deoxyguanosine[2] was recently reported by us[3] and others.[4] DHPG belongs to a class of relatively selective antiherpetic nucleoside analogues that are similar in their mechanism of action.[5] These analogues are first phosphorylated to a monophosphate by a virus-specified thymidine kinase present in infected cells.[6,7] If an analogue is a poor substrate for host kinases, selectivity is realized at this step. Cellular kinases convert the monophosphates to the triphosphate analogues.[7] The triphosphates inhibit the virus-specified DNA polymerase and thus virus replication.[8,9] Of this class, DHPG is potent and broadly active, being effective against not only herpes simplex virus types 1 (HSV-1) and 2 (HSV-2)[3,4a,6,10,11] but also human cytomegalovirus (HCMV),[4a,6,10,12] varicella zoster virus,[4a,13] and Epstein–Barr virus.[4a,10,14]

We have been synthesizing a number of analogues of DHPG in order to define the structure–activity relationships of antiviral activity and host toxicity and now report the synthesis and biological activity of the thio analogue (thio-DHPG, **2**). The substitution of sulfur for oxygen in order to obtain biologically active analogues has substantial precedent in medicinal chemistry. For instance, the thio analogue **3** was shown to exhibit one-fifth the in vitro potency of acyclovir **4**.[15] Another example is the 4′-thio analogue of the nucleoside antibiotic toyocamycin which retains antibiotic and antileukemic activity.[16] Although

Scheme I



5 X = OH
6 X = OTs
7 X = SAc
8 X = SH
9 X = SCH_2OAc

10 **11** **12** **13** **14** **2** **17** **15** **16**

McCormick and McElhenney have synthesized acyclic thio nucleosides,[17] their attempted synthesis of **2** failed.[18]

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(1) Contribution 200 from the Institute of Bio-Organic Chemistry, Syntex Research.

(2) The structural formulas of DHPG (**1**) and the related acyclic nucleoside analogues have been depicted in a "ribose-like" conformation only to draw attention to the similarity in structure between these compounds and 2′-deoxynucleosides. In accordance with this representation, the two terminal carbons of the glycerol moiety are referred to as the 3′- and 5′-positions and the central carbon as the 4′-position.

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Table I. Antiviral and Anticellular Activities of the Acyclic Nucleoside Analogues in Tissue Culture

	ID_{50},[b] μM				
virus or cell	2	15	16	1	4
HSV-1 (F)[a]	0.9	25	40	0.3	0.4
HSV-2 (G)	8.0			1.3	1.5
HCMV (AD 169)	80			6	100
Vero cells	900			500	1100

[a] The strain is given in parentheses. [b] Determined by plaque reduction assays in Vero (HSV) or MRC-5 (HCMV) cells or cell proliferation assays in uninfected cells.

Chemistry

The synthesis of **2** (Scheme I) parallels our earlier DHPG synthesis[3] and commenced from 1,3-di-*O*-benzylglycerol (**5**),[3] which was treated with *p*-toluenesulfonyl chloride to give tosylate **6** in 96% yield following chromatographic purification. Reaction of **6** with potassium thioacetate at 90 °C for 2 h in dimethylformamide gave a 94% yield of thio ester **7** that, upon treatment with methanolic ammonia (saturated at 0 °C) at room temperature for 16 h, afforded a 54% distilled yield of thiol **8**. Chloromethylation of **8** (HCl, paraformaldehyde) followed by reaction with sodium acetate gave the intermediate acetoxymethyl thioether **9**.

Condensation of **9** with N^2,9-diacetylguanine (**10**) in the presence of a catalytic amount of bis(4-nitrophenyl) phosphate in sulfolane (100 °C, 6 h) gave the desired N^9 isomer **11** in 11% yield along with a 9% yield of the N^7 isomer **12**. As indicated by TLC, the major product of this condensation reaction was the acetoxymethyl derivative **13**, which was only a minor product in the corresponding DHPG synthesis.[3] This result was probably due to the poorer stability of the intermediate methylene sulfonium ion as opposed to the more stable oxonium ion intermediate in the DHPG synthesis. The structural assignments of regioisomers **11** and **12** were made on the basis of comparison of their corresponding ^{13}C NMR spectra with those of known purines.[19]

Cleavage of the benzyl ether functionalities of **11** was first attempted by hydrogenolysis. Standard catalytic reduction (10% Pd/C, 50 psi H_2) or transfer hydrogenation (20% $Pd(OH)_2$/C, cyclohexene)[20] were unsuccessful, presumably because of inactivation of the palladium catalysts by sulfur. Sodium in ammonia reduction gave cleavage of the thioaminal functionality. Finally, the benzyl ether groups were cleaved by an acetolysis reaction (BF_3·OEt_2, acetic anhydride, room temperature, 7 h) to give triacetyl derivative **14** in 54% yield. Ammonolysis of **14** by treatment with methanolic ammonia (saturated at 0 °C) at room temperature for 16 h gave thio-DHPG (**2**) in 80% yield.

The sulfoxide and sulfone derivatives **15** and **16** were also synthesized. Initially, sulfide **14** was oxidized to sulfoxide **17** with *m*-chloroperoxybenzoic acid. Unfortunately, attempted deacetylation of **17** by ammonolysis led to decomposition. Direct oxidation of **2** with hydrogen peroxide afforded both **15** and **16**. The two products were isolated in pure form by fractional crystallization.

Table II. Effects of Subcutaneous Treatment with Thio-DHPG (**2**) and DHPG (**1**) on HSV-2 Induced Mortality in Mice

drug	dose,[a] mg/kg	survivors/ total	survivor inc[b]	mean survival time, days	mean survival time inc[c]
saline		1/16 (6)[d]		8.5 ± 1.2[e]	
DHPG (1)	30	12/16 (75)	<0.001	13.8 ± 1.9	<0.001
	10	7/16 (44)	<0.04	12.4 ± 2.6	<0.001
	3	4/16 (25)	NS[f]	11.2 ± 2.2	NS
thio-DHPG	100	8/15 (53)	0.01	13.0 ± 2.4	<0.001
(2)	30	2/16 (12)	NS	9.4 ± 1.4	NS
	10	1/16 (6)	NS	9.7 ± 1.4	NS

[a] Mice were infected intraperitoneally, and a subcutaneous dose was administered once daily (at 24-h intervals) for 4 days starting 24 h post-infection. [b] Probability (Fisher exact test). [c] Probability (Mann–Whitney u-test). [d] Percent survivors. [e] Standard deviation. [f] Not statistically significant ($p < 0.05$).

Biological Results and Discussion

The in vitro antiviral activities of thio analogue **2**, sulfoxide **15**, and sulfone **16** were compared to that of DHPG (**1**) and acyclovir (**4**) (Table I). Thio-DHPG (**2**) was half as active as both DHPG and acyclovir against HSV-1 but less effective against the type 2 virus. Derivatives **15** and **16** were only marginally active against HSV-1. Like acyclovir, thio-DHPG was not very effective against HCMV. As a measure of toxicity, the inhibition by these analogues of Vero cell growth was measured. In this assay, both thio-DHPG and acyclovir showed about half the toxicity of DHPG.

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We have previously found that the in vitro data of antiviral activity against HSV does not necessarily correlate well with in vivo activity. For instance, although DHPG and acyclovir are equally potent in vitro against HSV-2 (G strain), DHPG is approximately 50-fold more active against this virus in a mouse encephalitis model.[6] In order to more accurately evaluate its antiviral potency, the effects of thio-DHPG were studied in the above in vivo model. In this model, mice die of encephalitis after being infected intraperitoneally with 10 times the 50% lethal dose of HSV-2 (G strain) (Table II). Thio-DHPG (2) was effective at preventing death at a dose of 100 mg/kg per day. In fact, since 53% of the mice survived at this dose, 2 was more effective than oral acyclovir (4), which was shown in this model to have an ID_{50} of approximately 500 mg/kg per day.[6] These data show that although thio-DHPG (2) is less active than either DHPG (1) or acyclovir (4) against HSV-2 (G strain) in vitro, its potency in vivo is less than that of 1 and similar to that of 4.

Experimental Section

Nuclear magnetic resonance spectra were recorded on an EM-390 (^{1}H NMR, 90 MHz), a Bruker WH-90 (^{13}C NMR, 22.62 MHz), and a Bruker WM-300 instrument (^{1}H NMR, 300 MHz; ^{13}C NMR, 75.453 MHz), and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Spectroscopic data and elemental analyses were obtained by Syntex Analytical Research. All chromatographic purifications were carried out on silica gel. Melting points were determined on a hot-stage microscope an are corrected.

(1,3-Di-*O*-benzyl-2-*O*-*p*-toluenesulfonyl)glycerol (6). A solution of **5** (32.0 g, 117 mmol) and *p*-toluenesulfonyl chloride (28.0 g, 147 mmol) in pyridine (100 mL) was stirred at room temperature for 16 h and then evaporated to dryness. The residue was dissolved in dichloromethane, and the resulting solution was washed with water and saturated Na_2CO_3, dried over $MgSO_4$, and evaporated to a yellow oil. The oil was chromatographed (1:3 ethyl acetate/hexane) to give 48.2 g (96%) of **6** as a clear oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.75 (d, J = 8 Hz, 2 H, tosylate), 7.30 (s, 10 H, phenyl), 7.15 (d, J = 8 Hz, 2 H, tosylate), 4.74 (p, J = 6 Hz, 1 H, CH), 4.40 (s, 4 H, benzylic), 3.64 (d, J = 6 Hz, 4 H, CH_2), 2.33 (s, 3 H, CH_3). Anal. ($C_{24}H_{26}O_5S$) C, H, S.

1,3-Bis(benzyloxy)-2-(acetylthio)propane (7). A solution of **6** (48.0 g, 112 mmol) and potassium thioacetate (17.3 g, 151 mmol) in DMF (100 mL) was heated at 90 °C for 2 h and then cooled to room temperature. The solution was diluted with toluene, washed with water, saturated Na_2CO_3, and 10% HCl, dried over $MgSO_4$, and evaporated to give 34.8 g (94%) of **7** as a dark oil. An analytical sample was obtained by Kugelrohr distillation: bp 170–180 °C (0.1 torr); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.30 (s, 10 H, phenyl), 4.52 (s, 4 H, benzylic), 3.87 (p, J = 6 Hz, 1 H, CH), 3.67 (d, J = 6 Hz, 4 H, CH_2), 2.31 (s, 3 H, CH_3). Anal. ($C_{19}H_{22}O_3S$) C, H, S.

1,3-Bis(benzyloxy)-2-propanethiol (8). A solution of crude **7** (34.8 g, 142 mmol) in methanolic ammonia (200 mL, saturated at 0 °C) was stirred at room temperature for 16 h and then evaporated to dryness. The residue was eluted with dichloromethane through a small amount of silica gel and the eluent evaporated to dryness. The resulting oil was distilled on a Kugelrohr apapratus to give 17.2 g (54%) of **8** as a red oil: bp 170–185 °C (0.1 torr); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.30 (s, 10 H, phenyl), 4.54 (s, 4 H, benzylic), 3.63 (d, J = 6 Hz, 4 H, CH_2), 3.20 (m, 1 H, CH), 1.92 (d, J = 8 Hz, 1 H, SH). Anal. ($C_{17}H_{20}O_2S$) C, H, S.

N^2-Acetyl-9-[[1,3-bis(benzyloxy)-2-propylthio]methyl]guanine (11) and N^2-Acetyl-7-[[1,3-bis(benzyloxy)-2-propylthio]methyl]guanine (12). Hydrogen chloride gas (dried through concentrated H_2SO_4) was bubbled into a stirred mixture of paraformaldehyde (1.3 g, 43.3 mmol) and **8** (6.7 g, 23.2 mmol) in 1,2-dichloroethane (60 mL) at 0 °C for 1.5 h. The resulting solution was dried over $MgSO_4$ and evaporated to dryness. The resulting oil was treated with sodium acetate (3.8 g, 46.3 mmol) in DMF (10 mL) at room temperature for 2 h. The resulting suspension was filtered. The filtrate was diluted with dichloromethane, washed with saturated Na_2CO_3 and 10% HCl, dried over $MgSO_4$, and evaporated to give **9** as a pale yellow oil. A mixture of **9** from above, diacetylguanine **10** (5.4 g, 22.9 mmol), bis(4-nitrophenyl) phosphate (100 mg), and sulfolane (10 mL) was heated with stirring at 100 °C for 6 h and then at room temperature for 18 h. The resulting mixture was diluted with dichloromethane and filtered through Celite. In order to remove sulfolane and byproduct **13**, the filtrate was filtered through a short silica gel column, eluting with a gradient of dichloromethane to 5% methanol/dichloromethane. Selected fractions were pooled and chromatographed (3% methanol/dichloromethane) to give in order of elution 1.0 g (9%) of **12** and 1.2 g (11%) of **11** as white solids.

An analytical sample of **11** was obtained by recrystallization from ethyl acetate: mp 169–170 °C; UV λ_{max} 278 nm (ϵ 11 300), 260 (15 700) (methanol); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 8.08 (s, 1 H, H-8), 7.18–7.35 (m, 10 H, aromatic), 5.30 (s, 2 H, H-1′), 4.41 (s, 4 H, benzylic), 3.55 (m, 4 H, H-3′, H-5′), 3.32 (p, J = 6 Hz, 1 H, H-4′), 2.15 (s, 3 H, CH_3); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 173.60 (CO), 154.84 (C-6), 148.43 (C-4), 147.91 (C-2), 139.37 (C-8) 138.16, 128.22, 127.37 (phenyl), 120.28 (C-5), 72.07 (benzylic), 69.89 (C-3′, C-5′), 45.35 (C-1′), 44.11 (C-4′), 23.70 (CH_3). Anal. ($C_{25}H_{27}N_5O_4S$) C, H, N, S.

An analytical sample of **12** was obtained by recrystallization from ethyl acetate/hexane: mp 108–109 °C; UV λ_{max} 285 nm (ϵ 9700), 264 (13 000) (methanol); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 8.30 (s, 1 H, H-8), 7.20–7.35 (m, 10 H, phenyl), 5.53 (s, 2 H, H-1′), 4.40 (s, 4 H, benzylic), 3.51 (m, 4 H, H-3′, H-5′), 3.35 (m, 1 H, H-4′), 2.17 (s, 3 H, CH_3); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 173.43 (CO), 157.51 (C-4), 152.66 (C-6), 147.24 (C-2), 144.21 (C-8), 138.20, 128.22, 127.37 (phenyl), 110.86 (C-5), 72.07 (benzylic), 69.90 (C-3′, C-5′), 47.23 (C-1′), 44.83 (C-4′), 23.63 (CH_3). Anal. ($C_{25}H_{27}N_5O_4S$) C, H, N, S.

9-(Acetoxymethyl)-N^2-acetylguanine (13). On a smaller scale condensation, the more polar acetoxymethyl derivative **13** was isolated in 19% yield during the chromatographic purification of **11** and **12**. An analytical sample of **13** was obtained by recrystallization from ethanol/ethyl acetate: mp 245–247 °C; UV λ_{max} 280 nm (ϵ 10 400), 258 (13 900) (methanol); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 8.07 (s, 1 H, H-8), 5.99 (s, 2 H, CH_2) 2.18 (s, 3 H, NHAc), 2.06 (s, 3 H, OAc); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 173.63 (NCO), 169.86 (OCO), 154.81 (C-6), 148.83 (C-2), 148.44 (C-4), 140.15 (C-8), 120.02 (C-5), 65.21 (CH_2), 23.67 (NHAc), 20.35 (OAc). Anal. ($C_{10}H_{11}N_5O_4$) C, H, N.

N^2-Acetyl-9-[(1,3-diacetoxy-2-propylthio)methyl]guanine (14). Boron trifluoride etherate (0.80 mL, 6.5 mmol) was added to a stirred 0 °C solution of **11** (1.15 g, 2.33 mmol) in acetic anhydride (25 mL). The resulting solution was kept at room temperature for 7 h and then evaporated to dryness. The residue was triturated twice with ether and then chromatographed (3% methanol/dichloromethane) to give 0.50 g (54%) of **14** as a white solid. An analytical sample was recrystallized from ethanol: mp 199–201 °C; UV λ_{max} 280 nm (ϵ 11 200), 260 (15 000) (methanol); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 8.10 (s, 1 H, H-8), 5.32 (s, 2 H, H-1′), 4.10, 4.15 (ABX, J = 6 and 11 Hz, 4 H, H-3′, H-5′), 3.45 (p, J = 6 Hz, 1 H, H-4′), 2.19 (s, 3 H, NHAc), 1.96 (s, 6 H, OAc); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 173.60 (NCO), 170.09 (OCO), 154.84 (C-6), 148.44 (C-4), 147.98 (C-2), 139.33 (C-8), 120.28 (C-5), 63.13 (C-3′, C-5′), 43.79 (C-1′), 43.17 (C-4′), 23.73 (NHAc), 20.35 (OAc). Anal. ($C_{15}H_{19}N_5O_6S$) C, H, N, S.

9-[(1,3-Dihydroxy-2-propylthio)methyl]guanine (2). A solution of **14** (1.10 g, 2.76 mmol) in methanolic ammonia (saturated at 0 °C) was stirred in a stoppered flask at room temperature for 16 h and then evaporated to dryness. The residue was triturated twice with dichloromethane and then recrystallized from water to give 0.60 g (80%) of **2**: mp 221–222 °C; UV λ_{max} sh 278 nm (ϵ 8000) and 257 (11 100) (0.1 N HCl), 266 (10 600) (0.1 N NaOH); ^{1}H NMR (300 MHz, MeSO-d_6) δ 10.61 (s, br, 1 H, NH), 7.78 (s, 1 H, H-8), 6.49 (s, br, 2 H, NH_2), 5.14 (s, 2 H, H-1′), 4.78 (t, J = 5 Hz, 2 H, OH), 3.51 (t, J = 6 Hz, 4 H, H-3′, H-5′), 2.90 (p, J = 6 Hz, 1 H, H-4′); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 156.64 (C-6), 153.46 (C-2), 150.86 (C-4), 136.92 (C-8), 116.46 (C-5), 61.17 (C-3′, C-5′), 49.66 (C-4′), 42.81 (C-1′). Anal. ($C_9H_{13}N_5O_3S$) C, H, N, S.

N^2-Acetyl-9-[(1,3-diacetoxy-2-propylsulfinyl)methyl]guanine (17). A suspension of **11** (90 mg, 0.22 mmol) and *m*-

chloroperoxybenzoic acid (53 mg, 0.31 mmol) in methanol (2 mL)/dichloromethane (10 mL) was stirred at room temperature for 16 h and then evaporated to dryness. The residue was purified by preparative TLC (1:9 methanol/dichloromethane) to give 40 mg (43%) of **17**. An analytical sample was obtained by recrystallization from ethanol: mp 215–217 °C; UV λ_{max} 283 nm (ϵ 11 300), 260 (15 200), 255 (15 300) (methanol); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.99 (s, 1 H, H-8), 5.43, 5.53 (AB, J = 13 Hz, 2 H, H-1′), 4.28–4.52 (m, 4 H, H-3′, H-5′), 3.62 (p, J = 6 Hz, 1 H, H-4′), 2.20 (s, 3 H, NHAc), 2.06 (s, 6 H, OAc). Anal. ($C_{15}H_{19}N_5O_7S$) C, H, N, S.

9-[(1,3-Dihydroxy-2-propylsulfinyl)methyl]guanine (15). A suspension of **2** (120 mg, 0.44 mmol) in 30% H_2O_2 (4 mL)/H_2O (4 mL) was stirred at room temperature for 48 h and then evaporated to dryness. The residue was recrystallized twice from water to give 30 mg (27%) of **15**: mp 209–210 °C; UV λ_{max} sh 274 nm (ϵ 9300) and 256 (12 600) (0.1 N HCl), 262 (11 200) (0.1 N NaOH); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.66 (s, 1 H, H-8), 6.60 (s, br, 2 H, NH_2), 5.29, 5.40 (AB, J = 13 Hz, 2 H, H-1′), 5.21 (s, br, 1 H, OH), 5.07 (s, br, 1 H, OH), 3.68–3.93 (m, 4 H, H-3′, H-5′), 3.00 (m, 1 H, H-4′). Anal. ($C_9H_{13}N_5O_4S{\cdot}0.5H_2O$) C, H, N, S.

9-[(1,3-Dihydroxy-2-propylsulfonyl)methyl]guanine (16). Repeated recrystallizations from water of the mother liquors of **15** gave 13 mg (10%) of **16**: mp 262–263 °C; UV λ_{max} 265 nm (ϵ 11 400) (0.1 N NaOH); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.70 (s, 1 H, H-8), 6.55 (s, br, 2 H, NH_2), 5.50 (s, 2 H, H-1′), 5.22 (s, br, 2 H, OH), 3.87 (m, 4 H, H-3′, H-5′), 3.44 (p, J = 6 Hz, 1 H, H-4′). Anal. ($C_9H_{13}N_5O_5S$) C, H, N, S.

Plaque Assays. Experiments were conducted with Vero (for HSV-1 and HSV-2 infections) and MRC-5cells (for HCMV infections) that were treated with the nucleoside analogue as described previously.[6] Inhibitory doses ID_{50} are defined as doses causing a 50% reduction in plaque numbers compared to untreated controls.

Animal Studies. Swiss–Webster female mice (Simonsen Laboratories, Gilroy, CA), weighing approximately 20 g each, were infected intraperitoneally with 5×10^4 PFU of HSV-2 (strain G). This challenge was approximately equivalent to 10 50% lethal doses. DHPG and **2** were administered subcutaneously once a day for 4 days starting 24-h post-infection. Deaths were recorded for 21 days after infection.

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Structure–Activity Relationships of Some Pyridine, Piperidine, and Pyrrolidine Analogues for Enhancing and Inhibiting the Binding of (±)-[^{3}H]Nicotine to the Rat Brain P_2 Preparation†

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Previous studies have shown that (±)-[^{3}H]nicotine binds to multiple sites in the rat brain P_2 preparation. Using a series of pyridine, piperidine and pyrrolidine analogues, the present studies identified drugs with specificity for a separate up-regulatory site that increases the density of nicotine binding at another site. Of these compounds, (±)-2-methylpiperidine was the most specific. Some compounds inhibited without enhancing (±)-[^{3}H]nicotine binding, but none bound with the very high affinity exhibited by nicotine and none could be classified as specific in inhibiting binding at a specific site. Structural changes in the 1- and 2-positions of pyridine and piperidine appear to be important for conferring specificity for the up-regulatory site whereas 3-position changes may be important for binding specificity.

Nicotine can induce diverse pharmacological actions in the central nervous system including analgesia,[1-3] euphoria,[4] ganglionic stimulation, or depression, leading to many effects such as changes in blood pressure and cardiac rhythmicity,[5-7] effects that can also be produced by intracerebroventricular administration.[1,3,8,9] Both behavioral arousal and depression have been observed[1,3,10,11] as well as alterations in conditioned responses,[12,13] alterations in myoneural junction transmission,[14,15] changes in respiration,[1,16] facilitation of memory, and reduction of aggression,[17,18] nausea, vomiting, and hormonal changes.[6]

On the basis of studies employing classic cholinergic agonists and antagonists the data suggest that nicotine exerts some of its pharmacological effects through different receptors (or subsets of receptors). Some of the actions of nicotine are clearly cholinergic since they are blocked by cholinergic antagonists.[2,9,10,19-32] Some other central actions of nicotine, however, appear to be mediated through noncholinergic mechanisms.[33-36] Different effects on behavior and the EEG have been observed with different doses of nicotine injected into the lateral ventricle of the conscious dog, thus lending further support to the hypothesis that nicotine has multiple modes of action.[3,11]

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