# Synthesis, Properties, and Pharmacokinetic Studies of $N^2$-Phenylguanine Derivatives as Inhibitors of Herpes Simplex Virus Thymidine Kinases

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Two series of selective inhibitors of herpes simplex virus types 1 and 2 (HSV1,2) thymidine kinases (TK) have been developed as potential treatment of recurrent virus infections. Among compounds related to the potent base analog $N^2$-[*m*-(trifluoromethyl)phenyl]guanine (*m*$CF_3$PG), none was a more potent inhibitor than *m*$CF_3$PG itself. Compounds related to the nucleoside $N^2$-phenyl-2′-deoxyguanosine (PhdG), but with alkyl, hydroxyalkyl, and related substituents at the 9-position in place of the glycosyl group of PhdG, retained significant but variable inhibitory potencies against the HSV TKs. The most potent inhibitor of HSV1 TK among 9-substituted derivatives, 9-(4-hydroxybutyl)-$N^2$-phenylguanine (HBPG), was a competitive inhibitor with respect to the substrate thymidine but was not itself a substrate for the enzyme. Water solubilities and 1-octanol:water partition coefficients for the 9-substituted $N^2$-phenylguanines were linearly but oppositely related to the sum of hydrophobic fragmental constants ($\Sigma f$) of the 9-substituents. Four of the inhibitors were given as solutions to mice by iv and ip routes, and the time course of their plasma concentrations was determined by HPLC analysis of the parent compounds. HBPG was completely absorbed by the ip route, and the plasma concentration could be prolonged by use of suspension formulations. HBPG is a candidate for animal trials of the ability of TK inhibitors to prevent recurrent herpes virus infections.

Herpes simplex virus (HSV)-encoded thymidine kinase (TK) is not required for the efficient replication of virus in dividing cells[1] but may be important in reactivation of virus from the latent state.[2] A strategy for the control of herpetic recurrences would be to interfere with the reactivation process by targeting inhibitors against the virus-encoded TK. This concept has led to efforts by several groups to develop HSV TK inhibitors as antiviral drugs.

Several $N^2$-phenylguanines and their nucleosides display selective inhibitory activity toward the herpes simplex virus type 1 (HSV1) and herpes simplex virus type 2 (HSV2) TKs relative to the human enzyme *in vitro* and *in vivo*.[3–6] Two such compounds are $N^2$-[*m*-(trifluoromethyl)phenyl]guanine, **2** (*m*$CF_3$PG), and $N^2$-phenyl-2′-deoxyguanosine, **3** (PhdG) (Scheme 1). *m*$CF_3$-PG and PhdG were the most potent TK inhibitors among compounds substituted on the phenyl ring and at the 9-position of $N^2$-phenylguanine, 1 (PG), respectively. The action of the inhibitors is competitive with the enzyme substrates thymidine (TdR) and 2′-deoxycytidine. The two inhibitors were tested for their ability to inhibit the reactivation of HSV1 from explant cultures of latently infected murine trigeminal ganglia. Both compounds at 150 $\mu$M significantly diminished the frequency of reactivation compared with that of untreated control ganglia.[6]

Several animal models of herpes virus latency and reactivation have been established. These include murine eye[7] and ear[8] models for HSV1, rabbit[9] and squirrel monkey[10] eye models for HSV1, and a guinea pig vaginal model for HSV2.[11] In these models both spontaneous and induced viral reactivation can be studied. Two studies of TK inhibitors on spontaneous HSV recurrences *in vivo* have been reported. 5′-Ethynylthymidine was given intraperitoneally (ip) as an aqueous suspension to squirrel monkeys in which latent infections had been established by prior corneal inoculation with HSV1.[10] After 25 days of dosing, viral lesions in the corneas of animals treated with drug were compared with those of control animals. Recurrent lesions were found in 3/20 eyes of treated animals compared to 8/20 eyes of control animals ($P = 0.077$).[10] In another study, 9-[[(*Z*)-2-(hydroxymethyl)cyclohexyl]methyl]guanine, L-653,180, was administered *ad libitum* in food and drinking water to female guinea pigs starting 21 days after vaginal inoculation with HSV2.[11] Drug treatment continued to 45 days, and animals were observed for recurrent herpetic disease for 91 days postinfection. During the entire observation period (22–91 days), there were 10.3 mean lesion days for treated animals compared with 15.3 for control animals ($p = 0.06$).[11]

These initial results stimulated us to continue to develop $N^2$-phenylguanines for animal testing. However, among the TK inhibitors synthesized previously in our laboratory, there exist serious limitations related to water solubility and other properties. In general, inhibitors such as 5′-ethynylthymidine, L-653,180, and $N^2$-phenylguanines have poor water solubility, and high and persistent concentrations of the drug at the putative sites of action *in vivo*, the sensory nerve ganglia, may not be attained. Therefore, we decided to improve inhibitory and physicochemical properties of $N^2$-phenylguanines before selecting a compound for animal

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**Scheme 1**

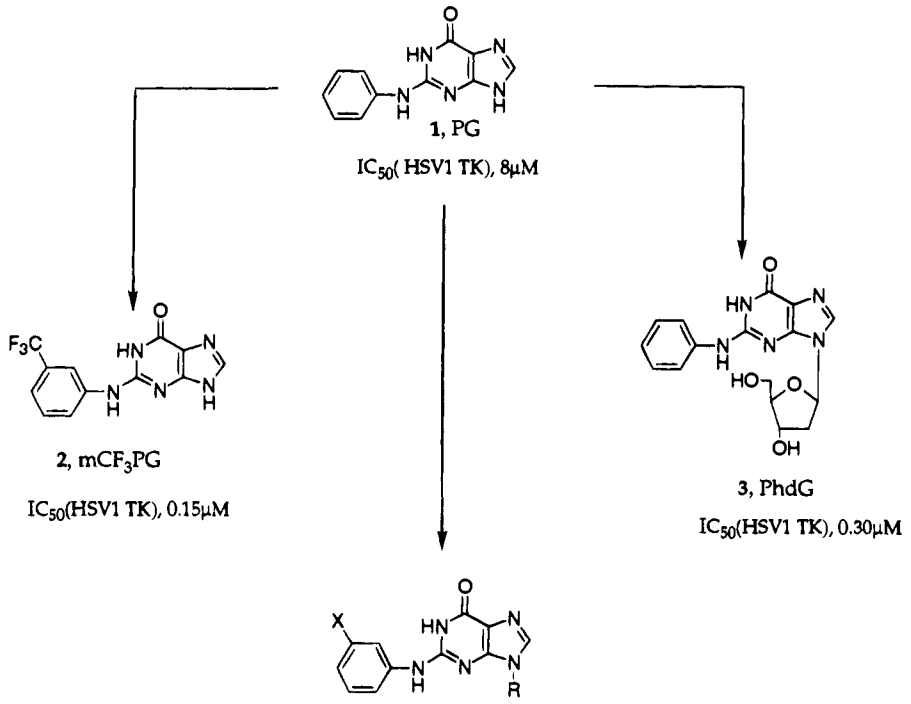



**Table 1.** Physical Data for $N^2$-Phenylguanine Derivatives

| compd | mp (°C) | yield (%) | crystn[a] | formula | $^1$H NMR (δ, ppm)[b] 2-NH | 8-H |
|---|---|---|---|---|---|---|
| **5** | 312–313 | 54 | A | $C_{13}H_{10}N_5OF_3 \cdot {}^1/_4H_2O$ | 8.85 | 7.88 |
| **6** | >350 | 62 | A | $C_{13}H_9N_5OCl_2F_2 \cdot {}^1/_2H_2O$ | 9.02 | 7.87 |
| **7** | 295–297 | 89 | A | $C_{12}H_8O_2N_5F_3 \cdot {}^1/_4EtOH$ | 9.04 | 7.91 |
| **8** | 295–296 | 75 | B | $C_{12}H_9ON_5F_2 \cdot {}^1/_8H_2O$ | 9.65 | 8.44 |
| **9** | >350 | 60 | A | $C_{13}H_8ON_5Cl_3$ | 8.90 | 7.92 |
| **10** | 347–349 | 54 | B | $C_{14}H_{12}O_2N_5F_3 \cdot {}^1/_8H_2O$ | 9.61 | 8.37 |
| **11** | 328–330 | 89 | B | $C_{15}H_{17}O_2N_5$ | 9.35 | 7.82 |
| **12** | 357–360 | 78 | B | $C_{14}H_{13}ON_5 \cdot {}^1/_4H_2O$ | 8.81 | 7.80 |
| **13** | 355–357 | 69 | B | $C_{14}H_{15}O_2N_5 \cdot {}^1/_8H_2O$ | 9.28 | 7.77 |
| **14** | 314–317 | 58 | C | $C_{16}H_{19}O_3SN_5$ | 8.82 | 7.72 |
| **15** | 342–344 | 61 | B | $C_{14}H_{15}O_3N_5 \cdot {}^1/_4H_2O$ | 8.82 | 7.74 |
| **16** | 338–339 | 73 | B | $C_{13}H_{13}O_2N_5 \cdot {}^1/_8H_2O$ | 8.80 | 7.75 |
| **17** | 312–315 | 82 | B | $C_{13}H_{11}O_3N_5 \cdot {}^1/_8H_2O$ | 9.44 | 7.77 |

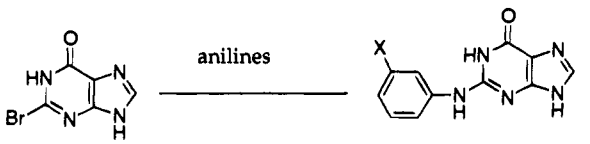
[a] A, ethanol. B, DMF/chloroform. C, methanol. [b] DMSO-$d_6$, TMS as internal reference.

study. In this paper we report results of efforts to increase potency of $N^2$-phenylguanines as HSV TK inhibitors, increase water solubility of active analogs, and maintain lipid solubility. We also describe physicochemical properties and pharmacokinetic studies in mice of several of these compounds. The results confirm that one compound, 9-(4-hydroxybutyl)-$N^2$-phenylguanine (HBPG, **11**), is a promising candidate for *in vivo* studies.

## Results

**Chemistry.** The *meta*-substituted $N^2$-phenylguanine derivatives **5**–**9** (Scheme 2) were prepared by reaction between 2-bromohypoxanthine and appropriate anilines in refluxing 2-methoxyethanol, as described previously.[3,12,13] Yields and properties of the new inhibitors are presented in Table 1. Certain anilines, such as 3-(difluoromethyl)-, 3-(2,2,2-trifluoroethyl)-, 3-(1,2-dichloro-2,2-difluoroethyl)-, and 3-(trichlorovinyl)anilines, were prepared by reduction of the corresponding nitro compounds, which were synthesized as described in the literature.[14,15] Synthesis and characterization of new anilines are described in the Experimental Section.

**Scheme 2**




**5**, X=$CH_2CF_3$

**6**, X=$CHClCF_2Cl$

**7**, X=$OCF_3$

**8**, X=$CHF_2$

**9**, X=$CCl{=}CCl_2$

The 9-substituted $N^2$-phenylguanine derivatives were synthesized by the two-step sequence shown in Scheme 3. First, sodium hydride was added to a suspension of 2-anilino-6-chloropurine in anhydrous acetonitrile at room temperature. The appropriate alkyl bromide was

**Scheme 3**

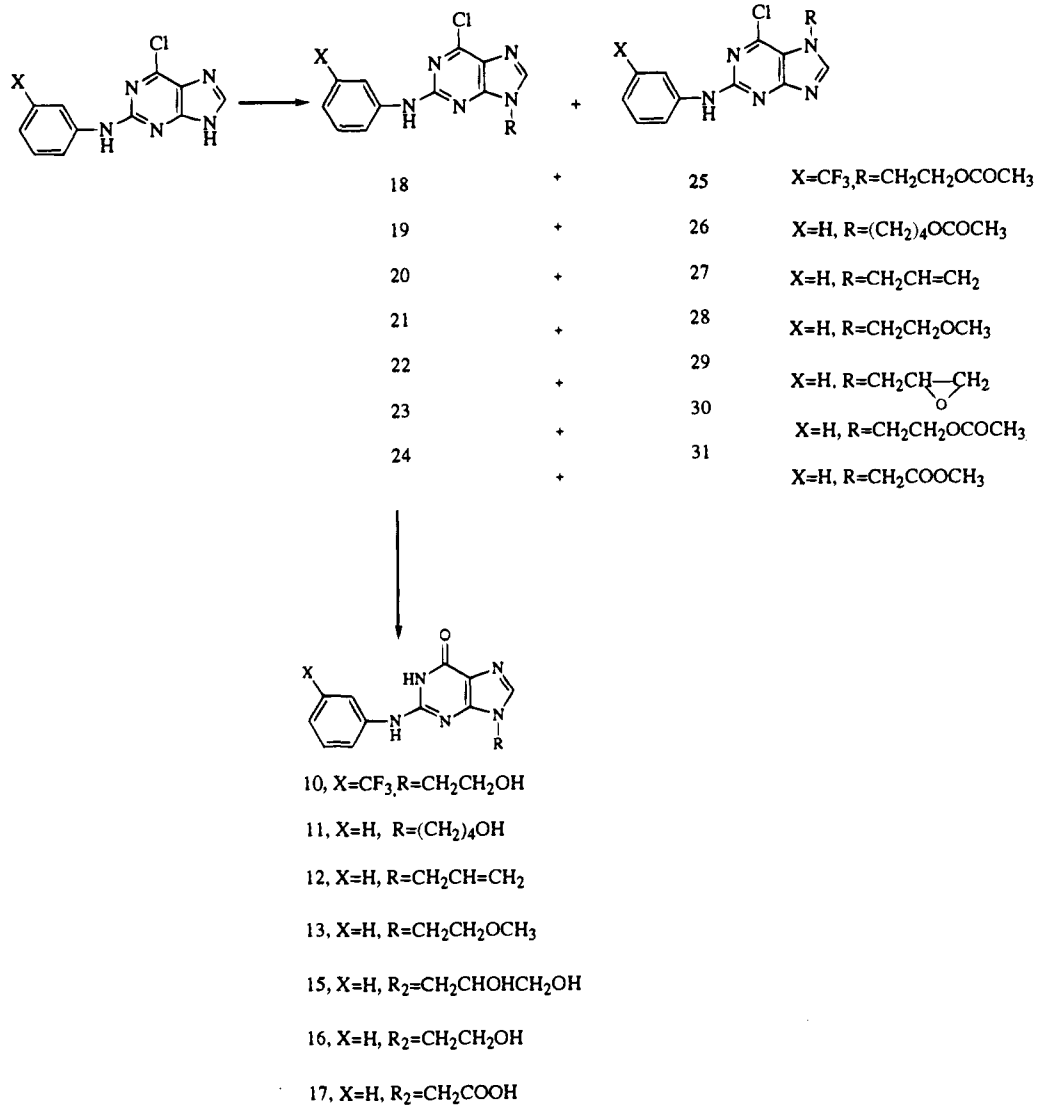



**Table 2.** Physical Data for 7- and 9-Substituted 6-Chloropurines

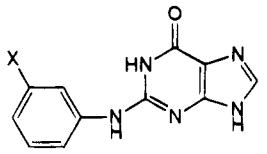
| compd | mp (°C) | yield[a] (%) | formula | $^1$H NMR ($\delta$, ppm)[b] 2-NH | 8-H | N-$CH_2$ |
|---|---|---|---|---|---|---|
| **18** | 212–213 | 30.2 | $C_{16}H_{13}O_2N_5ClF_3$ | 10.39 | 8.39 | 4.45 |
| **19** | 195–197 | 61 | $C_{17}H_{18}O_2N_5Cl$ | 9.98 | 8.32 | 4.21 |
| **20** | 218–220 | 48 | $C_{14}H_{12}N_5Cl$ | 9.90 | 8.26 | 4.82 |
| **21** | 149–153 | 34 | $C_{14}H_{14}ON_5Cl$ | 9.99 | 8.27 | 4.35 |
| **22** | 166–167 | 34 | $C_{14}H_{12}ON_5Cl$ | 9.97 | 8.24 | 4.48, 4.29 |
| **23** | 193–194 | 47 | $C_{15}H_{14}O_2N_5Cl\cdot{}^1/_4H_2O$ | 8.98 | 8.32 | 4.45 |
| **24** | 187–188 | 74 | $C_{14}H_{12}O_2N_5Cl\cdot{}^1/_8H_2O$ | 10.02 | 8.28 | 5.14 |
| **25** | 191–193 | 6.9 | $C_{16}H_{13}O_2N_5ClF_3$ | 10.19 | 8.60 | 4.66 |
| **26** | 152–153 | 4.4 | $C_{17}H_{18}O_2N_5Cl$ | 9.77 | 8.55 | 4.39 |
| **27** | 210–213 | 5.2 | $C_{14}H_{12}N_5Cl\cdot{}^1/_4H_2O$ | 9.78 | 8.54 | 5.03 |
| **28** | 146–148 | 7.5 | $C_{14}H_{14}ON_5Cl$ | 9.77 | 8.47 | 4.55 |
| **29** | 158–160 | 3.8 | $C_{14}H_{12}ON_5Cl$ | 9.78 | 8.45 | 4.69, 4.44 |
| **30** | 180–183 | 10 | $C_{15}H_{14}O_2N_5Cl\cdot{}^1/_2H_2O$ | 9.79 | 8.54 | 4.64 |
| **31** | 195–197 | 12 | $C_{14}H_{12}O_2N_5Cl\cdot{}^1/_8H_2O$ | 9.83 | 8.49 | 5.35 |

[a] All compounds were crystallized from chloroform. [b] DMSO-$d_6$, TMS as internal reference.

added, and the suspension was stirred at room temperature. Two products, the major 9-isomer and minor 7-isomer, were isolated in each case (Table 2). Formation of regioisomeric N-9 and N-7 substitution products is typically observed in alkylation reactions of purines.[16] The structure assignments for 9- and 7-isomers were based on characteristic chemical shift differences in their $^1$H NMR spectra. Signals from H-8 and N-$CH_2$ in 9-isomers are shifted upfield relative to the corresponding H-8 and N-$CH_2$ signals in 7-isomers. The 2-NH signals are shifted downfield in the 9-isomer relative to the corresponding 2-NH signals in the 7-isomer (Table 2). The chemical shift patterns are fully consistent with those observed in related 6-halopurines.[3-5,12,16] Hydrolysis of 9-isomers of the 6-chloropurines **18–24** in 0.5 N sodium hydroxide solution at reflux for about 2 h gave 9-substituted $N^2$-phenylguanine derivatives (**10–13** and **15–17**) in good yields. Compound **14** was obtained after prolonged reflux of compound **22** with 2-mercaptoethanol and sodium methoxide in methanol.[3] Yields and properties of 9-substituted $N^2$-phenylguanines are listed in Table 1.

Two disubstituted compounds were prepared to determine if substituent contributions to inhibitor activity were additive. The deoxyribonucleoside of $m$$CF_3$PG, i.e., compound **4**, was prepared by the reaction between 2-bromo-2′-deoxyinosine and $m$-(trifluoromethyl)aniline in ethanolic buffer at 120 °C as previously described.[5] Although the product was obtained in only 10% yield, this method avoided the multistep method described above to prepare similar compounds. A second disubstituted compound, **10**, was made by this multistep

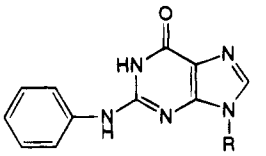
**Table 3.** Inhibition of HSV Thymidine Kinases by *meta*-Substituted PGs



| compd | X | $IC_{50}$ ($\mu$M)[a] HSV1 TK | HSV2 TK |
|---|---|---|---|
| **2**[b] | $CF_3$ | 0.15 | 0.10 |
| **5** | $CH_2CF_3$ | 0.45 | 1.0 |
| **6** | $CHClCF_2Cl$ | 0.65 | 0.47 |
| **7** | $OCF_3$ | 2.3 | 6.3 |
| **8** | $CHF_2$ | 2.8 | 3.9 |
| **1**[b] | H | 8.0 | 1.6 |
| **9** | $CCl{=}CCl_2$ | 70 | 70 |

[a] Concentration causing 50% inhibition of [$^3$H]thymidine phosphorylation, assayed as described in the Experimental Section. [b] From refs 3 and 5.

**Table 4.** Inhibition of HSV Thymidine Kinases by 9-Substituted PGs



| compd | R | $IC_{50}$ ($\mu$M)[a] HSV1 TK | HSV2 TK |
|---|---|---|---|
| **11** | $(CH_2)_4OH$ | 0.16 | 2.15 |
| **3**[b] | 2-deoxyribofuranosyl | 0.30 | 2.50 |
| **12** | $CH_2CH{=}CH_2$ | 0.45 | 0.18 |
| **13** | $CH_2CH_2OCH_3$ | 0.61 | 2.7 |
| **14** | $CH_2CHOHCH_2S(CH_2)_2OH$ | 1.6 | 1.6 |
| **15** | $CH_2CHOHCH_2OH$ | 1.7 | 3.5 |
| **16** | $CH_2CH_2OH$ | 2.9 | 1.2 |
| **1**[b] | H | 8.0 | 1.6 |
| **17** | $CH_2CO_2H$ | 12.4 | 9.8 |

[a] Concentration causing 50% inhibition of [$^3$H]thymidine phosphorylation, assayed as described in the Experimental Section. [b] From ref 3.

method. 2-[*m*-(Trifluoromethyl)anilino]-6-chloro-9-(2-acetoxyethyl)purine, **18**, was first obtained from the reaction between the sodium salt of 2-[*m*-(trifluoromethyl)anilino]-6-chloropurine and 2-acetoxyethyl bromide (Table 2). Alkaline hydrolysis of **18** was performed as described above giving 9-(2-hydroxyethyl)-$N^2$-[*m*-(trifluoromethyl)phenyl]guanine, **10**, whose yield and physical properties are summarized in Table 1.

**Thymidine Kinase Inhibition.** The thymidine kinases from HSV1- and HSV2-infected HeLa cells were isolated and purified as described previously[4] and assayed by measuring phosphorylation of [$^3$H]thymidine (TdR) using ATP as phosphate donor.[5] The results of inhibitor testing are presented in Tables 3–5 as $IC_{50}$ values, the concentrations of inhibitors necessary to cause 50% inhibition of phosphorylation of 1 and 2 $\mu$M [$^3$H]TdR by HSV1 and HSV2 TKs, respectively.

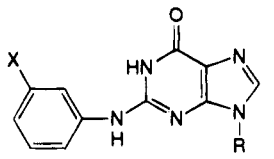
**Meta-Substituted $N^2$-Phenylguanines.** Table 3 summarizes the results of inhibition of HSV thymidine kinases by compounds **5**–**9**, analogs of the prototype inhibitor *m*$CF_3$PG (**2**). The data show that, with the exception of the trichlorovinyl derivative **9**, the newly synthesized compounds are potent inhibitors of both HSV1 TK and HSV2 TK. Although the compounds had similar potencies against both enzymes, none was more potent than the prototype inhibitor *m*$CF_3$PG.

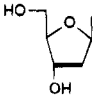
Compared with the unsubstituted $N^2$-phenylguanine, **1**, hydrophobic, electron-attracting substituents in the *meta* position generally increased inhibitory potency against the TKs. A previous quantitative structure–activity relationship study[13] showed that potencies of inhibitors were correlated positively with $\sigma$ and $\pi$ constants of *meta* substituents, and the results in Table 3 are in agreement with that study. One exception is compound **9**, which is dramatically less potent that **1**. At present, substituent contstants for the $CCl{=}CCl_2$ group of **9** are not available in the literature. On the basis of our observation that proton chemical shifts of 2-NH among $N^2$-phenylguanines are well correlated with $\sigma_m$ (correlation coefficient 0.812, $n$ = 12), we estimated a value of 0.30 for $\sigma_m$ of $CCl{=}CCl_2$. This compares with reported values of, for example, 0.43 for $CF_3$ and 0.38 for $OCF_3$.[17] Although $\pi$ is unknown for this substituent, it would be expected to be positive, and compound **9** would be predicted to be a highly potent inhibitor of both enzymes according to the QSAR equation of ref 13. Possibly a steric effect derived from the group size or the rigid double bond of the substituent may account for the weak activity of **9**.

**9-Substituted $N^2$-Phenylguanines.** The results of inhibition of HSV thymidine kinases by 9-substituted $N^2$-phenylguanines are summarized in Table 4. While most 9-substituents enhanced the potencies of the compounds against HSV1 TK compared to PG (**1**), many of the compounds were unchanged in potency against HSV2 TK. Compound **11**, "HBPG", is the most potent inhibitor of HSV1 TK among this series and more potent than the nucleoside prototype, PhdG (**3**). In contrast to PG itself, which is 5-fold more potent against HSV2 TK than against HSV1 TK, HBPG and PhdG are about 10-fold less potent against HSV2 TK than against HSV1 TK. It is interesting that 9-allylPG (**12**), although it contains no hydroxyl group in the side chain, shows very strong potency against both HSV1 and HSV2 TKs. This observation indicates that a hydroxyl group in the 9-substituent is not required for TK inhibition by this family of compounds and is also consistent with the finding that PhdG (**3**) was not a substrate for HSV1 TK.[3]

**Disubstituted $N^2$-Phenylguanine Derivatives.** The above data show that intoduction of appropriate substituents either at the *meta* position in the phenyl ring or the 9-position of the purine ring of $N^2$-phenylguanines enhances inhibitory potency against HSV TKs, especially the HSV1 enzyme. This led to the suggestion that a combination of substituents at both positions might increase the potency at least in an additive way. We combined the *m*-$CF_3$ group with two different 9-substituents, the 2-deoxyribofuranosyl and 2-hydroxyethyl groups, to test this hypothesis. Table 5 shows the results of inhibition of the TKs by these compounds, **4** and **10**. Unexpectedly, **4** and **10** had lower potencies against both enzymes than the monosubstituted parent compounds. For example, compound **10**, with $IC_{50}$ values of 4.7 $\mu$M (HSV1 TK) and 20.4 $\mu$M (HSV2 TK), is much less potent than either of its parents, **2** and **16** (see Tables 3 and 4). The potency of compound **4** is also significantly reduced compared with the monosubstituted prototypes. These particular combinations of substituents did not increase potency,

**Table 5.** Inhibition of HSV Thymidine Kinases by Disubstituted PGs



| compd | X | R | $IC_{50}$ ($\mu$M)[a] HSV1 TK | HSV2 TK |
|---|---|---|---|---|
| **4** | $CF_3$ |  | 4.0 | 2.0 |
| **10** | $CF_3$ | $CH_2CH_2OH$ | 4.7 | 20.4 |

[a] Concentration causing 50% inhibition of [$^3$H]thymidine phosphorylation, assayed as described in the Experimental Section.

implying that the geometry of the drug:enzyme complex may be different in the absence or presence of 9-substituents. This conclusion is supported by the very different patterns of sensitivities of the two enzymes to the 9-substituted PGs (Table 4).

**Selectivity of Inhibitors.** Members of the $N^2$-phenylguanine family have previously been shown to inhibit the herpes virus TKs without demonstrable effect on the human enzyme.[3,5] Several compounds reported in this paper were tested for their effect on TK isolated from the human, HeLa cell line by methods previously reported.[3] Neither *m*-substituted bases, **7** and **8**, nor 9-substituted compounds, **11** and **17**, inhibited the human TK activity at 1 mM concentrations. The disubstituted compound **10** had no effect on the human enzyme at 260 $\mu$M, the highest concentration tested.

**Inhibitory Mechanism of HBPG (11).** Among the newly synthesized compounds, HBPG (**11**) was both a potent inhibitor of HSV1 TK and likely to have solubility properties that would be appropriate for animal studies. We compared its mechanism with that of PhdG, **3**, known to be a competitive, nonsubstrate inhibitor of HSV1 TK.[3] Assay of HSV1 TK with HBPG at several concentrations of thymidine and plotting of the results by the method of Lineweaver and Burk indicated that the compound was competitive with the substrate (data not shown). The apparent $K_m$ for thymidine, 0.5 $\mu$M, increased in the presence of HBPG, but $V_{max}$ remained constant. The $K_i$ for HBPG was calculated to be 0.05 $\mu$M (see the Experimental Section).

The next experiment questioned if HBPG could serve as a substrate for HSV1 TK (see the Experimental Section). An ion exchange thin layer chromatographic method was used to detect phosphorylation with [$\gamma$-$^{32}$P]-ATP by measuring radioactivity at a position where nucleoside monophosphates migrate. In contrast to the appearance of labeled TMP upon incubation of HSV1 TK with 10 $\mu$M TdR, incubation of up to 80 times the $K_i$ concentration of HBPG produced no label at the monophosphate position. These experiments indicate that HBPG is, like PhdG (**3**), a competitive, nonsubstrate inhibitor of HSV1 TK.

**Physicochemical Properties.** A drug is often introduced for practical reasons into a part of the body remote from the target tissue. To be absorbed and distributed to the target organ, the drug molecule must be capable of diffusion into the body from the site of

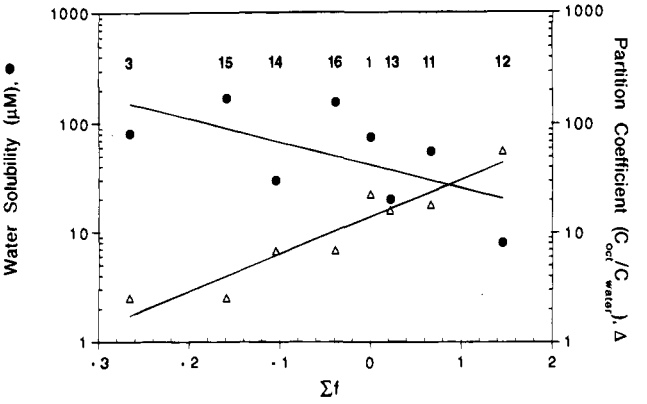
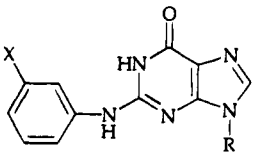



**Figure 1.** Relationships between sums of fragmental hydrophobic constants ($\Sigma f$) and water solubilities (●) and partition coefficients (△) of inhibitors. Compound numbers are given above each pair of data points. Correlation coefficients were, for logarithms of water solubilities, 0.369 and, for logarithms of partition coefficients, 0.958.

administration (absorption) and between different parts of the body (distribution). Aqueous diffusion and lipid diffusion require that the drugs have both good water solubility and a high degree of lipid solubility relative to aqueous solubility. The latter property is typically quantified as an organic solvent:water partition coefficient. Therefore, we measured water solubilities and partition coefficients of those 9-substituted $N^2$-phenylguanines with strong potencies as inhibitors of HSV1 and HSV2 TKs. The UV spectroscopic data required for calculation of compound concentrations in water and in 1-octanol are presented in Table 6.

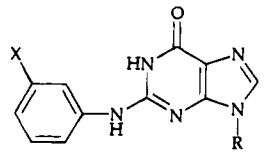
Water solubilities and 1-octanol:water partition coefficients for the inhibitors are summarized in Table 7. The trend in both parameters is qualitatively consistent with the relative hydrophilicities expected for the 9-substituents. In general, compounds with the lowest water solubilities had the highest 1-octanol:water partition coefficients. In an attempt to compare the solubilities on a quantitative basis, we calculated sums of hydrophobic fragmental constants ($\Sigma f$) for the 9-substituents. The hydrophobic fragmental constant ($f$) represents the lipophilicity contribution of a constituent part of a structure to the total lipophilicity, and common values have been derived from partition coefficients by statistical analysis.[18] For any organic group or compound, summation of the pertinent $f$ values will yield a $\Sigma f$ value that is a good indicator of the lipophilicity of this group or compound. The $\Sigma f$ value for each 9-substituent among the TK inhibitors was calculated from compiled $f$ values[18] as described in the Experimental Section, and these are listed in Table 7. The opposing correlations between $\Sigma f$ and water solubilities and $\Sigma f$ and partition coefficients are illustrated in Figure 1. Logarithms of the partition coefficients increased linearly with $\Sigma f$. Logarithms of water solubilities decreased linearly with $\Sigma f$, although with considerable scatter in the data points. Among the compounds studied, we noticed that compound **11** (HBPG) was located at the intersection of the two correlation lines, suggesting that, with substantial solubility in both water and 1-octanol, HBPG may possess optimal properties for efficient absorption and distribution in animals. The strong inhibitory potency of the compound against HSV1 TK ($IC_{50}$ = 0.16 $\mu$M), but not HSV2 TK ($IC_{50}$ = 2.15 $\mu$M)

**Table 6.** UV Spectral Data for $N^2$-Phenylguanine Derivatives



| compd | X | R | $\lambda_{max}$ (nm) $H_2O$ | $\lambda_{max}$ (nm) 1-octanol | $\epsilon_{max}$ ($cm^{-1}$ $M^{-1}$) $H_2O$ | $\epsilon_{max}$ ($cm^{-1}$ $M^{-1}$) 1-octanol |
|---|---|---|---|---|---|---|
| 1 | H | H | 275 | 278.5 | 18 600 | 19 400 |
| 2 | $CF_3$ | H | 275 | 278.5 | 18 200 | 21 000 |
| 3 | H | 2-deoxyribofuranosyl | 275 | 278 | 19 200 | 20 100 |
| 11 | H | $(CH_2)_4OH$ | 275.5 | 278.5 | 18 300 | 19 000 |
| 12 | H | $CH_2CH{=}CH_2$ | 275 | 278.5 | 18 500 | 19 300 |
| 13 | H | $CH_2CH_2OCH_3$ | 275.5 | 278.5 | 19 500 | 19 200 |
| 14 | H | $CH_2CHOHCH_2S(CH_2)_2OH$ | 276 | 278.5 | 21 100 | 22 300 |
| 15 | H | $CH_2CHOHCH_2OH$ | 275.5 | 278 | 19 200 | 17 700 |
| 16 | H | $CH_2CH_2OH$ | 275.5 | 278.5 | 16 300 | 19 100 |

**Table 7.** Properties of $N^2$-Phenylguanine Derivatives



| compd | X | R | $H_2O$ sol ($\mu$M)[a] | $\lambda$ (oct:$H_2O$)[b] | $\Sigma f$[c] |
|---|---|---|---|---|---|
| 12 | H | $CH_2CH{=}CH_2$ | 8.1 | 56.2 | 1.457 |
| 2 | $CF_3$ | H | 15.3 | 7.6 | – |
| 13 | H | $CH_2CH_2OCH_3$ | 20.2 | 15.8 | 0.22 |
| 14 | H | $CH_2CHOHCH_2S(CH_2)_2OH$ | 29.8 | 6.7 | −1.046 |
| 11 | H | $(CH_2)_4OH$ | 56.0 | 18.0 | 0.668 |
| 1 | H | H | 76.0 | 22.4 | 0 |
| 3 | H | 2-deoxyribofuranosyl | 81.0 | 2.5 | −2.654 |
| 16 | H | $CH_2CH_2OH$ | 156.3 | 6.8 | −0.386 |
| 15 | H | $CH_2CHOHCH_2OH$ | 170.6 | 2.5 | −1.59 |

[a] Solubility in distilled water at 25 °C. [b] Ratio of concentrations of compound in 1-octanol and water at 25 °C. [c] Calculated as described in the Experimental Section.

(Table 4), indicates that HBPG might be a promising candidate for inhibition of HSV1 reactivation *in vivo*.

**Pharmacokinetic Studies.** The pharmacokinetic properties of selected compounds were determined in mice after the development of HPLC analytical methods for measuring their concentration in plasma. We chose to study the potent prototype TK inhibitors $m$$CF_3$PG (**2**) and PhdG (**3**) and two of the water soluble 9-substituted $N^2$-phenylguanines, HBPG (**11**) and HEPG (**16**). Single doses of compounds were administered to animals by the intravenous and intraperitoneal routes, and samples of blood were collected at times following a likely period of redistribution, in order to characterize the time course of elimination of the drug from plasma. The data were used to estimate the drug concentration in plasma at time = 0 ($C_o$) as an indirect measure of drug concentration in target tissue and the plasma half-life ($t_{1/2}$) as an indicator of the rate of absorption and/or elimination. The detailed procedures are described in the Experimental Section, and the results are listed in Table 8. Figure 2 illustrates typical semilogarithmic plasma concentration:time curves for HBPG, **11**, administered under different conditions. Intravenous dosing required suitable vehicles to completely dissolve the compounds—concentrations of 10 mg/mL were achieved in saline at pH 11.4 (for **2** and **3**) and in 90% aqueous DMSO (for **11** and **16**).

**Table 8.** Pharmacokinetic Parameters of Inhibitors in Mice following Intravenous (40 mg/kg) and Intraperitoneal (100 mg/kg) administration

| compd | acronym | vehicle | route[a] | plasma $t_{1/2}$[b] (min) | $V_d$ (L/kg)[c] | $C_o$ ($\mu$M)[d] |
|---|---|---|---|---|---|---|
| 2 | $m$$CF_3$PG | saline, pH 11.4 | iv | 25 | 3.0 | 54.2 |
| | | saline, pH 11.4 | ip | 140 | | 10.2 |
| 3 | PhdG | saline, pH 11.4 | iv | 7.5 | 0.4 | 174 |
| | | saline, pH 11.4 | ip | 60 | | 11.7 |
| 11 | HBPG | 90% DMSO | iv | 14 | 2.7 | 49.5 |
| | | 90% DMSO | ip | 75 | | 30.5 |
| 16 | HEPG | 90% DMSO | iv | 15 | 3.3 | 44.7 |
| | | 90% DMSO | ip | 21 | | 100 |

[a] iv, intravenous; ip, intraperitoneal. [b] Plasma concentrations were measured by analytical HPLC on a C8 reverse phase column as described in the Experimental Section. [c] $V_d$, Volume of distribution. [d] Plasma concentration extrapolated to $t = 0$.

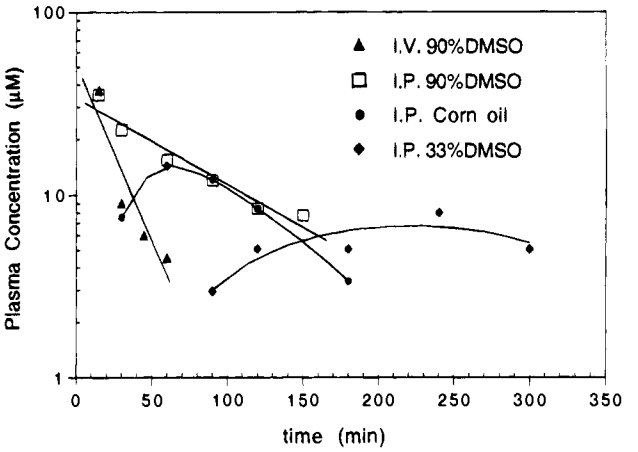



**Figure 2.** Time dependence of the plasma concentration of HBPG (**11**) in mice. Doses given in solution in 90% DMSO were 40 mg/kg (iv) and 100 mg/kg (ip). Doses given ip as suspensions in corn oil and in 33% DMSO were 200 mg/kg. Each data point is the average of duplicate assays from two independent experiments.

Following iv administration in the tail vein, the compounds were eliminated from plasma by apparent first-order kinetics with similar half-lives, varying from 7.5 min for PhdG (**3**) to 25 min for $m$$CF_3$PG (**2**) (Table 8). Three compounds had nearly identical volumes of distribution ($V_d$), about 3 L/kg, suggesting that the compounds are distributed widely in animal tissues and perhaps concentrated in body fat. In contrast, the nucleoside **3** was restricted to a volume of distribution

about that of total body water, 0.4 L/kg (Table 8). The lower $V_d$ and higher elimination rate of **3** may be a result of its low partition coefficient compared with those of the other compounds tested (Table 7) and not related to its water solubility. The three compounds with identical $V_d$ had very different water solubilities but higher partition coefficients than that of **3**.

Intraperitoneal dosing of the same compounds was done for comparison with the iv data, and because the ip route would more likely be used for anti-reactivation testing of compounds. The three compounds with almost identical pharmacokinetic properties after iv injection had very different apparent half-lives after ip injection (Table 8). For example, $m$CF$_3$PG (**2**) displayed an apparent $t_{1/2}$ after ip injection of 140 min, about 6-fold higher than that after iv injection (25 min). This finding and its much lower $C_o$ value suggest that **2** is only slowly absorbed from the peritoneal cavity. In fact, colorless particles observed in the peritoneal cavity of mice 24 h after ip injection of **2** proved to be the compound itself by thin layer chromatography. Precipitation of **2** in the peritoneal cavity is a likely result of its low water solubility (Table 7). In contrast, the most water soluble compound, HEPG (**16**), had nearly the same $t_{1/2}$ after both ip and iv injections, and its $C_o$ values were simply proportional to the two doses (Table 8), suggesting that **16** was rapidly and completely absorbed from the peritoneal cavity.

PhdG (**3**) and HBPG (**11**) had similar, increased apparent plasma $t_{1/2}$ values after ip injection. However, the absorption rate of PhdG was probably much lower than that of HBPG considering that the iv elimination rate of PhdG is twice that of HBPG. This conclusion is consistent with the very low $C_o$ of PhdG after ip injection, even in light of the low $V_d$ of this compound (Table 8). The estimated relative rates of absorption of these two compounds, both of which had similar water solubilities (Table 7), are proportional to their partition coefficients—18 for HBPG and 2.5 for PhdG.

The dependence of the plasma half-life of HBPG on route of administration suggested that the vehicle may be varied to affect the absorption rate after ip dosing. When HBPG was given ip as suspensions in corn oil and 33% aqueous DMSO, the times to peak plasma concentration were prolonged but the apparent elimination rates were similar to that of the solution formulation of the compound (Figure 2).

While we did not conduct specific studies on the toxicity of these compounds, it is significant that no signs of systemic toxicity were seen in any animal after treatment. In addition, the abdominal cavities of some animals receiving each compound by ip injection were examined, and no signs of local tissue irritation were seen.

## Discussion

Two series of $N^2$-phenylguanines, modified at the *meta* position of the phenyl ring and at the 9-position of the guanine ring of PG (**1**), have been synthesized. Most of these compounds show strong inhibition against HSV TKs. Among compounds modified at the *meta* position, the high potencies of all but compound **9** are generally consistent with a QSAR equation[13] previously developed for inhibitors of HSV1 TK, that is, hydrophobic, electron-attracting groups in the *meta* position increased potency. Modification at the 9-position of the purine ring generally enhanced the potency of $N^2$-phenylguanines against HSV1 TK but not against HSV2 TK (Table 4). For example, compound **11**, HBPG, the most potent inhibitor of HSV1 TK among this series, was 50 times more potent than PG itself against the HSV1 enzyme but approximately equipotent with PG against HSV2 TK. The only 9-substituent that increased potency against HSV2 TK was the hydrophobic allyl group of **12**. The fact that compounds bearing no hydroxyl group on the 9-substituents are equally or more strongly inhibitory to TKs indicates that hydroxyl groups are not an essential component of their binding to the enzymes.

We expected that appropriate substituents in both the phenyl ring and the 9-position could increase inhibitory potencies. However, results for the two test compounds (Table 5) seem not to support this hypothesis. Disubstituted $N^2$-phenylguanines, **4** and **10**, had lower potencies than each of their prototype monosubstituted compounds against both TKs.

The results of this paper indicate that the structure–activity relationship of *meta* substituents is similar for both enzymes but that of 9-substituents is very different. These observations suggest that the region of the enzymes that binds the $N^2$-phenyl ring is conserved but that to which 9-substituents are exposed is not.

This study shows that HBPG (**11**) is a competitive, nonsubstrate inhibitor of HSV1 TK, properties previously found for PhdG (**3**).[3] The inability of the enzyme to phosphorylate HBPG is interesting in view of the reported properties of the closely related compound 9-(4-hydroxybutyl)guanine (HBG).[19] The latter compound, an isostere of acyclovir, is a substrate for HSV1 TK and possesses antiherpetic activity. If it is assumed that both compounds bind viral TKs at the same site, the presence of the $N^2$-phenyl ring of HBPG has two consequences. First, the interaction of the phenyl ring with the enzyme increases the affinity of the compound—the $K_i$ of HBPG is 0.05 $\mu$M compared with a value of 2.06 $\mu$M for HBG.[19] This comparison is consistent with our previous conclusion that the $N^2$-phenyl ring is a major site of inhibitor:enzyme binding. Second, interaction of the phenyl group with the enzyme blocks the phosphorylation of the hydroxy group in the 9-substituent by an unknown mechanism. Compared with other inhibitors of HSV1 TK, we found that HBPG not only is a potent enzyme inhibitor but also exhibits favorable pharmacokinetics and lack of acute toxicity in mice via the ip route. This compound is being tested in animal models of HSV1 reactivation.

## Experimental Section

All new compounds were fully characterized by $^1$H NMR and elemental analysis (C, H, N). Proton NMR spectra were obtained at 300 MHz with a Varian Unity 300 instrument; chemical shifts are reported in parts per million ($\delta$) relative to internal tetramethylsilane. Partial spectral data are presented; all other NMR results (including $^{19}$F, where appropriate) were as expected. Elemental analyses were done by the Microanalysis Laboratory, University of Massachusetts, Amherst, MA, and agree to within ±0.4% of calculated values. Melting points were determined on a Mel-temp apparatus and are uncorrected. UV spectra were obtained with a Gilford Response spectrophotometer. Extinction coefficients ($\epsilon$) and $\lambda_{max}$ values for 9-substituted PGs were measured in both water and 1-octanol (Table 6). Aqueous solutions were prepared by

dissolving compounds in dilute sodium hydroxide at pH 12.0, and the solutions (ca. $10^{-5}$ M) were neutralized to pH 7.0 with 0.1 N HCl.

The analytical HPLC apparatus consisted of a Rabbit-HP pump (Rainin Instruments Inc.) equipped with a variable wavelength detector (Knauer) and a HP3396A chromatointegrator (Hewlett-Packard, Inc.). Separations were achieved at ambient temperature (ca. 23 °C) using a C8 reverse phase column (25 cm × 4.6 mm).

Male CD-1 mice weighing about 25 g were purchased from Charles River Breeding Laboratory. Mice had access to food and water at all times. Centrifugation of blood samples was done with a RC2-B Sorvall Superspeed centrifuge.

2-Bromohypoxanthine was obtained from ring closure of 5,6-diamino-2-thiouracil,[20] followed by replacement of the mercapto group by bromine with an aqueous methanolic solution of concentrated hydrobromic acid.[21] 2-Anilino-6-chloropurine was synthesized as described previously.[3] 2-[*m*-(Trifluoromethyl)anilino]-6-chloropurine was synthesized from compound **2** by a two-step method (phosphoryl chloride/DMF at reflux; concentrated ammonium hydroxide at room temperature) as described[3] in a yield of 65% (from ethanol): mp 188–190 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.29 (s, 8-H), 10.23 (s, 2-NH). Anal. ($C_{12}H_7N_5ClF_3$) C, H, N.

**General Procedure for the Preparation of *meta*-Substituted Anilines.** The starting nitro compounds, 3-(trichlorovinyl)nitrobenzene, 3-(1,2-dichloro-2,2-difluoroethyl)nitrobenzene, and 3-(2,2,2-trifluoroethyl)nitrobenzene, were synthesized as described by Ando et al.[14] 3-(Difluoromethyl)nitrobenzene was prepared by the method of Middleton.[15] Reduction of nitro compounds was done as follows: The nitro compound (3.77 mmol) and granulated tin (11.3 mmol) were treated with concentrated hydrochloric acid (3 mL). The mixture was stirred at room temperature for 0.5 h and then heated at reflux for 0.5 h. The cooled reaction mixture was made alkaline (pH 10–12) with 5 N NaOH and then extracted three times with an equal volume of diethyl ether or hexane. The extract was evaporated, and the residue was chromatographed on silica gel (230–400 mesh) with hexane:chloroform (2:1) as eluting solvent. Concentrated hydrochloric acid was added to the eluate, and the precipitate was collected by filtration. Crystallization of the product from acetone gave fine crystals of the aniline hydrochloride.

**3-(Difluoromethyl)aniline hydrochloride:** yield 76%; mp 235–240 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.08 (t, $J$ = 54.9 Hz, $CHF_2$), 7.41–7.59 (m, Ph). Anal. ($C_7H_8ClF_2N$) C, H, N.

**3-(2,2,2-Trifluoroethyl)aniline hydrochloride:** yield 83%; mp 242-245 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.73 (q, $J$ = 12.0 Hz, $CH_2$), 7.30–7.50 (m, Ph). Anal. ($C_8H_9ClF_3N{\cdot}^1/_6H_2O$) C, H, N.

**3-(1,2-Dichloro-2,2-difluoroethyl)aniline hydrochloride:** yield 82%; mp 233–236 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.3 (dd, $J$ = 8.7, 12.0 Hz), 7.18–7.49 (m, Ph). Anal. ($C_8H_8Cl_3F_2N$) C, H, N.

**3-(Trichlorovinyl)aniline hydrochloride:** yield 71%; mp 231–234 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.26–7.51 (m, Ph). Anal. ($C_8H_7Cl_4N$) C, H, N.

**$N^2$-Substituted guanines** were prepared from 2-bromohypoxanthine and the appropriate anilines or their hydrochlorides in refluxing 2-methoxyethanol as described.[12] The yields and properties of new compounds, **5–9**, are presented in Table 1.

**General Procedure for the Synthesis of $N^2$-Phenyl 9-Substituted Guanines.** a. Sodium hydride (60% suspension in mineral oil) was added in an equimolar amount to a suspension of 2-anilino-6-chloropurine (0.35 g/100 mL) in anhydrous acetonitrile at room temperature. After stirring for 1 h, the appropriate alkyl bromide (1 equiv) was added, and the suspension was stirred for 48 h at room temperature. An equal volume of chloroform was added, and after filtration through Celite, the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (230–400 mesh), and the products were eluted with chloroform to give the major 2-anilino-6-chloro-9-substituted purine and then the minor 2-anilino-6-chloro-7-substituted purine. Yields and properties of these products are listed in Table 2.

b. A suspension of the 9-isomer (from above) in 0.5 N sodium hydroxide solution was heated at reflux for 2 h. After neutralization with 0.5 N hydrochloric acid, the solution was placed in the refrigerator overnight. The colorless solid was collected and washed with water to give crude 9-substituted $N^2$-phenylguanines. Colorless crystals of products, **10–13** and **15–17**, were obtained after crystallization from DMF/chloroform. Properties of compounds are presented in Table 1.

**$N^2$-Phenyl-9-[2-hydroxy-3-[2-(hydroxyethyl)thio]propyl]-guanine, 14.** A solution of 2-anilino-6-chloro-9-(2,3-epoxypropyl)purine, **22** (440 mg, 1.22 mmol), in ethanol (280 mL) was treated with 2-mercaptoethanol (20 mL) and a solution of 1 N sodium methoxide in methanol (20 mL). After heating at reflux for 72 h, the solution was diluted with an equal volume of water and chilled overnight. The colorless precipitate was collected and washed with water to give crude product. The product was crystallized from methanol to give pure **14** (Table 1).

**$N^2$-[*m*-(Trifluoromethyl)phenyl]-2'-deoxyguanosine, 4.** A suspension of 2-bromo-2'-deoxyinosine[5] (100 mg, 0.3 mmol) and 3-aminobenzotrifluoride (2 g, 9 mmol) in a mixture of phosphate buffer (pH 7.0) and ethanol (2 mL each) was heated at 120 °C for 8 h. After evaporation of solvents, the residue was passed through a small silica gel column (230-400 mesh) and the product was eluted with 20% methanol in chloroform. Crystallization from aqueous methanol gave 12.9 mg (10%) of **4**: mp 245–247 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.09 (s, 8-H), 9.22 (s, 2-NH), 6.19 (t, 1'-H), all other resonances as expected. Anal. ($C_{17}H_{16}O_4N_5F_3{\cdot}^1/_4H_2O$) C, H, N.

**Enzyme Isolation and Assay.** The thymidine kinases from HSV1- and HSV2-infected HeLa cells were isolated by the use of a thymidine 3'-[(*p*-aminophenyl)phosphate] affinity column as described previously.[4] The enzymes were assayed with the DE-81 filter binding method as described with limiting concentrations of [$^3$H]TdR: 1 $\mu$M for HSV1 TK and 2 $\mu$M for HSV2 TK. The HeLa cell TK was isolated and assayed as described.[3] Stock solutions of inhibitors in DMSO were diluted into assay mixtures; control assays contained an identical concentration of DMSO.

**$K_i$ Determination.** HSV1 TK was assayed with four concentrations of HBPG, 11 (0.05, 0.1, 0.2, and 0.5 $\mu$M), in the presence of increasing concentrations of [$^3$H]TdR (0.4, 0.8, and 1.6 $\mu$M). Apparent velocities were determined as usual with the DE-81 filter binding method (see above). Apparent $K_m$ values for thymidine and $V_{max}$ values were calculated with the program Enzyme Kinetics (D. G. Gilbert, Indiana University); regression lines had correlation coefficients of 0.95 ± 0.03. $K_i$ values were calculated from the equation $K_i = I/[(K_m'/K_m) - 1]$, where $K_m'$ is the apparent $K_m$ in the presence of HBPG at concentration $I$. The $K_m$ for thymidine was 0.5 ± 0.1 $\mu$M, and the $K_i$ for HBPG was 0.05 ± 0.002 $\mu$M.

**Substrate Assay.** HSV1 TK was incubated for 30 min in the presence of 200 $\mu$M [$\gamma$-$^{32}$P]ATP and increasing amounts of HBPG ranging from $K_i$ to 80 × $K_i$ concentrations. Control reactions were performed with 10 $\mu$M thymidine as substrate. Aliquots (2 $\mu$L) were spotted on a PEI-cellulose TLC sheet. After development in 100% methanol and then in 44% methanol:1.1 M LiCl:0.37 M ammonium formate (pH 3.6) (to maximize the separation between unreacted ATP and phosphorylated products), the sheet was dried and exposed to X-ray film overnight at −80 °C. Radioactive spots were cut and counted. No detectable amount of radioactivity accumulated in the position of TMP when HBPG was the "nucleoside". Thus, HBPG was not detectably phosphorylated by HSV1 TK under these conditions.

**Measurement of Water Solubilities.** An excess of each compound was stirred in deionized water at 25 °C for 1 h. After removing undissolved compound by filtration, the absorbance of the solution at the $\lambda_{max}$ was measured and the concentration of the compound was calculated. Each measurement was done three times, and the results are summarized in Table 7.

**Determination of Partition Coefficients.** The partitioning of compounds between 1-octanol and water at 25 °C was measured by shaking 5 mL of a saturated aqueous solution at pH 7.0 with an equal volume of 1-octanol for 2 h. After centrifugation at 2000 rpm for 2 h to completely separate the

phases, the concentration of compound in each phase was measured by spectrophotometry as described above. The partition coefficients, $\lambda$, the ratio of concentrations in 1-octanol and water, are the average of three measurements for each compound and are summarized in Table 7.

**Pharmacokinetic Studies.** Two formulations suitable for treating animals by both the intravenous (tail vein) and intraperitoneal routes were used. Solutions of 10 mg/mL in isotonic saline in pH ca. 11.4 were prepared for **2** and **3**. Solutions of 10 mg/mL in 90% dimethyl sulfoxide were prepared for 11 and **16**. Doses for intravenous (iv) and intraperitoneal (ip) injections were 40 and 100 mg/kg, respectively. Suspensions of 11 in corn oil and in 33% DMSO were prepared by sonication.

Mice were sacrificed by decapitation at various times after dosing. Blood samples were collected in heparinized glass tubes and immediately centrifuged at 23K rpm for 20 min to obtain plasma, which was decanted and diluted 10-fold with deionized water. The dilute plasma was stored frozen at −21 °C until HPLC analysis.

An aliquot of 20 $\mu$L of diluted plasma was injected onto an analytical C8 column in a mobile phase of mixtures of deionized water, acetonitrile, and acetic acid at a flow rate of 1 mL/min at ambient temperature. Mobile phase compositions and retention times were for **2**, 50:49.8:0.2, $t_R$ 8.6 min; for **3**, 75.6:24.2:0.2, $t_R$ 16.9 min; for 11, 75:24.8:0.2, $t_R$ 6.5 min; and for **16**, 80.6:19.2:0.2, $t_R$ 15.0 min. Peaks were monitored by UV absorbance at 275 nm at a sensitivity of 0.005 AUFS. Blank plasma samples were analyzed showing the absence of peaks at the retention time of the compounds. To confirm the peak identity in plasma samples, authentic compound was added to the plasma resulting in expected increased intensity of the peak.

The calibration was performed by means of 10 standard control plasma samples containing added compound at concentrations between 0.11 and 56 $\mu$M (three measurements each). The calibration curves were linear over the above range with a correlation coefficient $\geq$0.999 and an intercept not significantly different from 0. The detection limit for the compounds was approximately 0.1 $\mu$M (signal/noise = 3), resulting in an estimated limit of quantitation of ca. 1 $\mu$M in plasma.

The plasma concentration at time = 0, $C_o$, and the first-order elimination rate constant, $k_e$, were estimated for each compound from a least squares regression line fit to the time after dosing and the logarithm of the corresponding plasma concentration of the compound. These calculations employed all data following iv administration and only from the terminal log-linear portion following ip administration. In addition, the half-life, $t_{1/2}$, was calculated as ln $2/k_e$, and the apparent volume of distribution upon iv administration, $V_d$, was estimated as dose/$C_o$. Typical experimental results are depicted in Figure 2 for compound 11, HBPG.

**Calculation of Hydrophobic Fragmental Constants, $\Sigma f$.** Each $f$ value was obtained from ref 18. The hydrophobic fragmental constants, $\Sigma f$, were calculated simply by summation of the pertinent $f$ values. As an example, for $(CH_2)_4OH$, $\Sigma f = 4 \times f_{(CH_2)} + f_{(OH)} = 4 \times 0.527 - 1.44 = 0.668$. The $\Sigma f$ values of all other organic fragments were calculated as described above and are listed in Table 7.

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