Inhibition of Herpes Simplex Virus Thymidine Kinases by 2-Phenylamino-6-oxopurines and Related Compounds: Structure−Activity Relationships and Antiherpetic Activity in Vivo

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · JULY 2005

Impact Factor: 5.45 · DOI: 10.1021/jm049059x · Source: PubMed

8 AUTHORS, INCLUDING:

Andrzej Manikowski
Norbrook Laboratories
15 PUBLICATIONS **168** CITATIONS
SEE PROFILE

Andrea Lossani
Italian National Research Council
10 PUBLICATIONS **78** CITATIONS
SEE PROFILE

George Wright
83 PUBLICATIONS **1,294** CITATIONS
SEE PROFILE

Published in final edited form as:
J Med Chem. 2005 June 2; 48(11): 3919–3929.

Inhibition of Herpes Simplex Virus Thymidine Kinases by 2-Phenylamino-6-oxopurines and Related Compounds: Structure-activity Relationships and Antiherpetic Activity *In Vivo*

Andrzej Manikowski[1], **Annalisa Verri**[2], **Andrea Lossani**[2], **Bryan M. Gebhardt**[3], **Joseph Gambino**[1], **Federico Focher**[2], **Silvio Spadari**[2], and **George E. Wright**[1,*]

1 *GLSynthesis Inc., One Innovation Drive, Worcester, MA 01605*

2 *Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche, Pavia, Italy 27100*

3 *Lions Eye Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112*

Abstract

Derivatives of the herpes simplex thymidine kinase inhibitor HBPG (2-phenylamino-9-(4-hydroxybutyl)-6-oxopurine) have been synthesized, and tested for inhibitory activity against recombinant enzymes (TK) from herpes simplex types 1 and 2 (HSV-1, HSV-2). The compounds inhibited phosphorylation of [^{3}H]thymidine by both enzymes, but potencies differed quantitatively from those of HBPG, and were generally greater for HSV-2 than HSV-1 TKs. Changes in inhibitory potency were generally consistent with the inhibitor/substrate binding site structure based on published x-ray structures of HSV-1 TK. In particular, several 9-(4-aminobutyl) analogs with bulky tertiary amino substituents, were among the most potent inhibitors. Variable substrate assays showed that the most potent compound, 2-phenylamino-9-[4-(1-decahydroquinolyl)butyl]-6-oxopurine, was a competitive inhibitor, with K_i values of 0.03 and 0.005 gM against HSV-1 and HSV-2 TKs, respectively. The parent compound HBPG was uniquely active in viral infection models in mice, both against ocular HSV-2 reactivation and against HSV-1 and HSV-2 encephalitis. In assays lacking [^{3}H]thymidine, HBPG was found to be an efficient substrate for the enzymes. The ability of the TKs to phosphorylate HBPG may relate to its antiherpetic activity *in vivo*.

INTRODUCTION

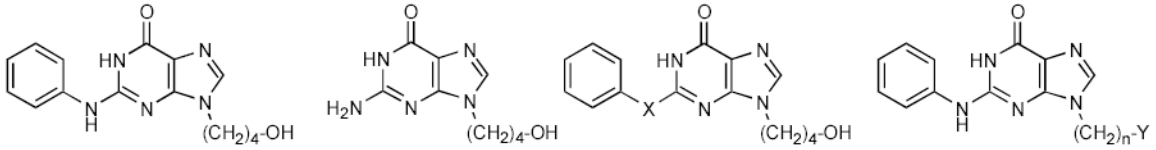
Herpes simplex viruses (HSV-1 and HSV-2) establish lifelong latency in neurons.[1] Latency is interrupted periodically by reactivation leading to recurrent disease, but a significant proportion of infected individuals may shed infectious virus without apparent signs of disease. Thus these individuals represent a real threat for venereal spread. In addition, herpes simplex viruses may cause life threatening encephalitis, both in neonates as a result of virus exposure during birth, and in immunocompromised adults. Herpetic encephalitis results in high mortality, and survivors are often severely handicapped. Herpetic encephalitis occurs in about 3000 newborns each year in the USA. If infection results in disseminated disease, CNS disease occurs in up to 50% of the infants and can result in 75% mortality. For the survivors of CNS involvement the outcome is bleak, with psychomotor retardation in 50 to 75% of the survivors. Acyclovir (ACV) and foscarnet (PFA) have been employed for HSV encephalitis and for therapy of newborns with localized and disseminated HSV disease. Although these treatments have reduced mortality from encephalitis to 15%, only 50% of survivors develop normally.[2]

*Author for correspondence: George E. Wright, Ph.D., GLSynthesis Inc., One Innovation Drive, Worcester, MA 01605, Phone 508 754-6700, FAX 508 754-7075, Email:george.wright@glsynthesis.com

Supporting Information Available: Lineweaver-Burke plots for inhibition of HSV-1 and HSV-2 TK by **12i**; elemental analysis results.

Unfortunately the overall mortality for disseminated neonatal herpes remains very high (40 to 65%) regardless of present antiviral therapy.[3] Clearly there is a need for an antiviral drug which can enter the CNS and effectively inhibit HSV infection.

Competitive inhibitors of HSV thymidine kinases (TK) prevent viral reactivation *in vitro* and *in vivo*, and expression of viral DNA in the infected nerve ganglia is reduced by treatment with TK inhibitors.[4] These results have demonstrated the validity of HSV TK as a target for antiviral therapy, and encouraged us to proceed with the optimization and development of these compounds for eventual clinical application. The first task was to make available significant quantities of the HSV enzymes, and this has been accomplished by expression of recombinant TKs. Secondly, we have synthesized several new candidate inhibitors to extend the structure-activity relationships of 2-phenylamino-6-oxopurines ("N^2-phenylguanines"), and have found highly potent inhibitors of both HSV-1 and HSV-2 TKs. Finally, we have found that a lead compound, 2-phenylamino-9-(4-hydroxybutyl)-6-oxopurine (HBPG, **1**), effectively prevents lethal encephalitis in mice, and is an efficient alternate substrate for the viral TKs.



1, HBPG **2**, HBG **3-5** **6-10**, **12a-s**

RESULTS

Synthesis of inhibitors.

2-Bromohypoxanthine served as common precursor of 2-phenylthio- and 2-phenoxy-6-oxopurine, as described previously for preparation of 2-phenylamino-6-oxopurine.[5] These compounds were converted into the corresponding 6-chloropurines with phosphoryl chloride (Scheme). Alkylation of the 6-chloropurines with 4-acetoxybutyl bromide and separation of the major 9 isomers, followed by alkaline hydrolysis, gave 2-phenylthio- (**3**) and 2-phenoxy- (**4**) analogs of **1** in high yields. A similar strategy with 2-phenylamino-6-chloropurine and other ω-substituted alkyl halides gave several related 9-hydroxyalkyl, 9-methoxybutyl and 9-carboxypropyl compounds. Methylation of the 6-chloro derivative of **1**, followed by alkaline hydrolysis, provided 2-(N-methyl-N-phenylamino)-9-(4-hydroxybutyl)-6-oxopurine (**5**). Conversion of **1** to the 9-(4-iodobutyl) derivative (**11**), followed by reaction of the latter intermediate with numerous secondary amines, provided a series of 9-(4-aminobutyl)-2-phenylamino-6-oxopurines, **12a-s** (Scheme).

Cloning and expression of HSV-1 and HSV-2 thymidine kinases.

The complete coding sequences of HSV-1 TK and HSV-2 TKs were expressed from the pTrcHis vector as N-terminal fusion proteins. The fusion proteins contain six tandem histidine residues that allow one-step purification of the enzymes with a nickel-chelating resin. Cloning and expression were performed as described in the Experimental Section. One liter of bacterial culture produced approximately 5 mg of tagged TK, which was eluted from a Ni-NTA Superflow column (1 mL) as a single sharp peak following step-elution with 250 mM imidazole.

In order to determine the behavior of recombinant HSV TKs, experiments were conducted *in vitro* to measure the K_m of each enzyme. Several concentrations of [^{3}H]thymidine (TdR) were incubated at 37 °C in the presence of a limiting amount of enzyme, and the reaction velocity

V was determined at each substrate (S) concentration. By plotting V and S in double-reciprocal format, we obtained the Lineweaver-Burke plots shown in Figure 1. These results indicate that recombinant HSV-1 and HSV-2 TKs had K_m values of 1 and 2 μM, respectively, identical to those reported for the enzymes purified from corresponding HSV-infected cells.[5,6]

Inhibition of HSV TKs by 9-substituted-6-oxopurines.

Screening of new compounds against the recombinant TKs was done by measuring the effect of each test compound on phosphorylation of [^{3}H]TdR, present at the K_m value for each enzyme. In this way, relative potencies, IC_{50}s, have been obtained, and these are summarized in Table 1. HBPG (**1**) inhibited the recombinant HSV-1 and HSV-2 TKs with IC_{50} values of 1.3 and 0.5 μM, respectively (Table 1). The activities are of the same order of magnitude, but of opposite potencies, as those reported for the enzymes isolated from virus-infected cells.[7] The significance of the N^2-phenyl substituent in this family of inhibitors is emphasized by the weak inhibition afforded by 9-(4-hydroxybutyl)guanine (**2**), a weak alternate substrate of HSV TK and antiherpetic compound.[8] The requirement of the 2-NH group of active compounds was probed by the assay of 2-phenylthio (**3**) and 2-phenoxy (**4**) analogs; both derivatives were potent inhibitors of HSV-1 TK, but less potent against HSV-2 TK. In contrast, the N-methyl analog **5** was weakly inhibitory to both TKs. Although no direct interactions of 2-NH were observed in the x-ray structure of **1** bound to HSV-1 TK,[9] the drastic reduction of affinity of **5** is consistent with a significant steric effect of the N-Me group.

The 9-(2-hydroxyethyl) (**6**) and 9-(3-hydroxypropyl) (**7**) homologs of **1** inhibited both TKs within a few-fold of **1**, but the 9-(5-hydroxypentyl) compound (**8**) was 12-fold and 108-fold weaker against HSV-1 and HSV-2 TKs, respectively. The congruence between the positions of the hydroxybutyl side chain of **1** and the the analogous 5'-hydroxymethyl and sugar ring atoms of TdR in the TK binding site[9] may explain the high affinity of **1** and the weak affinity of **8** to the enzymes.

Although the methyl ether (**9**) corresponding to **1** was 10–20-fold weaker than **1**, the carboxy isostere (**10**) was a potent inhibitor of both enzymes. In contrast, amino-substituted derivatives displayed a wide range of activities. In general, 9-(4-aminobutyl) compounds were less active against HSV-1 than HSV-2 TKs, but several derivatives were more potent than **1** against both enzymes. For example, the substituted piperidino compounds **12h**, **12i**, **12k** and **12q** had IC_{50} values that were 2–9-fold lower and 6–38-fold lower than **1** against HSV-1 and HSV-2 TKs, respectively. The bulky piperidino groups of these compounds suggested better packing of the sidechain at the 9 position compared with **1** or closely related analogs. Indeed, molecular modeling studies with the x-ray coordinates of the HBPG:HSV-1 TK complex[9] have revealed space in the binding site that can accommodate such bulky substituents. (Details of these studies will be presented elsewhere.)

Various 2-phenylamino-6-oxopurines and their 9-substituted derivatives such as **1** have been shown to be competitive inhibitors of HSV TKs.[5,7] In order to assure that the potent 9-(4-aminobutyl) derivatives were, in fact, binding at the active site of the viral TKs, the mechanism of the most potent compound **12i** was investigated. When the inhibitory activity was measured at various substrate concentrations and the results plotted in double reciprocal (Lineweaver-Burke) format (see Supporting Information), the results were consistent with competitive kinetics. The K_i values of **12i** obtained in this way were 0.03 and 0.005 gM for HSV-1 and HSV-2 TKs, respectively.

Effect of TK inhibitors on experimental HSV infections in mice.

HBPG (**1**) was previously shown to reduce the frequency of stress-induced reactivation of latent HSV-1 in mice[10] and HSV-1 ocular disease in squirrel monkeys.[11] In order to extend

these observations to latent disease caused by HSV-2 and to explore the potential of TK inhibitors in HSV encephalitis, corresponding experiments were conducted in mice with **1** and several new analogs.

Reactivation from latency.—Ocular infection of mice with HSV establishes latency in the trigeminal ganglia, and latent virus can be induced to reactivate by heat stress. In previous studies, multiple intraperitoneal (IP) doses of 200 mg/kg of **1** to HSV-1-latent mice reduced the presence of stress-induced virus in the eyes to 15% compared with 47% for vehicle-treated animals.[10] We subsequently found that a single IP dose of 200 mg/kg given 1 hour before heat stress reduced the number of eyes with infectious HSV-1 to 35% compared with 70% for vehicle-treated animals.[12] Consequently we tested **1** and various analogs and control antiherpetic compounds in latent HSV-2-infected mice under similar conditions (see Experimental Section), and the results are presented in Table 2. A single dose of **1** given 30 minutes pre-stress gave a significant reduction in infectious virus in eyes 24 hours later, i.e. 27% vs. 73% for controls. In contrast, none of the derivatives of **1** showed activity at the same dose. Thus, neither the phenylthio isostere **3** nor the hydroxyalkyl homologs **7** or **8** reduced reactivation of latent HSV-2. The carboxypropyl compound **10** and two aminobutyl compounds, **12f** and **12i**, were also devoid of activity at 200 mg/kg. Acyclovir (ACV) at 100 mg/kg had no effect on HSV-2 reactivation, but phosphonoformate (PFA), a pyrophosphate analog and moderately potent anti-HSV drug, had statistically significant activity, but only at 400 mg/kg (Table 2).

Herpetic encephalitis.—Ocular infection with HSV-1 (strain McKrae) or HSV-2 (strain G) with 10^6 plaque forming units per eye leads to lethal encephalitis rather than self resolving latency.[13] Under these conditions untreated mice die on average at 9 and 14 days post-infection with HSV-1 and HSV-2, respectively (see Table 3). These models were validated by testing the antiherpes drugs ACV and PFA. Both drugs had dose-dependent protective effects when given IP in corn oil suspension twice daily for five days, a common regimen in such studies.[14] ACV had ED_{50} values for both infections between 50 and 100 mg/kg, and modestly prolonged the mean day of death in each case. PFA was effective but weaker, having ED_{50}s of 200 mg/kg and above, and mean days of death were also prolonged.

HBPG (**1**) and representative TK inhibitors were tested in this model, by IP dosing in corn oil twice daily for five days. Compound **1** was effective against both HSV-1 and HSV-2 infections, with ED_{50} values between 100 and 200 mg/kg for HSV-1 and about 100 mg/kg against HSV-2. The highest dose of **1** was completely curative for the HSV-2 infection. The close hydroxyalkyl homologs, **7** and **8**, were ineffective at 100 mg/kg, and the potent aminoalkyl derivatives **12f** and **12i** were inactive at 200 mg/kg.

The findings that only **1** demonstrated significant anti-reactivation and anti-encephalitis activity in these models raised questions of the relevance of TK inhibition in this series to antiherpetic activity *in vivo*. The results suggested that compound **1**, a close relative of the weak antiherpes compound HBG (**2**) and its “isostere” ACV, may owe its *in vivo* activity to a different form of the compound, i.e. a metabolite, and/or a different enzymatic target.

HBPG is efficiently phosphorylated by HSV-1 and HSV-2 TKs.

To evaluate whether **1** is a non-substrate inhibitor or an alternate substrate of the viral enzymes, we incubated excess HSV-1 TK and HSV-2 TK with **1**, and, as control, with the substrate TdR, under conditions that maximized formation of the 5′-monophosphate of the natural substrate. For comparison, the incubations were also performed with the antiherpes drug ACV, a weak alternate substrate[15] for the enzymes. Reaction products were resolved and quantitated by HPLC, as described in the Experimental Section. Table 3 shows the retention times of

substrates and monophosphates, clearly indicating that peaks in the monophosphate region were observed in all cases. The identity of the peak at 31.8 min resulting from incubation of HBPG with TKs was confirmed by comparison with authentic monophosphate, HBPG-MP. The relative rates and extents of phosphorylation are seen in Figure 2 for HSV-1 TK (panel A) and HSV-2 TK (panel B). It is apparent that the rates of conversion of **1** to the monophosphate are similar to those of the substrate TdR for both enzymes, and that both are significantly better substrates than ACV. Thus, **1** is not a pure inhibitor of HSV TKs, but an efficient alternate substrate for the enzymes.

HSV-1 TK possesses thymidylate kinase (TMPK) activity, as determined by HPLC analysis of enzyme incubated with TMP, and we have shown that this activity is inhibited by HBPG and related, non-substrate inhibitors.[16] When authentic HBPG-MP was incubated with rec HSV-1 TK in the absence of substrate, no product consistent with a diphosphate was detected by HPLC, suggesting that the TMPK activity does not phosphorylate this monophosphate (data not shown).

DISCUSSION

A series of 2-phenylamino-6-oxopurine analogs related to HBPG (**1**) was synthesized and studied to investigate the structural basis of HSV TK inhibition, and to search for more potent inhibitors suitable for antiviral testing *in vivo*. Although we have identified highly potent, competitive inhibitors of HSV-1 and HSV-2 TKs, these "non-substrate" analogs did not show activity in relevant mouse infection models. The x-ray structure of the HBPG:HSV-1 TK complex[9] revealed that the inhibitor occupies the active, TdR-binding site in a manner similar to the binding modes of ACV and GCV.[17] Interestingly, the presence of the phenyl ring of **1** causes rotation of a tyrosine (Y132) by ca. 90° from its position in the TdR and ACV complexes, and close to that found in the BVdU:TK complex.[17] The space available for packing of the 9-substituents suggested that bulkier substituents might enhance affinity of compounds. Indeed, several 9-(4-aminobutyl) derivatives with bulky tertiary amino substituents were highly potent inhibitors of both HSV-1 and HSV-2 TKs (Table 1).

Early generation HSV TK inhibitors did not inhibit replication of virus in cell cultures,[18] consistent with the lack of requirement of the virus-encoded enzyme in virus growth in proliferating cells. Even **1**, now found to be a substrate for the enzyme, had weak activity (ED_{50} >100 μM) against HSV-1 and HSV-2 in human foreskin fibroblasts and no activity in systemic infection with HSV-2 (E. Kern, personal communication). However, HBPG (**1**) was previously shown to reduce the frequency of latent HSV-1 reactivation in mice[10] and squirrel monkeys.[11] In the present work we extended the studies of **1** to latent infection with HSV-2, and have demonstrated that the compound is effective in reducing the rate of reactivation of HSV-2 in mice (Table 2). We also asked if **1** and related TK inhibitors affected the course of experimental HSV-1 and HSV-2 encephalitis in mice. The present results demonstrate that **1** is an effective anti-encephalitic compound in both cases, nearly as potent as the standard drug ACV and more potent than the antiherpetic drug PFA (Table 3).

HBPG (**1**) was originally selected from a series of TK "inhibitors" because of its favorable balance of water solubility and octanol:water partition coefficient.[7] Our observations that **1** exerts antiherpetic reactivation and encephalitic activity *in vivo* and that it is an efficient substrate for the enzymes now suggest that TK inhibition alone is not responsible for the activities. Closely related 9-hydroxyalkyl homologs **7** and **8**, and very potent, non-substrate inhibitors, i.e. **12a-s**, albeit more polar compounds whose penetration into the nervous system is uncertain, did not show activity against HSV reactivation or encephalitis in mice (Tables 2 and 3). Interestingly, the 9-hydroxypropyl analog **7** was not apparently phosphorylated by the TKs under the assay conditions employed (data not shown). It is possible that it is the

monophosphate of **1** that is active *in vivo* or that further virus-specific metabolism of **1**, for example to the triphosphate, HBPG-TP, is responsible for that activity.

EXPERIMENTAL SECTION

All new compounds were fully characterized by ^{1}H NMR and elemental analyses or high resolution mass spectra (HR-MS). Elemental analyses (C,H,N) were obtained from the Microanalysis Laboratory, University of Massachusetts, Amherst, and agree to ±0.4% of calculated values. HR-MS were obtained from the Mass Spectrometry Facility, University of Massachusetts, Amherst, in EI mode. NMR spectra were determined in Me_2SO-d_6 with a Bruker Avance 300 instrument; chemical shifts are in ppm (d) from internal Me_4Si. All chemicals were reagent grade. 2-Bromohypoxanthine, 2-phenylamino-6-oxopurine, and 2-phenylamino-6-chloropurine were prepared as described in ref [5], and **1** and **6** were prepared as described in ref. [7]. HBG (**2**) was synthesized from 2-amino-6-chloropurine as described for **1**[7]; the compound had properties identical to those reported by Yamazaki.[18] Acyclovir (ACV) and phosphonoformate (PFA) were purchased from Sigma-Aldrich.

Bacterial media components were from Difco, and Ni-NTA Superflow resin was from QIAGEN. Restriction and modification enzymes were purchased from Promega, Sigma or Boehringer. IPTG was from Sigma, and [^{3}H-methyl]thymidine (TdR), 20 Ci/mmol, was from New England Nuclear.

9-(4-Hydroxybutyl)-2-phenylthio-6-oxopurine, 3.

2-Phenylthio-6-chloropurine, 3a.—A suspension of 2-bromohypoxanthine (3.0 g, 14 mmol) in 2-methoxyethanol (50 mL) was treated with thiophenol (4.3 mL, 3eq) and anhydrous K_2CO_3 (3.86 g, 2eq), and heated at 100 °C for 30 min. The brownish solution was brought to rt and neutralized with 10% aq HCl. The solution was extracted with hexane until the sulfur smell was gone, and the solvent was evaporated under vacuum to obtain 2-(phenylthio) hypoxanthine as an oil, used without further purification. Phosphoryl chloride (5.2 mL, 4eq) was added to a well stirred solution of 2-(phenylthio)hypoxanthine, triethylamine (1.94 mL, 2eq) and triethylamine hydrochloride (0.96 g, 0.5eq) in dry DMF (100mL). The mixture was heated in an oil bath at 70 °C for 2 h. The brown solution was evaporated under reduced pressure, and the residue was treated with water-ice, and a saturated solution of $NaHCO_3$ was added in small portions to bring the pH to ca. 7. The solution was extracted with $CHCl_3$ (3 x 80 mL), and the organic layer was dried over anhydrous Na_2SO_4. After evaporation of solvent under reduced pressure, the residue was purified on a silica gel column in a gradient of 0–4% MeOH in $CHCl_3$ to give 3.2 g (87%) of product as yellow solid foam. An analytical sample was crystallized from Et_2O, mp 177–179°C. ^{1}H NMR: δ 7.50 (m, 3H), 7.66 (m, 2H), 8.52 (s, 1H), 13.77 (s, 1H). Anal. ($C_{11}H_7ClN_4S$): C,H,N.

9-(4-Acetoxybutyl)-2-phenylthio-6-chloropurine, 3b.—4-Bromobutyl acetate (2.22 mL, 1.3 eq) and anhydrous K_2CO_3 (1.96 g, 1.2 eq) were added to a solution of 2-phenylthio-6-chloropurine (3.1 g, 11.80 mmol, 1 eq) in dry DMF (30mL), and the mixture was stirred overnight at 50 °C. Evaporation of solvent under reduced pressure gave a yellow oil, which was mixed with $CHCl_3$ and coevaporated with silica gel to dryness. The product was purified on a silica gel column in a gradient of 0–4% MeOH in $CHCl_3$ to yield 3.3 g (74%) of the 9-isomer as a yellow foam, mp 66–68 ºC (from Et_2O). ^{1}H NMR: δ 1.44, 1.78 (2xquint, 2H), 1.99 (s, 3H), 3.94 (t, 2H), 4.09 (t, 2H), 7.47 (m, 3H), 7.66 (m, 2H), 8.56 (s, 1H). Anal. ($C_{17}H_{17}ClN_4O_2S$): C,H,N.

A suspension of **3b** (3.25 g, 8.62 mmol) in 0.5 N aq NaOH (100 mL) was heated under reflux for 30 h. The pale yellow solution was brought to rt and treated with conc. acetic acid to bring the pH to ca. 5. Saturated aq NH_4Cl was added, and the solution was extracted with $CHCl_3$ (6

x 80 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified on a silica gel column in a gradient of 0–11% MeOH in $CHCl_3$ to give 2.55 g (93%) of **3** as white crystals, mp 184–186 °C (from MeOH). ^{1}H NMR: δ 1.19, 1.60 (2xquint, 2H), 3.28 (q, 2H), 3.82 (t, 2H), 4.37 (t, 1H), 7.49 (m, 3H), 7.63 (m, 2H), 7.94 (s, 1H), 12.66 (s, 1H). Anal. ($C_{15}H_{16}N_4O_2S$): C,H,N.

9-(4-Hydroxybutyl)-2-phenoxy-6-oxopurine, 4.

2-Phenoxy-6-chloropurine, 4a.—A mixture of 2-bromohypoxanthine (1.0 g, 4.65 mmol) and K_2CO_3 (1.93 g, 3eq) in phenol (15 mL) was heated under Ar at 160 °C for 20 h. The pale yellow solution was washed with Et_2O, and the white residue of crude 2-phenoxyhypoxanthine was used without further purification. Phosphoryl chloride (2.6 mL, 6 eq) was added to a well stirred solution of 2-phenoxyhypoxanthine, triethylamine (1.3 mL, 2 eq) and triethylamine hydrochloride (1.28 g, 0.5 eq) in dry DMF (20 mL). The flask was heated in an oil bath at 70 °C for 5 h. The solution was evaporated under reduced pressure, and the residue was treated with water-ice, and saturated aqueous $NaHCO_3$ was added in small portions to bring the pH to ca. 7. The solution was extracted with $CHCl_3$ (4 x 80 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4. After evaporation of solvent under reduced pressure, the residue was purified on a silica gel column in a gradient of 0–4% MeOH in $CHCl_3$ to give 0.66 g (57%) of product as a yellow solid, mp 192–195 °C (from MeOH). ^{1}H NMR: δ 7.28 (m, 3H), 7.46 (m, 2H), 8.49 (s, 1H), 13.70 (s, 1H). Anal. ($C_{11}H_7ClN_4O$): C,H,N.

9-(4-Acetoxybutyl)-2-phenoxy-6-chloropurine, 4b.—4-Bromobutyl acetate (0.48 mL, 1.3eq) and anhydrous K_2CO_3 (0.35 g, 1 eq) were added to a solution of 2-phenoxy-6-chloropurine (0.63 g, 2.55 mmol) in dry DMF (10 mL), and the mixture was heated at 50 °C for 20 h. The solvent was removed under reduced pressure to leave a yellow oil, which was mixed with $CHCl_3$:MeOH (1:1) and coevaporated with silica gel to dryness. The material was purified on a silica gel column in a gradient of 0–3% MeOH in $CHCl_3$ to give 0.59 g (64%) of product as a pale yellow solid, mp 75–77 °C (from Et_2O). ^{1}H NMR: δ 1.51, 1.85 (2xquint, 2H), 1.98 (s, 3H), 3.98 (t, 2H), 4.16 (t, 2H), 7.28 (m, 3H), 7.46 (m, 2H), 8.57 (s, 1H). Anal. ($C_{17}H_{17}ClN_4O_3$): C,H,N.

A suspension of **4b** (0.4 g, 1.11 mmol) in 0.25 N aqueous NaOH (50mL) was heated under reflux for 20 h. The pale yellow solution was brought to rt and treated with conc. acetic acid to bring the pH to ca. 5. Saturated aq NH_4Cl was added, and the solution was extracted with $CHCl_3$ (8 x 30 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified on a silica gel column in a gradient of 0–11% MeOH in $CHCl_3$ to give 0.24 g (72%) of **4** as a pale yellow solid, mp 185–188 °C (from Me_2CO). ^{1}H NMR: δ 1.23, 1.66 (2xquint, 2H), 3.29 (q, 2H), 3.85 (t, 2H), 4.35 (t, 1H), 7.30 (m, 3H), 7.46 (m, 2H), 7.91 (s, 1H), 12.57 (s, 1H). Anal. ($C_{15}H_{16}N_4O_3$): C,H,N.

9-(4-Hydroxybutyl)-2-(N-methyl-N-phenylamino)-6-oxopurine, 5.

A solution of t-BuOK in THF (20 mL, 1.3 eq) was added to a solution of 9-(4-hydroxybutyl)-2-phenylamino-6-chloropurine[7] (0.5 g, 1.57 mmol) in Me_2SO:THF (1:1, 10mL) at rt. After 0.5 h, MeI (1.3 eq) was added in one portion to the yellow solution, and the solution was stirred at rt for 4 h. A small volume of saturated aq NH_4Cl was added, and the solution was extracted with EtOAc (3 x 20 mL), the organic layer washed with saturated aq NH_4Cl, and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave 0.35 g (67%) of 9-(4-hydroxybutyl)-2-(N-methyl-N-phenylamino)-6-chloropurine as a pale yellow solid, used in the next step without further purification. ^{1}H NMR: δ 1.35, 1.83 (2xquint, 2H), 3.39 (q, 2H), 3.51 (s, 3H), 4.08 (t, 1H), 4.42 (t, 1H) 7.24 (m, 1H), 7.41 (m, 4H), 8.26 (s, 1H).

A suspension of the above intermediate (0.33 g, 1.01 mmol) in 10% aqueous NaOH (10 mL) was heated under reflux for 44 h. After cooling, the pH was brought to neutrality with conc. acetic acid. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$:MeOH (1:1, 30 mL) and coevaporated with silica gel to dryness. The residue was purified on a silica gel column in a gradient of 0–11% MeOH in $CHCl_3$ to give crude product. Crystallization from Me_2CO/Et_2O provided 0.14 g (44%) of product as pale yellow crystals, mp 163–165 °C. ^{1}H NMR: δ 1.35, 1.77 (2xquint, 2H), 3.37 (q, 2H), 3.40 (s, 3H), 3.96 (t, 1H), 4.42 (br s, 1H) 7.27 (m, 3H), 7.43 (m, 2H), 7.77 (s, 1H), 10.51 (br s, 1H). Anal. ($C_{16}H_{19}N_5O_2.0.25H_2O$): C,H,N.

9-(3-Hydroxypropyl)-2-phenylamino-6-oxopurine, 7.

9-(3-Acetoxypropyl)-2-phenylamino-6-chloropurine, 7a.—Potassium carbonate (3.4 g, 0.6 eq) was added in one portion to a solution of 2-phenylamino-6-chloropurine (10.0 g, 40.7 mmol, 1 eq) in dry DMF (250 mL) at rt. 3-Chloropropyl acetate (7.8 g, 1.4 eq) was added to the mixture, followed by NaI (0.6 g, 0.1 eq). The reaction mixture was stirred at 55 °C for 40 h. After cooling to rt, 20 mL of glacial acetic acid were added slowly to the reaction mixture. The solvents were evaporated under reduced pressure to leave a yellow slurry. Addition of water and extraction of the product with $CHCl_3$ (3 x 200 mL), drying of the combined organic extracts over Na_2SO_4, and evaporation gave a yellow solid. This material was stirred in 150 mL of EtOH at rt for 2 h, and the suspension filtered to give a white solid containing both 7- and 9-isomers (NMR). Purification of the mixture on a silica gel column in a gradient of 0–11% EtOH in $CHCl_3$ gave 6.8 g (48%) of the 9-isomer **7a** as a white solid, mp 181°-183° C. ^{1}H NMR: δ 1.91 (s, 3H), 2.21 (quint, 2H), 4.03 (t, 2H), 4.27 (t, 2H) 6.98 (t, 1H), 7.32 (t, 2H), 7.81 (d, 2H), 8.32 (s, 1H), 9.97 (s, 1H). Anal. ($C_{16}H_{16}ClN_5O_2$): C,H,N.

A suspension of **7a** (6.8 g, 19.7 mmol) in 10% aqueous NaOH (100 mL) was heated at reflux for 48 h. The pale yellow solution was brought to rt and treated with conc. acetic acid to bring the pH to ca. 5, producing a yellowish precipitate. Collection of the solid by filtration, and washing with water and Me_2CO gave crude product. Crystallization from aqueous NH_4OH/MeOH gave 3.7 g (72%) of **7** as yellowish crystals, mp >300°C. ^{1}H NMR: δ 1.95 (quint, 2H), 3.41 (q, 2H), 4.1 (t, 2H), 4.63 (t, 1H), 7.03 (t, 1H), 7.34 (t, 2H), 7.67 (d, 2H), 7.81 (s, 1H), 8.81 (s, 1H), 10.50 (s, 1H). Anal ($C_{14}H_{15}N_5O_2$): C,H,N.

9-(5-Hydroxypentyl)-2-phenylamino-6-oxopurine, 8.

2-(N-Formyl-N-phenylamino)-6-chloropurine, 8a.—Phosphoryl chloride (41 mL, 2 eq) was added dropwise to a well stirred suspension of 2-phenylamino-6-chloropurine (50 g, 0.22 mol, 1 eq), triethylamine (30.7 mL, 1 eq) and triethylamine hydrochloride (15.1 g, 0.5 eq) in dry DMF (150 mL). The solid disappeared almost immediately to give a dark solution which was heated in an oil bath at 60 °C. After 4 h, the suspension of yellow solid was brought to rt, and solvents were evaporated under reduced pressure to ca. 100 mL of yellow slurry. This residue was treated with water-ice, and a saturated solution of aqueous $NaHCO_3$ was added in small portions to bring the pH to ca. 7. After standing overnight, the yellow precipitate was collected by filtration, washed with water and dried to give 49.7 g (83%) of product as a yellow-orange solid. ^{1}H NMR: δ 7.30 (t, 2H), 7.40-7.54 (m, 3H), 8.53 (s, 1H), 9.72 (s, 1H), 13.81 (br s, 1H). HR-MS: Calcd for $C_{12}H_8ClN_5O$, 273.0417. Found 273.0437.

9-(5-Acetoxypentyl)-2-(N-formyl-N-phenylamino)-6-chloropurine, 8b.—Sodium hydride (1.60 g, 60% dispersion in mineral oil) was added in one portion to a solution of **8a** (10.0 g, 36.5 mmol, 1 eq) in dry DMSO:THF (1:1, 200 mL) at 0 °C. The mixture was stirred for 0.5 h at 0 °C, and then brought to rt. 5-Bromopentyl acetate (7.31 mL, 1.2 eq) was added to the mixture, followed by NaI (0.55 g, 0.1 eq). The reaction mixture was stirred at rt for 24 h. THF was removed under reduced pressure, and 100mL of water was added. The product

was extracted with EtOAc (3 x 200 mL), and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. Purification on a silica gel column in a gradient of 0–5% Me_2CO in $CHCl_3$ gave the 9-isomer as a yellow oil. Crystallization from Et_2O-petroleum ether gave, in two crops, 7.93 g (52%) of product as pale yellow crystals, mp 73°–75°C. ^{1}H NMR: δ 1.20, 1.54, 1.80 (3xquint, 2H), 1.97 (s, 3H), 3.93 (t, 2H), 4.14 (t, 2H), 7.30 (m, 2H), 7.50 (m, 3H), 8.58 (s, 1H), 9.77 (s, 1H). Anal. ($C_{19}H_{20}ClN_5O_3$): C,H,N.

A suspension of **8b** (7.29 g, 17.5 mmol) in 1N aqueous NaOH (400 mL) was heated under reflux for 41 h. The pale yellow solution was brought to rt and treated with conc. acetic acid to bring the pH to ca. 5, producing a pale pink precipitate. Collection of the solid by filtration and washing with water and Me_2CO gave the crude title compound. Crystallization from DMF gave 4.42 g (80%) of **8** as pale pink crystals, mp >300 °C. ^{1}H NMR: δ 1.29, 1.46, 1.82 (3xquint, 2H), 3.37 (q, 2H), 4.04 (t, 2H), 4.36 (t, 1H), 7.03 (t, 1H), 7.34 (t, 2H), 7.66 (d, 2H), 7.82 (s, 1H), 8.92 (br s, 1H), 10.59 (br s, 1H). Anal. ($C_{16}H_{19}N_5O_2$): C,H,N.

9-(4-Methoxybutyl)-2-phenylamino-6-oxopurine, 9.

9-(4-methoxybutyl)-2-phenylamino-6-chloropurine, 9a.—Potassium carbonate (2.14 g, 0.7 eq) was added to a solution of 2-phenylamino-6-chloropurine (5.43 g, 22.1 mmol, 1 eq) and 4-methoxybutyl bromide (4.8 g, 1.3 eq) in dry DMF (80 mL). The mixture was heated in an oil bath at 50 °C for 20 h. The solvent was removed under reduced pressure, $CHCl_3$:MeOH (1:1, 50mL) was added, and the solution was coevaporated with silica gel to dryness. The mixture was purified on a silica gel column in a gradient of 0–4% MeOH in $CHCl_3$ to give 4.35 g (59%) of **9a** as pale yellow crystals, mp 191–193 °C. ^{1}H NMR: δ 1.50, 1.91 (2xquint, 2H), 3.20 (s, 3H), 3.34 (t, 2H), 4.19 (t, 1H), 6.98 (t, 1H), 7.31 (t, 2H), 7.81 (d, 2H), 8.32 (s, 1H), 9.97 (s, 1H). Anal. ($C_{16}H_{18}ClN_5O{\cdot}0.5H_2O$): C,H,N.

A suspension of **9a** (4.3 g, 13.1 mmol) in 1N NaOH (100 mL) was heated under reflux for 40 h. After cooling, the pH was brought to neutrality with conc. acetic acid to yield a white precipitate. The solid was filtered and washed several times with water. The wet solid was treated with EtOH:Me_2CO (8:1) and stirred for 1 h at rt. The solid was filtered to give 4.0 g (58%) of **9** as a white solid, mp >300°C (from MeOH). ^{1}H NMR: δ 1.48, 1.85 (2xquint, 2H), 3.19 (s, 3H), 3.32 (t, 2H), 4.06 (t, 1H), 7.04 (t, 1H), 7.35 (t, 2H), 7.65 (d, 2H), 7.84 (s, 1H), 8.80 (s, 1H), 10.49 (s, 1H). Anal. ($C_{16}H_{19}N_5O_2$): C,H,N.

9-(3-Carboxypropyl)-2-phenylamino-6-oxopurine, 10.

Sodium hydride (60% dispersion in mineral oil, 1.2 eq)) was added in one portion to a solution of 2-phenylamino-6-chloropurine (3.0 g, 12.2 mmol) in dry DMF (100 mL) at 0 °C. After 0.5 h the reaction mixture was warmed to rt, ethyl 4-bromobutyrate (3.3 g, 1.4 eq) was added, and the mixture was stirred at rt for 26 h. The solvents were evaporated under reduced pressure, the residue was treated with MeOH:$CHCl_3$ (1:1, 50 mL), and the solution was coevaporated with silica gel to dryness. The mixture was purified on a silica gel column in a gradient of 0–15% Me_2CO in $CHCl_3$ to give 1.92 g (44%) of the 9-isomer of the ethyl ester, used in the next step without further purification. ^{1}H NMR: δ 1.10 (t, 3H), 2.14 (quint, 2H), 2.36 (t, 2H), 3.96 (q, 2H), 4.22 (t, 2H), 6.98 (t, 1H), 7.31 (t, 2H), 7.81 (d, 2H), 8.30 (s, 1H), 9.95 (s, 1H).

A solution of the above ester (1.72 g, 4.78 mmol) in a mixture of 2N aqueous NaOH (100 mL) and MeOH (15 mL) was heated for 40 h under reflux. The pale yellow solution was brought to rt and treated with conc. acetic acid to bring the pH to ca. 5, producing a white precipitate. Collection of the solid by filtration, and washing with water and Me_2CO gave 1.4 g (93%) of **10** as an amorphous white solid, mp >300°C. ^{1}H NMR: δ 2.07 (quint, 2H), 2.26, 4.09 (2xt, 2H), 7.03 (t, 1H), 7.35 (t, 2H), 7.67 (d, 2H), 7.82 (s, 1H), 8.80 (s, 1H), 10.49 (s, 1H), 12.15 (s, 1H). Anal ($C_{14}H_{15}N_5O_2$): C,H,N.

9-(4-Iodobutyl)-2-phenylamino-6-oxopurine, 11 (IBPG).

Trimethylsilyl iodide (13.9 g, 4 eq) was added to a stirred suspension of **1** (5.21 g, 17.4 mmol, 1 eq) in sulfolane (80 mL) at rt. The solid dissolved immediately, and the resulting pale brown solution was heated in an oil bath at 60 °C for 22 h. The reaction mixture was brought to rt and quenched with a saturated solution of Na_2SO_3. The resulting pale yellow solution was treated with water (100 mL) and stirred for 1 h to give a white precipitate. Filtration, washing with water and Me_2CO, and drying of the material gave 7.05 g (99%) of **11** as a white solid, mp >250 °C (dec). ^{1}H NMR: δ 1.75, 1.90 (2xquint, 2H), 4.08 (t, 2H), 7.04 (t, 1H), 7.35 (t, 2H), 7.64 (d, 2H), 7.83 (s, 1H), 8.78 (s, 1H), 10.48 (s, 1H). Anal. ($C_{15}H_{16}IN_5O$): C,H,N.

General method for synthesis of 9-(substituted-butyl-2-phenylamino-6-oxopurines, 12a-s.

The appropriate amine (4–10 eq) was added in one portion to a suspension of **11** (1 eq) in dry DMF. The reaction mixture was stirred at rt until all starting material was consumed (TLC, usually 3–72 h). After this time, the mixture was evaporated to dryness under reduced pressure to yield a white or pale yellow residue. The residue was treated with water-MeOH, and the solid was filtered and washed with Me_2CO. The solid was dissolved in aq 2–4N HCl to obtain a yellow solution. Coevaporation several times with EtOH or Me_2CO gave a white precipitate, which was filtered and washed with a small amount of cold EtOH or Me_2CO. Products were crystallized from EtOH or MeOH.

2-Phenylamino-9-[4-(N,N-dimethylamino)butyl]-6-oxopurine dihydrochloride, 12a.

The general method with 2N dimethylamine in MeOH gave 94% of product as white crystals, mp 260–262 °C (from MeOH). ^{1}H NMR: δ 1.70, 1.92 (2xquint, 2H), 2.66 (s, 6H), 3.05 (m, 2H), 4.22 (t, 2H), 7.08 (t, 1H), 7.38 (dd, 2H), 7.68 (d, 2H), 8.86 (s, 1H), 10.28 (s, 1H), 10.36 (s, 1H), 11.69 (s, 1H). Anal. ($C_{17}H_{22}N_6O\cdot 2HCl$): C,H,N.

2-Phenylamino-9-[4-(N,N-diethylamino)butyl]-6-oxopurine dihydrochloride, 12b.

The general method with diethylamine gave 75% of product, mp 249–255 °C (from MeOH). ^{1}H NMR: δ 1.14 (t, 6H), 1.68, 1.93 (2xquint, 2H), 3.00 (m, 6H), 4.23 (t, 2H), 7.08 (t, 1H), 7.39 (t, 2H), 7.68 (d, 2H), 8.86 (s, 1H), 10.18 (s, 1H), 10.29 (s, 1H), 11.70 (s, 1H). Anal. ($C_{19}H_{26}N_6O\cdot 2HCl$): C,H,N.

2-Phenylamino-9-[4-(N-ethyl-N-methylamino)butyl]-6-oxopurine dihydrochloride, 12c.

The general method with ethylmethylamine gave 79% of product as white crystals, mp 260–263 °C (from MeOH-water). ^{1}H NMR: δ 1.17 (t, 3H), 1.68, 1.92 (2xquint, 2H), 2.61 (s, 3H), 2.90–3.15 (m, 4H), 4.22 (t, 2H), 7.08 (t, 1H), 7.39 (t, 2H), 7.68 (d, 2H), 8.87 (s, 1H), 10.31 (s, 2H), 11.71 (s, 1H). An analytical sample was recrystallized from of MeOH/H_2O, mp 260–263 °C. Anal. $C_{18}H_{24}N_6O\cdot 2HCl\cdot 0.25H_2O$): C,H,N.

2-Phenylamino-9-{4-[N,N-bis-(2-hydroxyethyl)amino]butyl}-6-oxopurine dihydrochloride, 12d.

The general method with bis(2-hydroxyethyl)amine gave 85% of product, mp 240–246 °C (from EtOH). ^{1}H NMR: δ 1.73, 1.91 (2xquint, 2H), 3.13–3.24 (m, 6H), 3.72 (t, 4H), 4.21 (t, 2H), 7.08 (t, 1H), 7.38 (t, 2H), 7.68 (d, 2H), 8.79 (s, 1H), 9.64 (s, 1H), 10.23 (s, 1H), 11.64 (s, 1H). Anal. ($C_{19}H_{26}N_6O_3\cdot 2HCl$): C,H,N.

2-Phenylamino-9-[4-(N,N-diallylamino)butyl]-6-oxopurine dihydrochloride, 12e.

The general method with diallylamine gave the product in 62% yield, mp 237–235 °C (from MeOH). ^{1}H NMR: δ 1.73, 1.93 (2xquint, 2H), 2.99 (m, 2H), 3.62 (m, 4H), 4.22 (t, 2H), 5.44,

5.50 (2xd, 4H), 5.88, 6.03 (2xq, 2H), 7.09 (t, 1H), 7.37 (t, 2H), 7.67 (d, 2H), 8.90 (s, 1H), 10.34 (s, 1H), 11.01 (s, 1H), 11.74 (s, 1H). Anal. ($C_{21}H_{26}N_6O\cdot 2HCl\cdot 0.25H_2O$): C,H,N.

2-Phenylamino-9-[4-(1-piperidyl)butyl]-6-oxopurine dihydrochloride, 12f.

The general method with piperidine gave 97% of product, mp 280–284 °C (from MeOH). ^{1}H NMR: δ 1.31 (m, 1H), 1.70 (m, 7H), 1.91, 2.70 (2xquint, 2H), 3.00 (m, 2H), 3.30 (d, 2H), 4.22 (t, 2H), 7.09 (t, 1H), 7.39 (t, 2H), 7.67 (d, 2H), 8.84 (s, 1H), 10.18 (s, 1H), 10.27 (s, 1H), 11.68 (s, 1H). Anal. ($C_{20}H_{26}N_6O\cdot 2HCl$): C,H,N.

2-Phenylamino-9-{4-[4-(1-piperidyl)piperidyl]butyl}-6-oxopurine trihydrochloride, 12g.

The general method with 4-(N-piperidyl)piperidine gave 77% of product, mp 295–298 °C (from MeOH). ^{1}H NMR: δ 1.42 (m, 1H), 1.69–1.94 (m, 9H), 2.06–2.18 (m, 2H), 2.27 (m, 2H), 2.79–2.94 (m, 4H), 3.04 (m, 2H), 3.14–3.45 (m, 3H), 3.52 (m, 2H), 4.20 (t, 2H), 7.08 (t, 1H), 7.40 (t, 2H), 7.67 (d, 2H), 8.68 (s, 1H), 10.18 (s, 1H), 10.77 (s, 2H), 11.56 (s, 1H). Anal. ($C_{25}H_{35}N_7O\cdot 3HCl\cdot H_2O$): C,H,N.

2-Phenylamino-9-{4-[1-(2-hydroxyethyl)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12h.

The general method with 2-(2-hydroxyethyl)piperidine gave 62% of product, mp 225–232 °C (from MeOH). ^{1}H NMR: δ 1.34–2.02 (m, 12H), 2.84–3.54 (m, 7H), 4.20 (m, 2H), 7.08 (t, 1H), 7.38 (t, 2H), 7.67 (d, 2H), 8.64 (s, 1H), 9.75 and 9.93 (2xs, 1H), 10.00 (s, 1H), 11.45 (s, 1H). Anal. ($C_{22}H_{30}N_6O_2\cdot 2HCl$): C,H,N.

2-Phenylamino-9-[4-(1-decahydroquinolyl)butyl]-6-oxopurine dihydrochloride, 12i.

The general method with *cis,trans*-decahydroquinoline gave 96% of product, mp 198–206 °C (from MeOH). ^{1}H NMR: δ 0.86–2.09 (m, 17), 2.54–3.20 (4xm, 4H), 3.30 (d, 1H), 4.22 (t, 2H), 7.09 (t, 1H), 7.39 (t, 2H), 7.69 (d, 2H), 8.80 (s, 1H), 10.28 (s, 1H), 10.33 (s, 1H), 11.67 (s, 1H). Anal. ($C_{24}H_{32}N_6O\cdot 2HCl$): C,H,N.

2-Phenylamino-9-[4-(2,6-dimethylpiperazinyl)butyl}-6-oxopurine trihydrochloride, 12j.

The general method with 2,6-dimethylpiperazine and $NaHCO_3$ gave the product in 76% yield, mp 257–263 °C (from EtOH). ^{1}H NMR: δ 1.27, 1.29 (2xs, 6H), 1.78, 1.94 (2xm, 2H), 3.00–3.16 (m, 4H), 3.60–3.75 (m, 4H), 4.22 (t, 2H), 7.08 (t, 1H), 7.40 (t, 2H), 7.68 (d, 2H), 8.80 (s, 1H), 10.05 and 10.11 (2xs, 2H), 11.59 (s, 1H), 11.98 (s, 1H). Anal. ($C_{21}H_{29}N_7O\cdot 3HCl$): C,H,N.

2-Phenylamino-9-{4-[2-(1,2,3,4-tetrahydroisoquinolyl)]butyl}-6-oxopurine hydrochloride, 12k.

The general method with 1,2,3,4-tetrahydroisoquinoline and anhydrous K_2CO_3 gave the product in 98% yield, mp 257–262 °C (from MeOH). ^{1}H NMR: δ 1.84, 1.97 (2xm, 2H), 2.97 (m, 1H), 3.21 (m, 4H), 3.59 (m, 1H), 4.21 (m, 3H), 4.44 (m, 1H), 7.10 (m, 2H), 7.22 (m, 3H), 7.36 (t, 2H), 7.68 (d, 2H), 8.91 (s, 1H), 10.34 (s, 1H), 10.92 (s, 1H), 11.74 (s, 1H). Anal. ($C_{24}H_{26}N_6O\cdot HCl$): C,H,N.

2-Phenylamino-9-{4-[2-(1-piperidylmethyl)pyrrolidyl]butyl}-6-oxopurine trihydrochloride, 12l.

The general method with 2-(1-piperidylmethyl)pyrrolidine and $NaHCO_3$ gave the product in 74% yield, mp 178–188 °C (from MeOH). ^{1}H NMR: δ 1.35 (m, 1H), 1.68–1.94 (m, 12H), 2.41 (m, 1H), 2.89, 3.03 (2xm, 4H), 3.45 (m, 5H), 3.73 (m, 1H), 3.82 (m, 1H), 4.25 (t, 2H), 7.08 (t, 1H), 7.41 (t, 2H), 7.70 (d, 2H), 8.99 (s, 1H), 10.38 (s, 1H), 11.04 (br s, 1H), 11.34 (br s, 1H), 11.76 (s, 1H). Anal. ($C_{25}H_{35}N_7O\cdot 3HCl\cdot 0.25H_2O$): C,H,N.

2-Phenylamino-9-{4-[1-(4-hydroxy)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12m.

The general method with 4-hydroxypiperidine gave 84% of product, mp 294–297 °C (from aq EtOH). ^{1}H NMR (two conformers): δ 1.70 (m, 4H), 1.89 (m, 4H), 2.77 (m, 1H), 3.02 (m, 3H), 3.16 (m, 1H), 3.32 (m, 1H), 3.52+3.90 (2xm, 1H), 4.22 (t, 2H), 7.09 (quint, 1H), 7.39 (t, 2H), 7.67 (d, 2H), 8.85 (d, 1H), 10.24 (d, 2H), 11.67 (s, 1H). Anal. ($C_{20}H_{26}N_6O_2 \cdot 2HCl \cdot H_2O$): C,N; H, calc 6.38; found 5.94.

2-Phenylamino-9-{4-[1-(3-hydroxy)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12n.

The general method with 3-hydroxypiperidine gave 90% of product, mp 275–279 °C (from MeOH). ^{1}H NMR (two conformers): δ 1.20+1.68 (2xm, 5H), 1.91 (m, 3H), 2.41–2.93 (4xm, 2H), 3.01 (m, 2H), 3.22 (m, 2H), 3.81+3.99 (2xm, 1H), 4.19 (t, 2H), 7.08 (t, 1H), 7.38 (h, 2H), 7.67 (d, 2H), 8.60+8.71 (2xs, 1H), 9.18+10.46 (2xbr s, 1H), 10.07+10.12 (2xs, 1H), 11.52 (br s, 1H). Anal. ($C_{20}H_{26}N_6O_2 \cdot 2HCl$): H,N; C, calcd 52.75, found 53.10.

2-Phenylamino-9-{4-[1-(4-methoxy)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12o.

The general method with 4-methoxypiperidine hydrochloride and equivalent anhydrous K_2CO_3 gave 73% of product, mp 241–251 °C (dec) (from EtOH). ^{1}H NMR (two conformers): δ 1.72 (m, 3H), 1.92 (m, 5H), 2.85 (m, 2H), 3.05 (m, 2H), 3.16–3.52 (m, 6H), 4.23 (t, 2H), 7.09 (t, 1H), 7.40 (t, 2H), 7.68 (d, 2H), 8.98 (d, 1H), 10.43 (s, 1H), 10.60 (br s, 1H), 11.82 (s, 1H). Anal. ($C_{21}H_{28}N_6O_2 \cdot 2HCl \cdot 0.5H_2O$): C,H,N.

2-Phenylamino-9-{4-[1-(3-methoxy)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12p.

The general method with 3-methoxypiperidine hydrochloride and equivalent K_2CO_3 gave 86% of product, mp 239–246 °C (from aq MeOH). ^{1}H NMR (two conformers): δ 1.18–2.08 (4xm, 8H), 2.55–3.62 (m, 10H), 4.24 (t, 2H), 7.09 (t, 1H), 7.39 (h, 2H), 7.69 (d, 2H), 8.95+9.04 (2xs, 1H), 9.22+10.98 (2xbr s, 1H), 10.52+10.58 (2xs, 1H), 11.87 (br s, 1H). Anal. ($C_{21}H_{28}N_6O_2 \cdot 2HCl \cdot 0.75H_2O$): C,H,N.

2-Phenylamino-9-{4-[1-(2-phenyl)piperidyl]butyl}-6-oxo-purine dihydrochloride, 12q.

The general method with 2-phenylpiperidine and anhydrous K_2CO_3 gave 53% of product, mp 190–196 °C (from aq EtOH). ^{1}H NMR: δ 1.60–2.08 (m, 8H), 2.12 (m, 2H), 2.71 (m, 2H), 2.96 (m, 1H), 3.55 (m, 1H), 4.13 (m, 3H), 7.10 (t, 1H), 7.38 (m, 5H), 7.63 (m, 4H), 8.82 (s, 1H), 10.31 (s, 1H), 11.02 (s, 1H), 11.76 (s, 1H). Anal. ($C_{26}H_{30}N_6O \cdot 2HCl \cdot H_2O$): C,H,N.

2-Phenylamino-9-{4-[1-(4-cyano-4-phenyl)piperidyl)]butyl}-6-oxopurine dihydrochloride, 12r.

The general method with 4-cyano-4-phenylpiperidine and equivalent $NaHCO_3$ gave 69% of product, mp 265–270 °C (from EtOH). ^{1}H NMR: δ 1.80 (m, 2H), 1.96 (m, 2H), 2.46 (t, 2H), 2.61 (t, 2H), 3.10–3.24 (2xm, 4H), 3.66 (m, 2H), 4.23 (t, 2H), 7.08 (t, 1H), 7.38–7.56 (m, 7H), 7.67 (d, 2H), 8.80 (s, 1H), 10.03 (s, 1H), 11.24 (s, 1H), 11.55 (s, 1H). Anal. ($C_{27}H_{29}N_7O \cdot 2HCl$): H,N; C, calc 60.00; found 60.45.

2-Phenylamino-9-{4-[4-hydroxy-4-(4-chloro-3-trifluoromethylphenyl)piperidyl]butyl}-6-oxopurine dihydrochloride, 12s.

The general method with 4-hydroxy-4-[(4-chloro-3-trifluoromethyl)]phenylpiperidine and equivalent K_2CO_3 gave 87% of product, mp 246–250 °C (from MeOH). ^{1}H NMR: δ 1.78 (m, 4H), 1.96 (m, 2H), 2.45 (m, 2H), 3.14+3.37 (2xm, 6H), 4.23 (t, 2H), 7.08 (t, 1H), 7.40 (t, 2H), 7.67 (d, 2H), 7.78 (d, 2H), 7.92 (s, 1H), 8.80 (s, 1H), 10.07 (s, 1H), 10.75 (s, 1H), 11.57 (s, 1H). Anal. ($C_{27}H_{28}ClF_3N_6O_2 \cdot 2HCl$): C,H,N.

2-Phenylamino-9-(4-phosphoryloxybutyl)-6-oxopurine, 13 (HBPG-MP).

Phosphoryl chloride (553 mg, 3.61 mmol) was added to a cold slurry of **1** (400 mg, 1.34 mmol) in trimethyl phosphate (5 mL), and the mixture was kept at 0 °C for 4.5 h. Cold water (15 mL) was poured into the reaction mixture, and it was neutralized with triethylamine (1.09 g, 10.8 mmol). After stirring for 1 h, the white precipitate was collected and washed with water to give 487 mg (1.28 mmol, 96%) of **13** as the free acid. HR-MS: calc. 378.0968, found 378.1034. A portion of the acid (20 mg) was neutralized with triethylamine, dissolved in water (5 mL), and purified by ion exchange chromatography (DEAE-Sephadex, 20 x 2.2 cm). Elution was carried out in a linear gradient of triethylammonium bicarbonate, 0.02-1.0 M (pH 7.9), during 8 h at a flow rate of 4 mL/min with detection at 260 nm. The fractions containing the desired product were combined and evaporated under vacuum at 30 °C. The residue was dissolved in water and passed through 15 mL of Dowex 50Wx8 (Na+ form), and the aqueous solution was lyophilized to give 15 mg of the title compound as the sodium salt. ^{1}H-NMR (D_2O) δ 1.59–1.72 (m, 2H), 1.86–2.00 (m, 2H), 3.92 (q, 2H), 4.06 (t, 2H), 7.08 (m, 2H), 7.32 (m, 2H), 7.49 (m, 2H), 7.83 (s, 1H); ^{31}P-NMR (121 MHz, D_2O) δ 2.22 (s).

Cloning and expression of HSV thymidine kinases.

Construction of recombinant bacterial expression vector for HSV-1 TK.— Eukaryotic expression vector pMC1 (kindly provided by Dr. L. Magrassi, IGM-CNR, Pavia, Italy), which contains the complete 1131 bp coding sequence of HSV-1 TK, was amplified by using the following primers: 5'-GCTATGGCTAGCTACCCCGGCCATCAGCACGCGTCTGCG-3' (primer 1, sense) and 5'-CGTGAATTCTCAGTTAGCCTCCCCCATCTCCC-3' (primer 2, antisense), containing the *NheI* and *EcoRI* restriction sites, respectively. The amplified region (1143 bp long) containing the complete TK coding sequence was inserted into the multiple cloning site of pTrcHisA (Invitrogen), restricted with *NheI* and *EcoRI*, to give the recombinant bacterial expression vector pHisHSV-1-TK containing the sequence which encodes for the complete 376 amino acid enzyme with a 6-His tag at its NH_2- terminus.

Construction of recombinant bacterial expression vector for HSV-2 TK.—Plasmid pYCA2 (kindly provided by Dr. John Bishop, Center for Genome Research, University of Edinburgh), which contains the complete 1128 bp coding sequence of HSV-2 TK, was digested with *SacI* and *HindIII*. The 1916 bp fragment containing the HSV-2 TK coding sequence was amplified by using the following primers: 5'-GGTATGGCTAGCCACGCCGGCCAACAGC-3' (primer 1, sense) and 5'-CGTGAATTCTAAACTCCCCCCACCTCGC-3' (primer 2, antisense), containing the *NheI* and *EcoRI* restriction sites, respectively. The amplified region (1139 bp long) containing the complete TK coding sequence was inserted into the multiple cloning site of pTrcHisA, restricted with *NheI* and *EcoRI*, to give the recombinant bacterial expression vector pHisHSV-2-TK containing the sequence which encodes for the complete 386 amino acid enzyme with a 6-His tag at its NH_2- terminus.

Expression and purification of recombinant HSV TKs from bacterial cells.— Expression and purification of the HSV-1 and HSV-2 TK were carried out as described by the manufacturer of the Ni-NTA Superflow resin (Qiagen). Briefly, a fresh overnight saturated culture of *E. coli* (DH5α strain) transformed with pHis-HSV-1 TK or pHis-HSV-2 TK was diluted 1:100 in 2 L of 2x YT (yeast tryptone) broth containing ampicillin (60 μg/mL) and incubated at 37° with shaking. At 0.6 OD_{600}, isopropylthio-β-D-galactopyranoside (IPTG, Sigma) was added to a final concentration of 1 mM, and the culture was incubated for a further 4 h at 37°. The bacterial cell pellet was resuspended in four volumes of lysis buffer (50 mM sodium phosphate (pH 8.0), 300 mM NaCl, 10 mM imidazole, 1 mM PMSF and 1 mg/mL lysozyme) and incubated on ice for 30 min. Cells were then sonicated on ice, and the lysate

was centrifuged at 10,000 x *g* for 30 min at 4 °C. The supernatant was loaded on a Ni-NTA Superflow column (1 mL) at a flow rate of 0.25 mL/min. The column was first washed with lysis buffer and then with 50 mM sodium phosphate buffer (pH 8.0) containing 300 mM NaCl and 20 mM imidazole. The protein was then step-eluted with 250 mM imidazole in 50 mM sodium phosphate buffer (pH 8.0) containing 300 mM NaCl at a flow rate of 0.5 mL/min. Fractions were collected for enzymatic activity analysis. The enzyme, collected from peak fractions, was dialyzed against 50 mM Tris-HCl (pH 7.5) containing 20% glycerol and 1 mM dithiothreitol (DTT), and then frozen in liquid nitrogen until used.

Thymidine kinase assays.

Recombinant HSV TKs were assayed at 37 °C for 15 min in 25 μL of a mixture containing 30 mM HEPES K+, pH 7.5, 6 mM $MgCl_2$, 6 mM ATP, 0.5 mM DTT, and 1 or 2 μM (*ca.* K_m concentration) of [^{3}H-methyl]TdR (2200 cpm/pmol). The reaction was terminated by spotting 20 μL of the incubation mixture on a 23 mm DEAE paper disk (DE-81, Whatman). The disk was washed twice in an excess of 1 mM ammonium formate, pH 5.6, in order to remove unconverted nucleoside and finally in ethanol. Filters were dried, and radioactivity was counted in 1 mL of Betamax (ICN) scintillation fluid. One unit (U) is the amount of enzyme which phosphorylates 1 nmol of TdR to TMP in 1 h at 37°C. *Inhibitor assay.* Stock solutions of inhibitors in DMSO were serially diluted into enzyme assay mixtures. Enzymes were assayed with at least five inhibitor concentrations in duplicate assays to yield IC_{50}, inhibitor concentration reducing activity by 50% in the above assay. When inhibitor K_is were measured, the assay was carried out at several concentrations of [^{3}H]TdR. *Substrate assay.* When nucleoside analogs were tested as possible substrates of HSV TKs, each compound (200 μM) was incubated at 37 °C for 30 min in 25 μL of a mixture containing 30 mM HEPES-K^+, pH 7.5, 6 mM $MgCl_2$, 6 mM ATP, 0.5 mM DTT and approximately 2 U of HSV-1 TK or 8 U of HSV-2 TK. Samples were then heated at 100°C for 5 min and centrifuged for 15 min at 10,000 rpm in a microcentrifuge. Supernatants were transferred to a new tube for subsequent HPLC analysis. *HPLC separation of nucleosides and nucleotides.* Reverse phase chromatography employing a SHIMADZU HPLC system was used to separate nucleosides from nucleotides. A 4.6 mm x 25 cm ALLTIMA C18-NUC 100A 5U (Alltech) column was used at rt in the following conditions: injection volume, 20 μL; detection, UV 260 nm; eluents, buffer A (20 mM KH_2PO_4, pH 7.5), buffer B (20 mM KH_2PO_4, pH 5.2, 60% MeOH); linear gradient, 30 min from 0% to 100% buffer B; 20 min 100% buffer B; flow rate, 0.5 ml/min.

In vivo models.

BALB/c female mice at 6 weeks of age were used in these experiments. The animals were provided food and water *ad libitum* and were maintained in an AAALAC accredited animal care facility. The McKrae strain of HSV-1 was propagated in Vero cells, titered on the same cells, and stored frozen as a stock until used to infect animals. The G strain of HSV-2 was obtained from the American Type Culture Collection, Manassas, VA, propagated and titered on Vero cells, and stored frozen until use. *Reactivation.* The establishment of latency and hyperthermic reactivation in mice were performed as described.[10] Groups of 15 animals were treated intraperitoneally with drugs suspended in corn oil or corn oil alone, subjected 30 min later to hyperthermic stress consisting of immersion in 43 ^oC water for 10 min, dried, and returned to their cages. After 24 h, the eyes of all animals were swabbed for the determination of infectious virus at the ocular surface. *Encephalitis.* Groups of 10 mice each were anesthetized with a mixture of ketamine and xylazine, their corneas lightly scratched with a 27 gauge needle, and 10^6 plaque forming units of infectious virus in tissue culture medium were placed on each cornea. The animals were returned to their cages for recovery from anesthesia. Following the infection and recovery, groups of ten animals were given 0.1 ml intraperitoneal injections of test compounds as suspensions in corn oil or corn oil alone. Treatments were continued twice daily for five days. Animals were observed daily for 30 days, and the day of death of each

animal recorded. Statistical analysis of results was done with Student's t test comparing analysis of variance between groups.

Acknowledgements

This work was supported by small business grant AI43170 from the National Institutes of Health (to G.W.) and by FIRB grant no. RBAU01LSR4_001 (to F.F.). The authors thank Dr. Andrew Maioli for synthesis of HBPG-MP.

References

1. Wildy, P.; Field, H.J.; Nash, A.A. (1982) in Virus Persistence, Mahy, B.W.H., Minson, A.C. and Darby, G.K., eds., Cambridge University Press, Cambridge, pp. 134–167.
2. Whitley RJ, Lakeman F. Herpes Simplex Virus Infections of the Central Nervous System: Therapeutic and Diagnostic Considerations. Clin Infect Dis 1995;20:414–420. [PubMed: 7742450]
3. McGrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome. J Neurol Neurosurg Psychiatry 1997;63:321–326. [PubMed: 9328248]
4. Wright GE, Focher F, Spadari S, Sun H. Inhibitors of Herpes Simplex Thymidine Kinases. Drugs of the Future 1997;22:531–537.
5. Focher F, Hildebrand C, Freese S, Ciarrocchi G, Noonan T, Sangalli S, Brown NC, Spadari S, Wright G. N_2-phenyldeoxyguanosine: a novel selective inhibitor of Herpes simplex thymidine kinase. J Med Chem 1988;31:1496–1500. [PubMed: 2840499]
6. Hildebrand C, Focher F, Gambino J, Spadari S, Wright G. Structure-activity Relationships of N^2-Phenylguanines as Selective Inhibitors of HSV-1 and HSV-2 Thymidine Kinases. J Med Chem 1990;33:203–206. [PubMed: 2153203]
7. Xu H, Maga G, Focher F, Smith E, Spadari S, Gambino J, Wright GE. Synthesis, Properties, and Pharmacokinetic Studies of N^2-Phenylguanine Derivatives as Inhibitors of *Herpes Simplex* Virus Thymidine Kinases. J Med Chem 1995;38:49–57. [PubMed: 7837239]
8. Larsson A, Alenius S, Johansson NG, Öberg B. Antiherpetic activity and mechanism of action of 9-(4-hydroxybutyl)guanine. Antiviral Res 1983;3:77–86. [PubMed: 6312878]
9. Bennett MS, Wien F, Champness JN, Batuwangala T, Rutherford T, Summers WC, Sun H, Wright G, Sanderson MR. Structure to 1.9 Å resolution of a complex with herpes simplex virus type-1 thymidine kinase of a novel, non-substrate inhibitor: X-ray crystallographic comparison with binding of aciclovir. FEBS Lett 1999;443:121–125. [PubMed: 9989588]
10. Gebhardt BM, Wright GE, Xu H, Focher F, Spadari S, Kaufman HE. 9-(4-Hydroxybutyl)-N^2-phenylguanine, HBPG, a thymidine kinase inhibitor, suppresses herpes virus reactivation in mice. Antiviral Res 1996;30:87–94. [PubMed: 8783801]
11. Kaufman HE, Varnell ED, Wright GE, Xu H, Gebhardt BM, Thompson HW. Effect of 9-(4-hydroxybutyl)-N^2-phenylguanine (HBPG), a thymidine kinase inhibitor, on clinical recurrences of ocular herpetic keratitis in squirrel monkeys. Antiviral Res 1996;33:65–72. [PubMed: 8955854]
12. Wright GE, Gambino JJ, Sun H, Gebhardt BM. Status of inhibitors of herpes simplex thymidine kinases. Curr Op Anti-inf Invest Drugs 1999;1:541–546.
13. Gebhardt, B.M.; Varnell, E.D.; Hill, J.M.; Kaufman, H.E. Animal Models of Ocular Herpes Simplex Virus Infections (Rabbits, Primates, Mice). Chapter 110 in Handbook of Animal Models of Infection. Academic Press, 1999, pp 919–926.
14. Kern ER, Glasgow LA, Overall JC Jr, Reno JM, Boezi JA. Treatment of Experimental Herpersvirus Infections with Phosphonoformate and Some Comparisons with Phosphonoacetate. Antimicr Agents Chemother 1978;14:817–823.
15. Keller PM, Fyfe JA, Beauchamp L, Lubbers CM, Furman PA, Schaeffer HJ, Elion GB. Enzymatic Phosphorylation of Acyclic Nucleoside Analogs and Correlations with Antiherpetic Activities. Biochem Pharmacol 1981;30:3071–3076. [PubMed: 6279102]
16. Maga G, Focher F, Wright GE, Garbesi A, Bendiscioli A, Spadari S. Kinetic Studies with N^2-phenylguanines and with L-thymidine indicate that *Herpes simplex* virus type-1 thymidine kinase and thymidylate kinase share a common active site. Biochem J 1994;302:279–282. [PubMed: 8068016]

17. Champness JN, Bennett MS, Wien F, Visse R, Summers WC, Herdewijn P, deClercq E, Ostrowski T, Jarvest RL, Sanderson MR. Exploring the Active Site of Herpes Simplex Virus Type-1 Thymidine Kinase by X-Ray Crystallography of Complexes With Aciclovir and Other Ligands. PROTEINS: Struc Func Genet 1998;32:350–361.

18. Leib DA, Ruffner KL, Hildebrand C, Schaffer PA, Wright GE, Coen DM. Specific Inhibitors of Herpes Simplex Virus Thymidine Kinase Diminish Reactivation of Latent Virus from Explanted Murine Ganglia. Antimicrob Agents Chemother 1990;34:1285–1286. [PubMed: 2168150]

19. Yamazaki A. Synthesis of 9-(4'-hydroxybutyl)-2-amino-6-hydroxypurine 4'-phosphate. Chem Pharm Bull 1969;17:1268–1270.

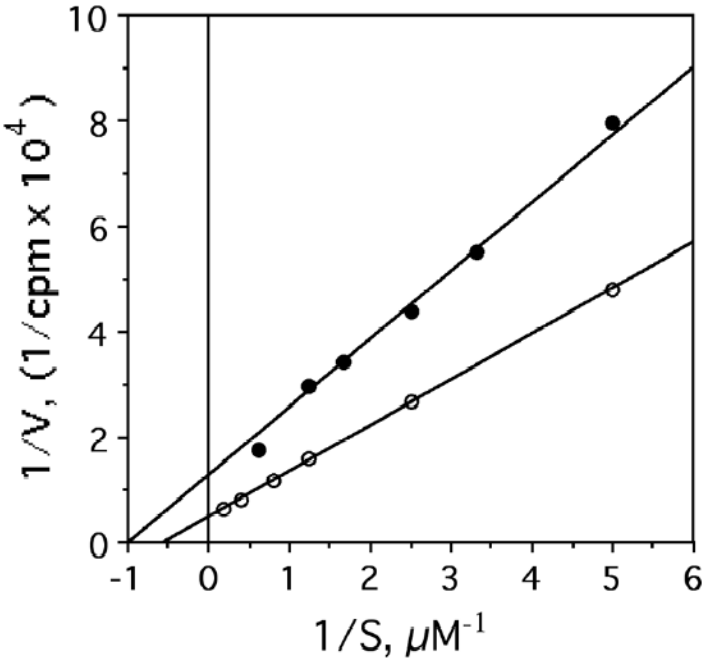


Figure 1.
Determination of K_m values of recombinant HSV TKs. Lineweaver-Burke (double reciprocal) plots of the activity of recombinant HSV TKs in the presence of increasing concentrations of substrate, [3H]TdR, assayed as described in the Experimental Section. HSV-1 TK (○), HSV-2 TK (•).

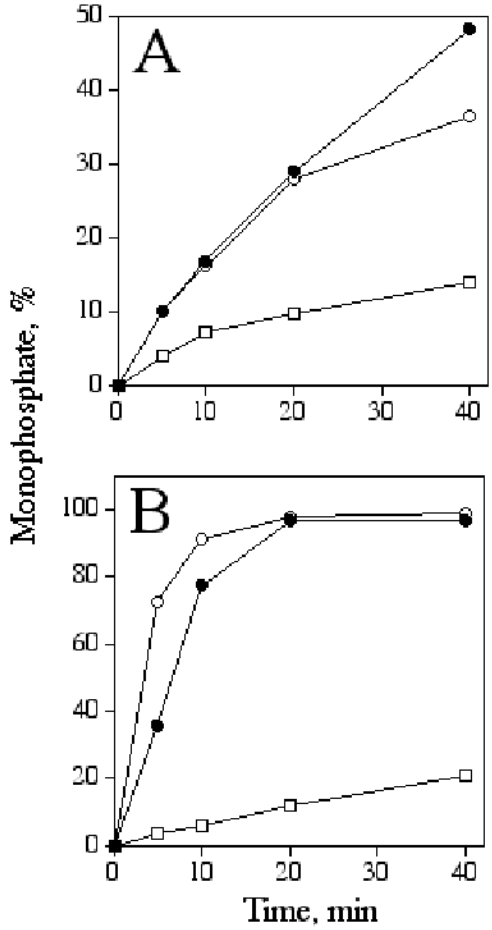
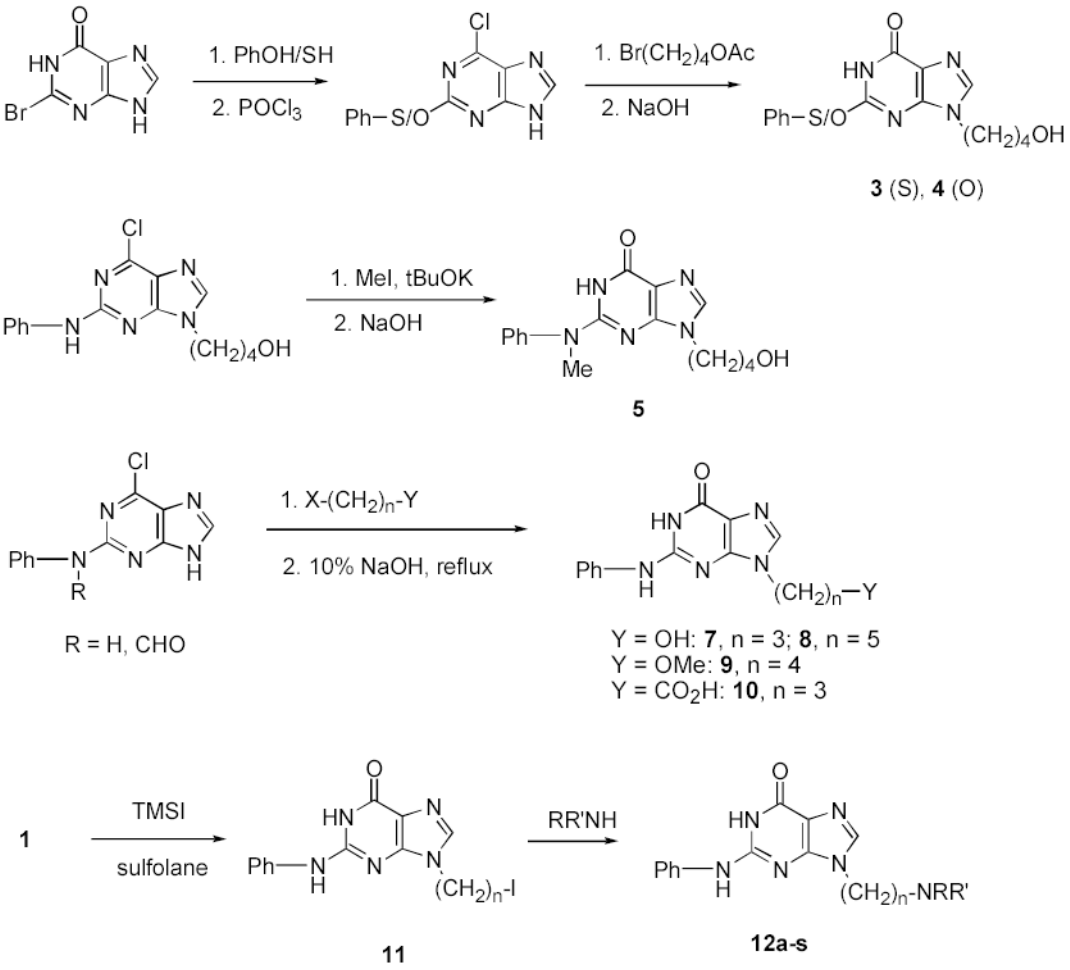


Figure 2.

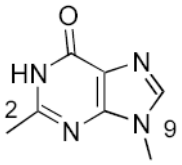
Kinetics of phosphorylation of nucleoside analogs by HSV TKs. Panel A, HSV-1 TK. Panel B, HSV-2 TK. TdR (o), **1** (•) and ACV (□). Two U of enzyme were used in the HSV-1 TK assay, but 8 U in the experiments with HSV-2 TK (to allow a detectable phosphorylation of ACV). Each nucleoside (200 μM) was incubated for the indicated time, and the products of the reaction were resolved by HPLC as described in the Experimental Section. (100% corresponds to 5 nmoles of substrate converted to monophosphate).

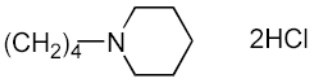
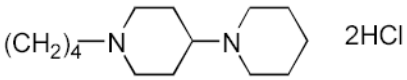
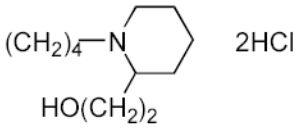
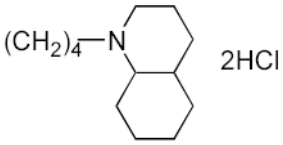
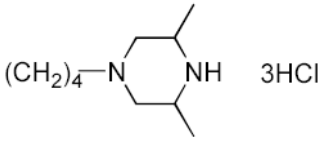
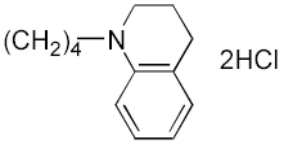


Scheme.

Table 1

Inhibition of HSV TKs by 9-substituted-6-oxopurines



Cpd	2-subst	9-subst	IC_{50} (μM) HSV-1 TK	IC_{50} (μM) HSV-2 TK
1	PhNH	$(CH_2)_4OH$ [HBPG]	1.3	0.5
2	NH_2	" [HBG]	60	160
3	PhS	"	0.5	2.0
4	PhO	"	0.75	9.0
5	PhNMe	"	16	37
6	PhNH	$(CH_2)_2OH$	4.4	2.2
7	"	$(CH_2)_3OH$	2.0	1.5
8	"	$(CH_2)_5OH$	15.0	54.0
9	"	$(CH2)_4OMe$	12.0	14.0
10	"	$(CH_2)_3CO_2H$	1.0	0.7
12a	"	$(CH_2)_4NMe_2 \cdot 2HCl$	3.6	0.62
12b	"	$(CH_2)_4NEt_2 \cdot 2HCl$	4.5	0.23
12c	"	$(CH_2)_4NMeEt \cdot 2HCl$	4.7	0.38
12d	"	$(CH_2)_4N(CH_2CH_2OH)_2 \cdot 2HCl$	5.2	0.34
12e	"	$(CH_2)_4N(CH_2CH{=}CH_2)_2 \cdot 2HCl$	2.1	0.42
12f	"		1.6	0.22
12g	"		16.5	1.0
12h	"		0.77	0.065
12i	"		0.15 [K_i 0.03]	0.013 [K_i 0.005]
12j	"		7.1	0.80
12k	"		0.35	0.047

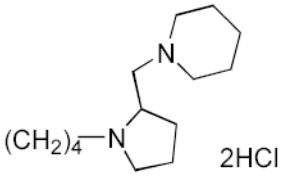
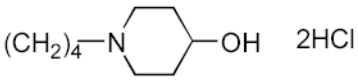
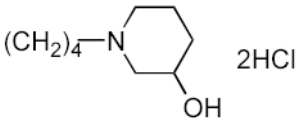
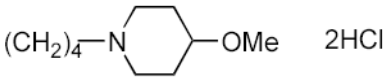
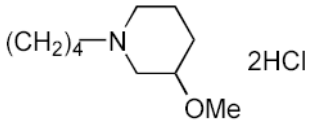
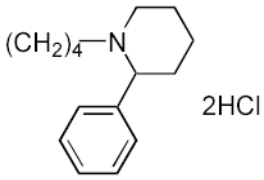
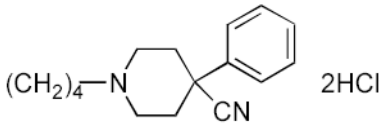
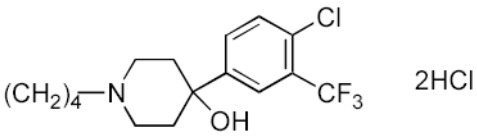
Cpd	2-subst	9-subst	IC_{50} (μM) HSV-1 TK	HSV-2 TK
12l	“		1.0	0.13
12m	“		8.2	0.71
12n	“		5.7	0.31
12o	“		2.3	0.14
12p	“		2.5	0.15
12q	“		0.55	0.09
12r	“		0.41	0.22
12s	“		2.0	0.2

Table 2

Effect of compounds on ocular HSV-2 reactivation in mice.[1]

Cpd	Dose, IP (mg/kg)	HSV-2-positive cultures/15 mice
-	(corn oil)	11
1	200	4[2]
7	200	9
8	200	8
3	200	10
10	200	11
12f	200	11
12i	200	10
ACV	100	10
PFA	400	7[3]

[1] Heat stress of latent HSV-2 infected mice, treatment with compounds, and assay for infectious virus were done as described in the Experimental Section.

[2] $p<0.027$.

[3] $p<0.128$.

Table 3

Effect of compounds on HSV encephalitis in mice.[1]

Cpd	Dose, IP (mg/kg)[2]	HSV-1		HSV-2	
		Survivors/10	Mean day of death	Survivors/10	Mean day of death
- (corn oil)	-	0	9.0	0	14.1
1 (HBPG)	100	3	9.5	4	15.7
“	200	6*	11.8	9*	18
“	400	9*	11	10*	-
7	100	1	9.7	0	10.7
8	100	0	10.7	1	11.2
12f	200	nt	-	0	15.2
12i	200	nt	-	0	15.4
ACV	25	1	10	0	14.2
“	50	3	11	2	15.0
“	100	7	13	6	17.2
PFA	100	1	9.2	0	15.2
“	200	5*	10.4	4*	17.8
“	400	7*	12.3	6*	18.5

[1] Infection and treatment with compounds were done as described in the Experimental Section. Animals alive at 30 days post-infection were “survivors”, and the day of death was recorded for the remainder.

[2] Twice daily for five days. nt, not tested.

* $p<0.05$

Table 4

Conversion of nucleoside analogs to monophosphates (MP) by HSV TKs.

Cpd	HPLC R_t, min[1]		Relative % MP[2]	
	Parent	MP	HSV-1 TK	HSV-2 TK
1 (HBPG)	38.5	31.8	100[3]	100[3]
ACV	25.2	14.6	32	20
TdR	20.7	17.5	70	100

[1] R_t = retention time; HPLC conditions in Experimental Section.

[2] Cpds were assayed at 200 μM with excess TK as described in the Experimental Section.

[3] 100% for HBPG corresponds to 25% conversion to HBPG-MP for both enzymes under the assay conditions.