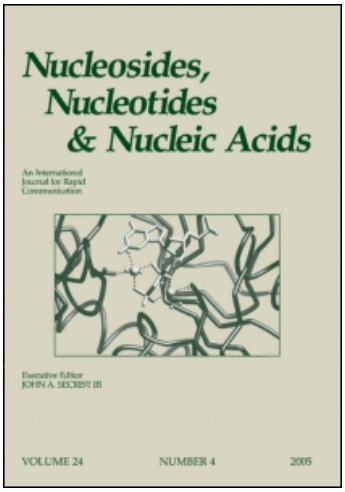

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A Potent, Selective, Non-Substrate Inhibitor of HSV-I Thymidine Kinase: (±)-9-[[(Z)-2-(Hydroxymethyl)Cyclohexyl]Methyl]Guanine and Related Compounds

W. T. Ashton[a]; L. C. Meurer[a]; R. L. Tolman[a]; J. D. Karkas[a]; R. Liou[a]; H. C. Perryt; S. M. Czelusniak[b]; R. J. Klein[b]
[a] Merck Sharp & Dohme Research Laboratories, Rahway, NJ [b] New York University Medical Center,

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A POTENT, SELECTIVE, NON-SUBSTRATE INHIBITOR OF HSV-1 THYMIDINE KINASE: (±)-9-[[(Z)-2-(HYDROXYMETHYL)CYCLOHEXYL]METHYL]GUANINE AND RELATED COMPOUNDS

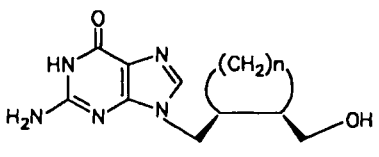
W.T. Ashton*, L.C. Meurer, R.L. Tolman, J.D. Karkas, R. Liou, H.C. Perry†, S.M. Czelusniak‡ and R.J. Klein†

Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065 and †West Point, PA 19486, and ‡New York University Medical Center, New York, NY 10016

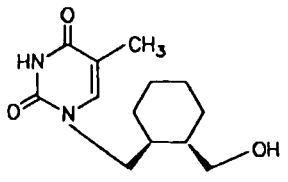
Abstract: The title compound was prepared and found to be a potent and selective inhibitor of HSV-1 thymidine kinase. This compound delayed the reactivation of latent virus from explanted mouse ganglia but exacerbated the primary HSV-1 infection in mice.

The well-known guanine acyclonucleosides acyclovir and ganciclovir are selective substrates of herpes simplex virus thymidine kinase (HSV TK) and are dependent on phosphorylation by this enzyme to exert their activity against HSV infections[1,2]. Until recently[3,4], no highly effective inhibitor of HSV TK had been reported. However, the viral TK may be important for the establishment or maintenance of latent infections in sensory ganglia by HSV or for the reactivation of virus from latently infected ganglia[5].

Consequently, we screened a series of acyclonucleoside analogs as inhibitors of HSV-1 TK. The most interesting of these proved to be 9-[[(Z)-2-(hydroxymethyl)cycloalkyl]methyl]guanines 1. While the cyclopropane compound 1a[6] was an effective substrate (45% conversion to monophosphate[6]) and a moderately good inhibitor (IC_{50}=1.7 μM), expansion of the ring to cyclobutane (1b) markedly improved the inhibition (IC_{50}=0.18 μM) and reduced substrate activity (7% conversion to monophosphate). Further increase in ring size to cyclohexane (1c) gave the most potent inhibitor (IC_{50}=0.07 μM). Furthermore, 1c was not a substrate for HSV-1 TK and did not inhibit the TK from uninfected HeLa cells, even at 800 μM.



1a, n=1
b, n=2
c, n=4



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In MRC-5 cell culture, **1c** failed to inhibit the replication of HSV-1 at the highest concentration tested (100 μg/ml). However, the protection of MRC-5 cells against HSV-1 challenge by the TK-dependent agent ganciclovir (at 0.62 μg/ml) was completely abolished by **1c** at 6.2-12.5 μg/ml and above. In contrast, the antiviral activity of the TK-independent ganciclovir cyclic phosphate[7] was unaffected by **1c**, even at 100 μg/ml.

In explant cultures of mouse trigeminal ganglia treated with **1c** (100 μg/ml; removed after 7 days), reactivated virus appeared in the medium after a mean of 8 days *vs.* 4 days for control cultures. Similarly, titration of virus released into the explant medium indicated that ganglia treated with **1c** (100 μg/ml) had a 40-fold reduction in reactivated virus relative to controls after 7 days.

Surprisingly, mice inoculated orofacially with HSV-1 and treated orally with **1c** at 50 or 150 mg/kg per day rapidly developed lesions which were significantly more severe than those of the control animals. Death also occurred earlier in the treated mice compared to the controls, although the final mortality rates were not significantly different. All surviving mice in these groups appeared to have latent infections, based on evidence of reactivated virus in explant cultures of their trigeminal ganglia.

Compounds **1b** and **1c** were prepared from the corresponding (*Z*)-1,2-cycloalkanedimethanols by: 1) monobenzoylation; 2) conversion of the free OH to Br or tosylate; 3) alkylation of 2-amino-6-benzyloxypurine[8] and chromatographic separation from 7-isomer; and 4) stepwise deprotection. Analog **2** (IC_{50}= 4 μM) was prepared by a similar route involving direct alkylation of thymine with the protected alkyl bromide. Isomeric assignments were fully supported by UV and NMR spectral data.

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