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Herpes Simplex Virus Type 1 Helicase-primase: Inhibition by N^2-Phenylguanines, 2-Anilinoadenines and Related Compounds. G.E. Wright and J. Gambino, Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA,; J.J. Crute and I.R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA.

Herpes simplex virus type 1 (HSV-1) encodes a heterotrimeric helicase-primase comprised of the products of three of the seven DNA replication-specific genes. The critical role this enzyme performs in viral DNA synthesis suggests that the enzyme may be a novel target for the development of antiviral drugs. We tested several N^2-phenylguanines and 2-anilinoadenines as potential inhibitors in assays measuring the intrinsic DNA-dependent NTPase activity of the HSV-1 helicase-primase. Several dihalo compounds, specifically 3,4- and 3,5-dichloro, 3,4-difluoro and 3-chloro-4-fluorophenyl derivatives, were strongly inhibitory in this assay, but alkyl and unsubstituted analogs were weak or inactive. Mechanistic studies revealed that 1) dose-response curves for inhibition of both DNA-dependent ATPase and GTPase activities were identical, and 2) inhibition was mixed or non-competitive with respect to NTP or DNA cofactor, respectively. These results suggest that the inhibitors bind an allosteric effector site on the enzyme. The primase activity of the enzyme was strongly inhibited by 3,4- and 3,5-dichloroanilino derivatives of adenine and 2-aminopyrimidines. Certain compounds were cytotoxic to mammalian cells in culture, suggesting that specificity of the inhibitors needs to be improved before antiviral compounds employing helicase and/or primase inhibition can be developed.

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Inhibitors of Herpes Simplex Thymidine Kinase Suppress Reactivation of Latent Infection *in vitro*.

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Considerable evidence indicate that mutants of herpes simplex virus (HSV) deleted in the thymidine kinase (TK) gene, though replication competent *in vitro* are attenuated *in vivo*. Furthermore, a specific requirement for the viral coded TK has been advocated for reactivation of virus from latency. This has led to the design of inhibitors of HSV TK as a potential approach to suppress reactivation of latent HSV infection. As a result a number of derivatives of 5-ethyl-2'-deoxyuridine with potent *in vitro* activity against partially purified HSV-1 and HSV-2 TK have been identified. The compounds are competitive with respect to thymidine and display marked selectivity for viral TK with respect to its cellular homologue. *In vitro*, several compounds inhibit the reactivation of HSV-2 from explant cultures of latently infected murine dorsal root ganglia. The mechanism of suppression appears to occur via specific inhibition of HSV TK, since it can be overcome by competition with thymidine. These results demonstrate a potential chemotherapeutic strategy for controlling recrudescent HSV infection.