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Inhibitors of Cellular Cyclin-Dependent Kinases as Antivirals.
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In a search for cellular proteins required for HSV replication, two inhibitors of cyclin-dependent kinases (cdks), Roscovitine (Rosco) and Olomoucine (Olo), were shown to inhibit HSV replication efficiently. Since Rosco and Olo target cellular proteins, it was not surprising that efforts to isolate drug-resistant mutant viruses were unsuccessful. In contrast, mutants resistant to PAA, which targets the HSV encoded DNA polymerase, were readily isolated in parallel experiments. HSV replication was shown to be inhibited by Rosco at multiple stages including the transcription of IE genes, the transcription of E genes (even in the presence of IE proteins), and synthesis of viral DNA (even in the presence of E viral DNA replication proteins). Thus, cdk inhibitors are the only drugs known to block expression of all HSV proteins/antigens, including cytotoxic IE proteins. Notably, Rosco was also shown to inhibit explant-induced reactivation of HSV from neuronal latency at a point *prior* to the detection of viral proteins. Because the explant process per se was shown to induce expression of Rosco-sensitive cdks, we postulate that inhibition of the activities of these cdks blocks reactivation before viral gene functions are activated. Based on 1) the ability of Rosco and Olo to inhibit HSV replication at multiple stages, 2) our failure to isolate drug-resistant mutants, 3) the ability of these inhibitors to block all viral protein/antigen expression, 4) the inhibition of reactivation from latency by Rosco prior to the expression of detectable viral proteins, and 5) the known lack of toxicity of cdk inhibitors in vivo, we propose that cdk inhibitors may be useful antiviral drugs. If so, a major advantage of their use includes the fact that multiple cellular proteins activated by cdks are (presumably) required for viral replication, such that mutations in the viral genome would not result in resistance to these drugs.

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Anatomical Distribution of HSV-1 During Antiviral Chemotherapy.
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Previously, we compared valaciclovir (VACV) and famciclovir (FCV) in the murine ear pinna infection model and showed little difference between their effects on infectious HSV in the nervous system. When tested several months later, however, FCV appeared to markedly reduce the number of LAT expressing neurons in the trigeminal ganglia (TG) and, to a lesser extent, cervical ganglia (CIII). The present experiment studied the effects of therapy on anatomical distribution of HSV in the nervous system during acute infection. A different model was used (neck scarification); infected neurons were detected using a recombinant HSV with β-gal under the IE110 promoter (Lachmann et al, 1999). Drugs were provided in drinking water from 1-9 days p.i. The results were clear-cut and revealed several interesting findings: left and right CIII were colonised 1-2 days earlier than TG. Neither compound prevented or delayed the anatomical distribution of HSV to ganglionic neurons including contralateral ganglia. However, both drugs reduced HSV $^{+ve}$ cells by approx. 70% or 30% for FCV or VACV respectively. There was a transient recurrence of blue neurons, two days after VACV therapy. At 10 months p.i., the number of LAT^{+ve} neurons was reduced in mice that had been treated and notably, a few neurons continued to express β-gal, including those mice that had been treated. We conclude that FCV or VACV given *ad lib* in water, suppress virus replication in neurons and reduce latent foci but neither compound blocks the dissemination of HSV through the nervous system during therapy nor completely prevents the establishment of latency.

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Novel bicyclic fluorescent nucleosides as potent and selective inhibitors of VZV.
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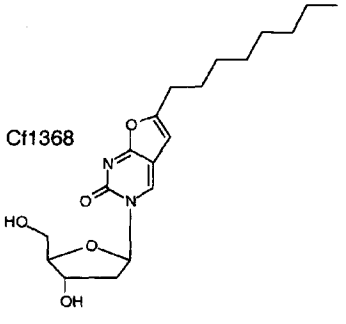
We report the discovery of an entirely new category of potent antiviral agents based on novel deoxynucleoside analogues with unusual bicyclic, fluorescent base moieties. Target structures, previously known as by-products in Pd catalysed coupling of terminal alkynes with 5-iodo-nucleosides, are recognised herein for the first time to be potent and selective inhibitors of varicella-zoster virus (VZV) in vitro. As an unusual structure activity relationship we noted the absolute requirement of a long alkyl side-chain, with an optimum length of C8-C10, for antiviral activity. We report the synthesis and characterisation of a series of chain-modified analogues, and their extensive in vitro evaluation. The lead compounds have a ca. 300-fold enhancement in anti-VZV activity over the reference compound acyclovir, and with no detectable in vitro cytotoxicity. In the presentation we will describe the discovery, synthesis, in vitro profile, and SAR of these novel agents.



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Surveillance for Resistant Herpes Simplex Virus Type 1 (HSV-1): a Collaborative Survey in the US of the General Population with Recurrent Herpes Labialis.
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Background. Penciclovir (PCV) and acyclovir (ACV) and their respective oral prodrugs are widely used to treat herpesvirus infections. PCV is approved in the US as a topical treatment for recurrent herpes labialis (RHL), and both ACV and PCV are available over the counter (OTC) in some countries for RHL. The survey focuses on the prevalence of resistant HSV-1 in subjects with RHL and was undertaken to prepare for Rx to OTC switch in the US. Previous surveillance has generally focused on genital herpes isolates, and shown that the prevalence of resistant HSV in the immunocompetent population remains low and stable. **Methods.** The study was pharmacy-based in 8 cities across the US. Subjects with an episode of RHL, which had not been treated with topical antiviral, were recruited from the general population. Histories of HSV disease and antiviral treatment were collected. Lesions were swabbed and specimens shipped to the testing center for virus isolation and typing. Susceptibilities to PCV and ACV were determined by the plaque reduction assay in two different cell lines, MRC-5 and Vero, respectively, to be compatible with institutional databases. Cell type affects the absolute IC_{50} values for these two drugs. **Results.** Between Sept '98 and March '99, 1803 subjects with RHL were sampled and 1088 HSV-1 isolates obtained (isolation rate = 60.3%). No isolate was scored as resistant to penciclovir. Two isolates had elevated IC_{50}s against ACV (2/1003 = 0.2%; IC_{50} = 3.2 and 2.4 ug/ml respectively) and will be investigated further. **Conclusions.** The prevalence of ACV^R and PCV^R HSV-1 is very low and consistent with previous surveys for ACV^R HSV in immunocompetent patients with genital herpes, which indicate no change in resistance patterns over the past 20 years. The data provide a baseline to monitor for any change in prevalence of resistant virus in subjects with RHL. Despite increasing antiviral pressure, resistant HSV-1 in the general population is very rare.