virus is due to inhibition of viral DNA synthesis. Interestingly, both vidarabine and prodrugs were more active against vaccinia ($IC_{50}$'s = 2.5–6 μM) than cowpox ($IC_{50}$'s = 8–54 μM). Both the D- and L-val prodrugs were stable at three pH's but D-val was more stable in intestinal and liver homogenates and in plasma. These properties make the D-amino acid prodrugs good candidates for further study as orally bioavailable anti-pox virus agents.

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## Antiviral Activity of (−)-D-Carbocyclic Cytosine (Carbodine) Against Avian Influenza Virus (H5N1)

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Natural and synthetic carbocyclic nucleosides are well known for their interesting biological properties, including antitumor as well as antiviral activities against a wide variety of RNA and DNA viruses. The carbocyclic analogue of cytosine (carbodine) was previously prepared as a racemic mixture and has been shown to possess inhibitory activity against human influenza type-A virus, measles, vesicular stomatitis virus and herpes simplex viruses. These interesting biological properties of carbodine, prompted us to develop an efficient synthetic method for the enantiomerically pure (−)-D-carbodine for further antiviral evaluations. Herein, we report the antiviral activity of carbodine against various strains of avian influenza virus (H5N1). Anti-influenza activity of (−)-D-carbodine was evaluated in comparison to its (+)-L- as well as (+)-DL analog against Duck, Gull, Hong Kong/2003 and Vietnam/2004 (H5N1) strains in vitro, and the results indicate that (−)-D-carbodine demonstrated potent antiviral activity with EC50 values of 0.57, 0.27, 0.18 and 0.18 μM, respectively, while the (+)-L-analog was inactive. Further biological and biochemical studies of (−)-D-carbodine as a potential antiviral agent for H5N1 virus are warranted.

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## Application of the Phosphoramidate Protide Approach to the Antiviral Drug Ribavirin

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Ribavirin is a nucleoside analogue with antiviral activity against a number of DNA and RNA viruses in vitro and in vivo (Sidwell et al., 1972). Ribavirin has been approved for the treatment of respiratory syncytial virus and for the treatment of hepatitis C virus in association with interferon. The bioactivation of ribavirin involves intracellular phosphorylation to its 5′-monophosphate mediated by adenosine kinase followed by further phosphorylation to the di- and tri-phosphate. Its broad spectrum of antiviral activity prompted us to design and synthesise a new series of ribavirin phosphoramidates in order to investigate its activity against different viruses. The synthesis and the biological evaluation for these compounds will be reported.

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## Design, Synthesis and Biological Evaluation of Novel Acyclovir ProTides

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Acyclovir and its prodrug valacyclovir are currently the treatments of choice for herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Intracellular conversion of acyclovir to its active triphosphate form is severely limited by the first phosphorylation step, which is carried out by a herpes virus encoded thymidine kinase (Elion et al., 1977). Further conversions to the di- and triphosphate are mediated by cellular guanosine monophosphate kinase and nucleoside diphosphate kinase respectively. Importantly, the activation of the compound by the viral nucleoside kinase is a target for drug resistance in both HSV and VZV strains (Larder et al., 1983). Our phosphoramidate ProTide approach was applied to acyclovir as a means to bypass the limiting step of its activation. However, no signifi-

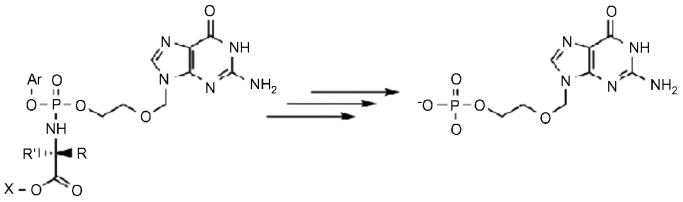

Fig. 1. Bioactivation of acyclovir ProTides.

cant improvement in antiviral activity was observed (McGuigan et al., 2000). In the present work, a new series of optimised acyclovir ProTides with an enhanced biological profile is reported (Fig. 1).

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## 103
## Vidarabine Prodrugs as Potential Inhibitors of Adenosine Deaminase

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Vidarabine (9-(β-D-arabinofuranosyl)adenine or ara-A) is active against herpes simplex virus (HSV) types 1 and 2, cytomegalovirus, and chronic hepatitis B virus (HBV) infections through inhibition of viral DNA synthesis. Although supplanted by acyclovir and other analogs for most applications, vidarabine is still an alternative therapy for acyclovir-resistant HSV and varicella-zoster virus infections. We recently reported that vidarabine was three- to fivefold more active than cidofovir against cow pox and vaccinia viruses in plaque reduction assays [Antiviral Res. 70 (2006) A14]. However, despite the proven efficacy against a variety of viruses, vidarabine suffers some limitations. First and most importantly, it is readily metabolized by adenosine deaminase (ADA) to 9-(β-D-arabinofuranosyl)hypoxanthine (ara-H), which has very low antiviral activity. Secondly, as a nucleoside, vidarabine has low lipophilicity and thus has low intestinal membrane permeability. Finally, it is also poorly soluble in aqueous solutions, thereby limiting options for both parenteral and peroral formulations. To address these concerns, vidarabine 5′-amino acid prodrugs were synthesized and, when perfused intestinally, resulted in increased plasma concentrations. Since it was previously discovered that vidarabine 5′-valerate inhibits metabolism of ara-A to ara-H by ADA [Mol. Pharm. 14 (1978) 366], we confirmed this inhibition ($K_i = 9.5$ μM) and demonstrated that this prodrug was more active verses vaccinia virus in vitro. In contrast, unlike its fatty acid counterpart, the vidarabine 5′-amino acid prodrugs did not inhibit the metabolism of ara-A to ara-H. We conclude that despite certain advantages over vidarabine, the amino acid prodrugs do not inhibit the deamination of the active compound.

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## Megaribavirin Aerosol for the Treatment of Influenza A Virus Infections in Mice

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Clinical efficacy of ribavirin aerosol treatment in mice translated well to natural influenza infections in college students. While newer neuraminidase inhibitors have been developed to treat influenza A and B infections, emergence of drug resistance poses potential problems. Because of this resistance, combination drug treatments have been evaluated. Ribavirin alone or in combination would be an ideal antiviral since it inhibits both influenza A and B viruses and drug resistance has not occurred in the clinical setting. To make ribavirin aerosol treatment a quicker process, limited to once or twice daily treatments, and be more cost effective, a MegaRibavirin formulation (reservoir concentration, 100 mg of ribavirin/mL) was developed that when used with the more efficient nebulizer, the Aerotech II nebulizer, was effective in preventing death in a lethal influenza A/HK/8/68 (H3N2) virus-mouse model. Aerosol generated with the MegaRibavirin formulation using the Aerotech II nebulizer flowing at 10 L of air/min produced aerosol droplets that contained 2.3 mg of ribavirin/L with a mass median aerodynamic diameter of 1.8 μm and a geometric standard deviation of 2.6. Thus, compared to the standard (20 mg/mL) or "high dose" (60 mg/mL) ribavirin concentrations used with the SPAG2-6000 nebulizer that has been used for both influenza and RSV infections, ribavirin aerosol generation was twice as efficient with the Aerotech II nebulizer while maintaining the same aerosol characteristics. Using this system for treatment, a single daily 30-min exposure on days 1–4 produced a survival rate of greater than 90% compared to 0% of animals without treatment. In addition, delaying the start of aerosol treatment for 48 or 72 h and treating just once daily for 30 min for only 2 days (days 2–3 and 3–4, respectively) still significantly increased the rate of survival and mean time to death. For the treatment of influenza in general and specially for pandemic avian influenza, the MegaRibavirin-Aerotech II method of aerosol treatment allows for short treatment periods (30 min once or twice daily), minimizes environmental issues and should