been attributed to hPepT1-mediated transport. In electrophysiology experiments on *Xenopus laevis* oocytes over-expressing hPepT1, we have not detected a significant signal with Val-Ser-OMe cHPMPC amino acid L/D stereoisomers, although stereoisomers having an L-configuration at the N-terminal amino acid potently inhibited Gly-Sar binding. Single side-chain ester-linked amino acid conjugates of cHPMPC have now been synthesized and investigated for transport and affinity in the oocyte model. An L-Val L-Val dipeptide analogue of acyclovir was also evaluated. The 'monopeptide' conjugates exhibited little or no hPepT1-mediated transport in the model, and had reduced affinity compared to dipeptide analogs. In some cases, TFA salts of the analogues produced weak positive signals in the model, whereas the HCl salts gave no signals, indicating the importance of using the latter form of the prodrug in these assays. The results suggest that one or more alternative mechanisms play a role *in vivo* to facilitate transport of the cHPMPC dipeptide prodrugs.

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## Compounds Designed to Bind Conserved Regions of Human Papillomavirus (HPV) DNA show Broad-spectrum Activity Against High-risk Genotypes

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Cervical infections by the "high-risk" human papillomaviruses (HPVs), including HPV16 and 18, are usually not treated upon their discovery, but are flagged for later "follow-up." Traditional approaches to antiviral design for HPV have failed for a variety of reasons including the lack of traditional antiviral targets. Therefore, novel antivirals designed to specifically reduce viral persistence are needed. A series of pyrrole-imidazole polyamides was optimized via medicinal chemistry based on an original lead compound designed against a sequence within the ori of HPV16. A set of improved polyamides was prepared, including compounds that potently reduced both HPV16 and HPV31 copy number (compared with vehicle-control) in cells maintaining these genomes as episomes. Keratinocytes maintaining either HPV16 or HPV31 episomes were treated with increasing concentrations of polyamide or vehicle-control for 48 h in order to study dose–response behavior. Loss of episomal DNA was measured by Q-PCR. Of the 46 polyamides tested, including 16 control polyamides not derived from our core lead structure, 12 gave pseudo-IC50s 200 nM against both genotypes, while 4 reduced HPV16 and HPV31 episomal DNA copy number to undetectable levels. Southern blot analysis confirmed these decreases. Broad-spectrum activity is likely achieved due to high conservation in A-T rich regions among high-risk HPV genotypes and the binding degeneracy of polyamides. Treatment of cells with a lead polyamide, followed by removal of compound and passage of cells, resulted in a moderate rebound of viral DNA that did not return to control levels after 6 additional days in culture. Extension of the polyamide treatment period resulted in a remarkably effective delay and inhibition of episomal DNA rebound. These results illustrate that targeting of the HPV ori with polyamides has the potential for potent and long-lasting effects on HPV DNA load.

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## Synthesis and Biological Studies of Mutagenic Ribonucleoside Analogues as Potential Inducers of *Error Catastrophe* of Riboviruses

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The development of effective treatments against riboviruses (causing many human diseases, i.e. common cold, haemorrhagic fever, AIDS, Hepatitis C, and SARS) is hampered by their ability to rapidly adapt by mutation and to acquire resistance to antiviral drugs. Riboviruses exhibited an extremely high mutation frequency, and this suggests that the viral population exists near the threshold for viral viability. Maintaining such a high mutation frequency is dangerous for the virus. An increase in mutation could result in a lethal increase in the already high proportion of defective viruses.

An antiviral strategy called *lethal mutagenesis* attempts to exploit this high mutation frequency by increasing the mutation rate even further and driving the virus population into "error catastrophe" (lethal accumulation of errors). This new strategy was validated with the demonstration that virus extinction can be achieved with the mutagenic nucleoside analogue ribavirin. Therefore, RNA virus mutagens may represent a promising new class of antiviral drugs.

We describe here the synthesis and biological studies of potential mutagenic ribonucleosides that may be incorporated into the viral genome during replication and, by mispairing, induce lethal mutagenesis. These ribonucleosides bear universal bases with ambiguous hydrogen bonding properties. We have documented various degrees of inhibition of the replication of foot-and-mouth disease virus (FMDV), encephalomyocarditis virus and lymphocytic choriomeningitis virus in BHK-21 cells by several base and ribonucleoside analogues.

VPg uridylylation (initiation of FMDV RNA synthesis) is inhibited by halogenated pyrimidine-triphosphates. The inhibitory activities cannot be accounted for by the toxicity of the drugs on BHK-21 cells. We are currently carrying out experiments to identify the steps in the life cycle of these viruses that may be affected by the drugs.

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## Antiflogistics as Viral Inhibitors

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Virus infection of cells induces cytokines production. Inflammatory cytokines increase a permeability of cell membranes and promote virus penetration. Antiflogistics hamper a synthesis of proinflammatory interleukines and therefore may show antiviral properties. (Iso)nicotinic acids derivatives such structure: $C_5H_4N$–3(4)-$CONH(CH_2)_m$–$(NHCO)_n$–Ar, where $m$, $n = 0$ or 1 are known as antiflogistics. In this series we found that compounds display antiviral activity against several viruses if they inhibit carrageneen oedema not less than 30%. Weak antiflogistics does not

possess antiviral activity. Two active antiflogistics are not virus inhibitors. It may be supposed antiviral activity of antiflogistics in part is due to an influence at the virus penetration. It is affirmed indirectly by nonspecific antiviral activity of compounds mentioned above. 1-Methyl-4-(N-benzylcarbamido)-pyridinium iodide (amizon) is effective in treatment of herpes, influenza, and measles. Three amizon analogs are active against several viruses as well.

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## Synthesis, Antiviral Activity, and Cytotoxicity of Some Novel 2-Phenyl-3-disubstituted Quinazolin-4(3H)-ones

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Quinazoline is the versatile lead molecule for designing potential antiviral agents. Its derivatives have been reported to possess broad spectrum antiviral activity. In the current study, 2-phenylbenzoxazin-4-ones was condensed with selected primary amines to form the corresponding 2,3-disubstituted quinazolin-4(3H)-ones. Their chemical structure was elucidated by means of spectral (FT-IR, $^1$H-NMR, MS) and elemental analysis. Antiviral activity of the compounds was evaluated in plaque assays using herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HMCV), vaccinia virus (VV) and cowpox virus (CPV). Cytotoxicity was determined in stationary human foreskin fibroblasts (HFF) and logarithmically growing KB cells. Compounds AA-1, AA-2, DBR-2, and MBR-2 had modest activity ($IC_{50}$ = 20–60 μM) against VV and/or CPV, similar to cidofovir. Little cytotoxicity was observed at concentrations up to 100 μM except for MBR-2. It was cytotoxic to both HFF and KB cells at 30–40 μM thereby implying that antiviral activity was a manifestation of cytotoxicity. Similar conclusions were reached for activity of MBR-2 against HSV-1 and HCMV. The other seven compounds had modest activity against HSV-1 (25–40 μM) but with some cytotoxicity noted at 100 μM. In contrast, in an experiment using a low multiplicity of infection with HSV-1, DBR-1 and MBR-1 were active in a low micromolar range suggesting the possibility of specific antiviral activity.

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## Expression of Infectious Bursal Disease Virus (IBDV) Polyprotein and VP4 Protease

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Infectious bursal disease virus consists of a bisegmented double-stranded RNA and infects the B-cells in the bursa of Fabricius of young chickens. It causes Gumboro disease, that is of considerable importance in the poultry industry. Although vaccines are available, their use is compromised by maternally derived antibodies and the emergence of very virulent strains. A promising approach is to develop anti-viral compounds that target the virus-encoded VP4 protease, which autocatalytically cleaves a 110 kDa viral polyprotein ($NH_2$-$pVP_2$-$VP_4$-$VP_3$-COOH) into pVP2, VP4 and VP3. VP2 and VP3 are necessary for the appropriate assembly of the infectious viral particles. It was initially suggested that VP4 cleaves between $Arg^{458}$-$Arg^{459}$ and $Lys^{722}$-$Arg^{723}$ but site-directed mutagenesis later identified $Ala^{512}$-$Ala^{513}$ and $Ala^{755}$-$Ala^{756}$ as the cleavage sites and VP4 ($Ala^{513}$-$Ala^{755}$) as the mature protease. We propose that this VP4 form is a product of the autocatalytic activity of the integral VP4 and that it is not the mature protease. The aim of the study was to determine the sequence required for the autocatalytic activity of VP4 and hence the sequence of the mature VP4. Thus constructs for the expression of full-length ($Met^1$-$Glu^{1012}$), truncated ($Ile^{227}$-$Trp^{891}$) polyprotein and three forms of VP4 with an alternative N- or C-terminus, namely VP4-RA ($Arg^{459}$-$Ala^{752}$), VP4-RK ($Arg^{459}$-$Lys^{722}$) and VP4-AA ($Ala^{513}$-$Ala^{755}$) were prepared. We also prepared anti-peptide antibodies against VP4-RK and VP4-AA. We report here on the expression of these various constructs in pGEX-4T-1 and pET 32a and their detection with anti-peptide antibodies towards elucidation of the sequence of the mature VP4.

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## Synthesis and SAR of 9-Arylpurines as Novel Inhibitors of Enterovirus Replication

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Enteroviruses cause often mild and self-limiting infections, but may also be involved in more serious conditions, which can be life-threatening, such as pancreatitis, meningitis, encephalitis or myocarditis. There are no drugs approved for the treatment of enterovirus infections (De Palma et al., 2008). Here we report on a novel class of enterovirus inhibitors that structurally can be described as 9-arylpurines. Interestingly, scarce examples of such kind of chemical structures are reported in the literature. Two synthetic strategies were used to obtain these compounds: (i) the coupling reaction between arylboronic acids and purines catalyzed by copper salts and (ii) a two-step classical protocol based on the reaction of chloropyrimidines with anilines followed by cyclization. For this second approach, we have set up a new microwave-assisted procedure that has significantly reduced the reaction time, therefore allowing the synthesis of a considerable number of compounds in a short period of time (Aguado et al., submitted for publication). The most selective compounds in this series inhibited viral (Coxsackie B Virus 3) replication with $EC_{50}$ values in the range 4–8 μM, and $EC_{90}$ values around 7–10 μM. $CC_{50}$ values were >250 μM. The most potent compounds in this series were shown to inhibit a selection of enterovirus but lacked activity against polio and rhinoviruses replication. This family of compounds is characterized by its simplicity in structure, synthetic accessibility and selective inhibitory activity against various enteroviruses replication.

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