and (*R*)-PMP-DAP, ANPs lacking a hydroxymethylene functionality. The new (*S*)-HPMPC and (*S*)-HPMPA prodrugs have IC_{50} values *vs* cowpox virus, vaccinia virus, HCMV and HSV-1 similar to or better than that of the parent drugs while exhibiting low cytotoxicity, good stability characteristics and efficient metabolic conversion pathways. *In vitro* antiviral evaluation of PMEA and (*R*)-PMP-DAP derivatives will be presented in comparison. The effect on pharmacokinetic properties and antiviral potency of phosphonate ester stereochemistry in diastereomeric prodrugs has been an intriguing question. To address it, a convenient synthetic procedure allowing preparation of the individual diastereomers was elaborated, allowing their absolute configurations to be unambiguously established based on X-ray diffractometry. *In vitro* antiviral and *in vivo* transport evaluation (murine model) of the individual diastereomers of L-Tyr-NH-*i*Bu cHPMPA were performed. The (S_p)-diastereomer demonstrated significant enhancement of prodrug oral bioavailability over the parent (*S*)-HPMPA (39% *vs* <5%). SAR studies exploring the effect of structural modifications in the amido tyrosine promoiety (C-terminal amide alkyl groups, amino acid stereochemistry) on *in vitro* antiviral activity, lipophilicity and solubility will also be presented.

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17

Cyclopropavir Inhibits the Normal Function of the Human Cytomegalovirus UL97 Kinase

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Cyclopropavir (CPV, MBX400) is active against human cytomegalovirus (CMV) as well as both variants of human herpes virus 6 and human herpesvirus 8. The mechanism of action of CPV against CMV is similar to that of ganciclovir (GCV) in that it is phosphorylated initially by the CMV UL97 kinase and the triphosphate metabolite is thought to inhibit the viral polymerase. Resistance to CPV maps to the UL97 kinase, but is associated primarily with H520Q mutations and thus retains good antiviral activity against most GCV-resistant isolates. An examination of infected cultures treated with CPV revealed an unusual cell morphology associated with the absence of UL97 kinase activity. A surrogate assay for UL97 kinase activity confirmed that CPV inhibited UL97 kinase activity and its activity was similar to that of maribavir (MBV) in this assay. Deep sequencing of a CPV-resistant laboratory resistant isolate confirmed the H520Q mutations associated with resistance. In a subpopulation of viral genomes, a mutation in the active site of the UL97 kinase was also identified (V356G). This mutation is located near the active site of the enzyme and is in the same region as those that confer resistance to MBV. Since MBV inhibits the UL97 kinase, it is thought to reduce the activation of GCV and has been reported to antagonize its antiviral activity. Combination studies using real time PCR confirmed these results and indicated that CPV exhibited a similar level of antagonism against GCV. We conclude that the mechanism of action of CPV against CMV is complex and involves both the inhibition of DNA synthesis as well as the inhibition of the normal activity of the UL97 kinase.

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18

Structural and functional characterization of human cytomegalovirus terminase leads to a new antiviral target

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Human cytomegalovirus (HCMV) is one of the eight human herpesviruses. HCMV infection in the healthy host is usually silent but it can have serious consequences in immunocompromised individuals. Current anti-HCMV drugs (ganciclovir, foscarnet and cidofovir), which inhibit the viral DNA polymerase, have considerable side effects and some patients develop resistance to them. New antiviral compounds targeting other viral proteins may overcome these drawbacks.

HCMV replicates its DNA via concatamers, a long molecule of DNA with several copies of the genome, which has to be cut in single genome units. This packaging process is facilitated by the terminase complex, which is composed by main protein subunits UL89 and UL56. Viral encapsidation has no counterpart in mammalian cells, thus implying that the proteins involved might be selective antiviral targets.

Here we used the high-throughput screening method ESPRIT to identify a soluble domain of UL89 from a library of 18,432 randomly truncated constructs. The soluble domain, called UL89-C, corresponds to the C-terminus domain of UL89. UL89-C was purified and crystallized and its three-dimensional structure was solved. The structure showed that UL89-C has the RNase H fold. Other proteins with a similar fold are the E. coli RuvC resolvase, the HIV and ASV integrases and some transposases. We demonstrated that UL89-C corresponds to the nuclease domain of the terminase and that its function is strongly dependent on Mn^{2+} ions.

We tested the effect of various HIV integrase inhibitors on the function of UL89-C. Raltegravir – one of the most recently approved drugs for the treatment of AIDS – inhibited the nuclease function at micromolar levels. Our study opens the way for the development of new inhibitors against herpesvirus.

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19

In Vivo Efficacy of N-methanocarbathymidine (N-MCT) against Herpes simplex Virus Type 2 in Neonatal Guinea Pigs

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The outcome of neonatal infections, even after therapy with high dose acyclovir (ACV), is not optimum. We therefore evaluated N-methanocarbathymidine [N-MCT], a nucleoside analogue with in vivo antiviral activity against herpesviruses and orthopoxviruses, in our guinea pig model of neonatal herpes as this model mimics many aspects of Herpes simplex virus type 2 (HSV-2) disease in newborn infants. Newborn guinea pigs were inoculated intranasally within 48 h of birth with 8.7×10^5 pfu of HSV-2, MS strain. Intraperitoneal treatment of ACV (60 mg/(kg day)) and N-