target which forms at 4 °C upon culture of cells and virus. Resistant viruses possess mutations in gp120 and gp41 consistent with the entry inhibition mechanism. However, the compounds do not block gp120-CD4 binding, chemokine receptor interactions, or the attachment of virus to target cells. They also do not inhibit SIV or SHIV-Env and do not inhibit entry or fusion in assays in which only isolated envelope glycoproteins and CD4 are involved (HL2/3 + MAGI cell fusion inhibition assay). Thus, inhibition of entry requires replication competent virus. Mutations in resistant viruses would suggest that gag and env proteins are included in the complex target recognized by the compounds. The contribution of gag proteins may also be responsible for the enhanced sensitivity of the pyrimidinediones (5–10-fold) to multi-drug resistant (MDR) viruses from experienced patients. The mechanistic results confirm that the pyrimidinediones represent a new and highly novel class of HIV inhibitors with an antiviral profile highly favorable for IND-directed investigation and clinical development.

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Development of the Dual-Acting Pyrimidinedione IQP0528 as a Vaginal Topical Anti-HIV Microbicide

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IQP0528 is a highly potent dual-acting HIV inhibitor targeting both reverse transcription and virus entry. The compound is non-toxic to all tested cell lines *in vitro*, including primary human cells, and the normal vaginal flora *Lactobacillus*. In standard *in vitro* assays, IQP0528 was active against all clinical strains of virus in the nanomolar to sub-nanomolar concentration range in PBMCs, dendritic cells and monocytes macrophages with therapeutic indices greater than one million. Equivalent or greater activity was observed when IQP0528 was evaluated in the presence of additives such as mucopolysaccharides or simulated vaginal and seminal fluids. The activity of the compound was not affected in cell-based entry assays mimicking the transition from low to neutral pH that occurs at the time of ejaculation. IQP0528 inhibited both cell-free and cell-associated virus transmission to CD4 expressing cells in virus transmission inhibition assays and was highly active in the microbicide transmission and sterilization assay (MTSA). IQP0528 was not active against several viral, bacterial or fungal STI-causing organisms. In preformulation studies, IQP0528 was soluble in a variety of solvents and was stable at ambient temperature and at pH's less than 8. Acute toxicology evaluations determined the compound to be non-toxic up to 1000 mg/(kg day) when dosed intravenously. Genotoxicology evaluations were all negative. IQP0528 is a novel candidate for a vaginal topical microbicide based on its dual mechanism of action, high level of potency, lack of toxicity, compatible formulation profile and toxicology profile. As a microbicide, IQP0528 would potentially inhibit two steps in virus replication which occur prior to reverse transcription and could be effectively used in combination with other microbicide products. IQP0528 is currently being formulated in an intravaginal ring for delivery of the compound.

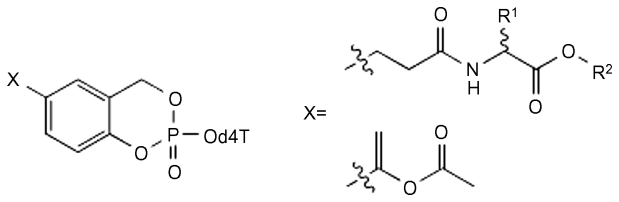

Fig. 1.

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Enzyme-triggered CycloSal-Pronucleotides

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CycloSal-nucleosylphosphate triesters are pronucleotides that deliver antiviral nucleotides into cells. The transport of these compounds across cellular membranes is basically achieved by passive diffusion, which is due to the high lipophilicity of the prodrug. As the release of the nucleotides is triggered by a pH-dependent hydrolysis cascade, cleavage may occur inside as well as outside cells. This unselective process may be influenced by attaching a trigger to the pronucleotide sensitive to esterases. The activated trigger is supposed to keep the prodrug inside the cell, leading to enrichment of the *cyclo*Sal-pronucleotide (trapping concept). To achieve this goal, different amino acid esters were linked to the aromatic ring of the *cyclo*Sal-masking unit via a carboxylic acid linker (see Fig. 1). In addition, enol esters were attached to the cycloSal-mask. In the presence of e.g. (carboxy)esterases, the aminoacyl or enol esters are transformed to the carboxylic acid or ketones, respectively, resulting in higher polarity, and thus decreasing ability of the prodrug to diffuse passively across membranes. In the case of the formed ketones, a fast hydrolysis reaction of the triesters released the nucleotide. The compounds were examined concerning their buffer stability, cell extract stability, cytotoxicity and antiviral activity in CEM/0 and TK-deficient cell lines.

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