Oral Session 3: Retroviruses

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A Strong Dominant Negative Mutation in the HIV-1 Gag Protein Defines a New Drug Target

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Small-molecule CCR5 Ligands that may Spare the CCR5 Function: Opportunity for New Antiviral Discovery?

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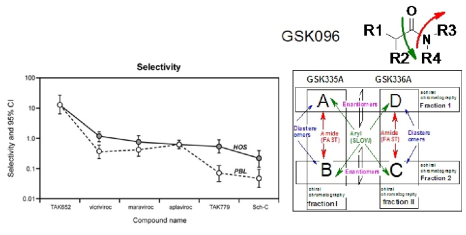
Discovery of novel antiviral drugs acting through host-cell receptors has been a subject of intense research, however such interactions may interfere with receptors' normal physiological functions, modulated by their endogeneous ligands, but now antagonized by the drug. Interactions of a host-cell receptor CCR5 and its endogeneous ligands CCL3L1, RANTES, MIP-1α and MIP-1β regulate a number of cellular events, such as chemotaxis and receptor internalization. CCR5 is also used by the M-tropic HIV to enter cells. Maraviroc and clinical small-molecules: TAK652, vicriviroc, aplaviroc, TAK779 and Sch-C utilize CCR5 to block HIV, however some undesirable consequences of blocking the CCR5 function have been proposed in the literature. We have recently discovered (Kenakin et al. Mol. Pharm. in press) that ratios (expressed in log scale as ordinates, Fig. 1A) of IC_{50} values for CCL3L1-induced CCR5 internalization in presence of inhibitors vs. their HIV inhibition in HOS cells (solid lines) and PBL (dotted lines) cover a surprisingly wide range from ∼10 to ∼0.1. In parallel, our internal medicinal chemistry programs discovered potent and bioavailable CCR5-based candidates for further development, GSK929 and in particular its analogue GSK096. Detailed chromatographic analysis of pure GSK096 unexpectedly revealed that it exists as a mixture of four atropisomers A, B, C and D, owing to restricted rotation around (CO)-aryl and (CO)–(NH) bonds, Fig. 1B. Chiral chromatography allowed to separate both slow-equilibrating (solution $t_{1/2}$ = 37 days at 37 °C) enantiomers GSK335 (diastereomers A + B) and GSK336 (diastereomers C + D). Surprisingly, in contrast to GSK096, GSK335 and other CCR5 compounds, GSK336 demonstrated a substantial separation of its antiviral and anti-chemotaxis activity (≫60-fold). We will detail the design, synthesis and SAR leading to the discovery of an allosteric ligand GSK336, which may "spare" some CCR5 function and thus has the potential for an improved safety profile.



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Development of Hexadecyloxypropyl Tenofovir (CMX157) for HIV: Potential for Use as a Microbicide and Therapeutic

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CMX157, a novel lipid conjugate of tenofovir (TFV), has been evaluated *in vitro* to determine its primary pharmacological effects as an antiviral agent; cytotoxicity, genotoxicity, and secondary pharmacological effects of CMX157 have also been determined. Additionally, 28-day repeat-dose toxicology studies were conducted in rodent and non-rodent species *in vivo*. *In vitro* CMX157 has activity against a wide range of wild-type and antiretroviral drug-resistant HIV viruses in different cell systems with potencies consistently >300-fold better than TFV, e.g., the CMX157 IC_{50} for M41L/L210W/T215Y mutants averaged 6.3 nM versus 2240 nM for TFV. The increase in potency is attributable to more intracellular active anabolite (TFV-diphosphate) as exemplified by the >30× higher levels observed in unifected human PBMCs incubated with 1 μM CMX157 versus 1 μM TFV (human C_{max} for TFV). CMX157 displayed no antagonism in combination with any approved antiretroviral and had an excellent cytoxicity profile. The dose-limiting toxicity in 28-day rat and cynomolgus monkey studies was gastric with NOAELs of 200 mg/(kg day)$^{-1}$ in both species. *In vitro* passaging of CMX157 and TFV in parallel showed no resistance-associated mutations emerging for CMX157 at later passages than those where TFV selected K65R. Of particular interest for use as a microbicide, CMX157 associated directly with HIV and subsequently reduced viral production in untreated target cells, suggesting virus exposed to CMX157 will carry the drug into diverse cellular and anatomical compartments, potentially including those currently considered privileged. These results suggest that CMX157 can be safely administered to humans at doses that are expected to effectively treat wild-type and antiretroviral resistant HIV, including strains that fail to respond to all approved nucleoside reverse transcriptase inhibitors. The combination of nanomolar potency, a high genetic barrier to resistance, and direct binding to HIV at levels affecting subsequent viral replication could make CMX157 a uniquely useful NRTI.

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Toward Unsymmetrical CADA Analogs as Novel Down-modulators of the CD4 Receptor

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Cyclotriazadisulfonamide (CADA) specifically down-modulates the CD4 receptor expression on the surface of lymphocytes and monocytes/macrophages. As CD4 is the primary receptor utilized by HIV for infection of its target cells, CADA also inhibits the entry of HIV into cells. Moreover, a strict correlation between the CD4 down-modulating and antiviral potencies of many CADA analogs has been described (Bell et al., 2006, J. Med. Chem., 49, 1291–1312). Structural modifications of CADA have been made to increase potency, reduce toxicity, and improve physical properties. However, unsymmetrical analogs having two different arenesulfonyl side-arms have not been fully explored. Based on initial molecular modeling stud-