Oral Session 3: Retroviruses

87

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

Ronald Swanstrom, Ph.D.

The University of North Carolina, Chapel Hill, NC, USA

doi:10.1016/j.antiviral.2009.02.092

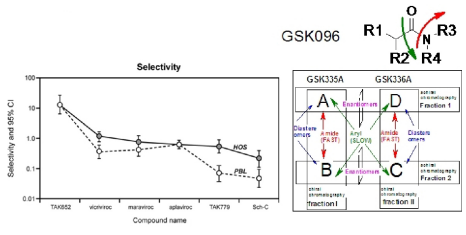
88

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

Wieslaw Kazmierski*, Vanessa Muniz-Medina, Susan Danehower, Stacey Jones, Terry Kenakin

GlaxoSmithKline, Research Triangle Park, USA

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.



doi:10.1016/j.antiviral.2009.02.093

89

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

Randall Lanier*, Bernhard Lampert, Lawrence Trost, Merrick Almond, George Painter

Chimerix Inc., Durham, USA

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.

doi:10.1016/j.antiviral.2009.02.094

90

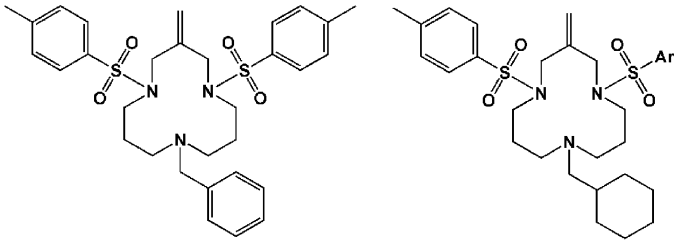
Toward Unsymmetrical CADA Analogs as Novel Down-modulators of the CD4 Receptor

Violeta Demillo[1,*], Sreenivasa Anugu[1], Kurt Vermeire[2], Dominique Schols[2], Thomas Bell[1]

[1] *Department of Chemistry, University of Nevada, Reno, USA;* [2] *Rega Institute for Medical Research, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium*

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-

ies and on the potencies of two known unsymmetrical CADA analogs, decreased symmetry may likely lead to improved activity of the compounds. To fully explore the potential of the unsymmetrical analogs as antiviral agents, a new synthetic route was developed towards their production. One of the synthetic modifications involves a new macrocyclization method using palladium as a catalyst. This technique avoids large solvent volumes, long reaction times, and polymer side products associated with the conventional, Richman–Atkins macrocyclization method. The anti-HIV and CD4 down-modulation activities of the novel CADA compounds will be presented.



CADA Target CADA analogs

doi:10.1016/j.antiviral.2009.02.095

91

Pradimicin-S is a Highly Soluble Non-peptidic Small-size Carbohydrate-binding Antibiotic that may Qualify as a Potential Drug Lead for HIV Treatment

J. Balzarini[1,*], K. François[1], K. Van Laethem[1], B. Hoorelbeke[1], J. Auwerx[1], S. Liekens[1], Y. Igarashi[2], T. Oki[3], D. Schols[1]

[1] *Rega Institute for Medical Research, K.U. Leuven, B-3000 Leuven, Belgium;* [2] *Biotechnology Research Center, Toyama Prefectural University, Toyama 939-0398, Japan;* [3] *Shoda, Sakae, Yokohama 247-0022, Japan*

Pradimicin-S (PRM-S) is a highly water-soluble negatively charged derivative of the antifungal antibiotic PRM-A in which the terminal xylose moiety has been replaced by 3-sulfated glucose. PRM-S does not prevent HIV adsorption, but inhibits virus entry into its target cells. It inhibits a wide variety of HIV-1 laboratory strains, HIV-1 clade isolates, HIV-2 and SIV in various cell cultures (50% effective concentration ranges in the lower micromolar range; 50% cytostatic concentration higher than 100 μM). It blocks syncytium formation between persistently HIV-1- and SIV-infected cells and uninfected T-lymphocytes, and prevents DC-SIGN-mediated HIV-1 and SIV capture and subsequent virus transmission to T-cells. Alike PRM-A, PRM-S strongly binds to gp120 in a Ca^{++}-dependent manner at a K_D in the lower micromolar range. Dose-escalating exposure of PRM-S to HIV-1-infected cells led to the isolation of mutant virus strains that had multiple deleted N-glycosylation sites in the envelope gp120. There was a strong preference for the deletion of high-mannose-type glycans. Genotypic resistance occurred slowly, and significant phenotypic resistance occurred only after the sequential appearance of more than 3–5 mutations in gp120, pointing to a relatively high genetic barrier of PRM-S. A variety of virus strains that are resistant to other anti-HIV drugs kept sensitivity to the inhibitory effects of PRM-S. The antibiotic is non-toxic against a variety of tumor cell lines, not mitogenic, not (anti)-angiogenic, and does not markedly trigger cytokines and chemokines in drug-exposed peripheral blood mononuclear cells. Therefore, PRM-S may qualify as a potential anti-HIV drug candidate for extended (pre)clinical studies.

doi:10.1016/j.antiviral.2009.02.096

92

Highly Potent and Dual-acting Pyrimidinedione Inhibitors of HIV-1 Possess a High Genetic Barrier to Resistance

Robert Buckheit*, Karen Watson, Tracy Hartman, Lu Yang

ImQuest BioSciences, Inc., Frederick, USA

With the increasing incidence of HIV drug-resistant viruses in the HIV-infected population, it is critical that a new generation of highly safe and potent drugs be developed to address this issue. Among a SAR series of 68 dual-acting pyrimidinedione compounds, a number were found to potently inhibit viruses with typical NNRT-resistance engendering mutations (Y181C, L100I, and K103N), suggesting that the molecules may interact with the RT in a manner resulting in a higher genetic barrier to resistance. The series of compounds are also highly active against multidrug-resistant viruses obtained from patients failing prolonged courses of RT and PI therapies. In order to further evaluate this hypothesis, viruses resistant to the antiviral effects of the lead compounds were selected in cell culture using both serial dose escalation and fixed dose resistance selection methods, as well as through the evaluation of the activity of the pyrimidinediones against biologically selected and site-directed viruses with defined NNRTI-resistance mutations. These studies confirmed that the pyrimidinediones required the complex accumulation of multiple mutations in the RT and Env in order to develop high level NNRTI resistance. Antiviral assays with drug resistant and multidrug-resistant viruses indicated that the compounds were able to effectively inhibit viruses with NNRTI-resistance mutations and exhibited enhanced sensitivity to multidrug-resistant viruses obtained from patients failing long courses of PI therapy as well as RT/PI therapy. Additional studies were performed with NNRTI-resistant viruses with the entire SAR series of molecules in an effort to define molecules with specific capability of inhibiting highly resistant viruses such as those with the Y181C, L100I, and K103N (alone and in combination) as well as with MDRs with resistance phenotypes/genotypes to RT inhibitors, PI inhibitors and both RT and PI inhibitors. These results would indicate that the pyrimidinediones possess a high genetic barrier to resistance based on both their dual mechanism of action as well as their low intrinsic level of resistance to individual RT amino changes.

doi:10.1016/j.antiviral.2009.02.097

93

Design, Synthesis and Anti-HIV-1 Evaluation of Novel Arylazolylthioacetanilides as Potent NNRTIS

Xinyong Liu[1,*], Peng Zhan[1], Christophe Pannecouque[2], Erik De Clercq[2,*]

[1] *Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012 Jinan, China;* [2] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, Leuven, Belgium*

Despite the demonstrated clinical efficacy of combination antiviral regimens using HIV-1 NNRTIs, the emergence of clinical resistance has become a key issue for this class of compounds and has become a major cause of treatment failure. Therefore, to search for the novel NNRTIs with potent and broad spectrum antiviral activity, as well as with safe and good pharmacokinetics profiles is urgently needed. Recently, from high-throughput screening (HTS) of compound libraries, several interesting sulfanyltriazole- and sulfanyltetrazole-type leads (A and B) were identified as novel HIV-1 NNRTIs, which have a simple, yet distinctively different chemical