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Anti-HIV-1 Activity of A3G is Enhanced by HSP70

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By an elucidation the action mechanism of a host factor to HIV-1, it is thought that it is applicable to the medical treatment of HIV-1. Our study focused on A3G which controls a virus duplicate to negative. A3G has cytidine deaminase activities that modify RNA or DNA. A3G exhibit varying degrees of inhibitory activity against HIV. In the absence of the Vif, A3G are packaged into HIV-1 particles through an interaction with Gag protein molecules and the help of cellular 7SL RNA and/or viral genomic RNA. Virion-packaged A3G mediates cytidine deamination in the viral minus-strand DNA during new target cell infection. Virion-packaged A3G can also reduce the accumulation of viral DNA by inhibiting reverse transcription processes or inducing viral DNA degradation. HIV-1 Vif suppresses the activity of A3G by assembling a viral-specific E3 ubiquitin ligase through its interaction with cellular Cullin5-ElonginB-ElonginC. Vif induces polyubiquitination of A3G and tags them for proteasome-mediated degradation. We thought that heat shock protein 70 (Hsp70) would have the action which protects A3G. Hsp70 family members facilitate assembly and disassembly of oligomeric protein complexes as well as their folding and intracellular transport. Hsp70 as a constituent of the HIV-1 virion and shown that the HIV-1 Gag polyprotein is sufficient for Hsp70 incorporation. There, it studies for the purpose of the elucidation the action of Hsp70 to the anti-HIV-1 activity of A3G. As a result, it was shown clearly that Hsp70 and A3G, and HIV-1 Gag are observed localization interaction with cytoplasm. Further, the amount of A3G incorporation into HIV-1 virions increased when Hsp70 was overexpressed. Thereby, the infectivity of HIV-1 declined. Showing possibility that over expression of Hsp70 could lead to inhibition of HIV-1 and we can suggest the possibility of HIV-1 therapy.

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Immediate and Persistent Anti-HIV-1 Activity of the Biguanide-based Compound NB325 Involves Specific Interactions with the Viral Co-receptor CXCR4

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Experiments designed to determine the mechanism by which NB325 (also known as polyethylene hexamethylene biguanide or PEHMB) inhibited HIV-1 infection suggested the involvement of cell surface molecules that participate in HIV-1 attachment and entry. In experiments involving flow cytometry, antibodies with distinct epitope specificities were used to detect CXCR4 at the cell surface and internalization induced by NB325 exposure. Competitive binding assays with the CXCR4 ligand, CXCL12, and peptides derived from extracellular domains of CXCR4 were used to examine interaction specificity. Inhibition of CXCR4-induced chemotaxis was also assessed. NB325 altered the detection of CXCR4 on primary human CD45RO+CD4+ T lymphocytes in an epitope-specific manner. These results were consistent with the lack of inhibition of CXCL12 binding, potent inhibition of CXCL12-induced chemotaxis, and the lack of CXCR4 internalization after NB325 exposure. Peptide competition experiments supported the hypothesis that NB325 interacts with CXCR4 extracellular loop 2 (ECL2). Additional mechanistic experiments were performed to support the observation that NB325 provides antiviral memory, which persistently protects cells from subsequent HIV-1 infection. Experiments demonstrated decreased detection of CXCR4 ECL2 up to 24 h after removal of NB325 from the media, as well as reductions in CXCL12-induced chemotaxis after pre-exposure of cells to NB325. These results indicate that NB325 inhibits X4 HIV-1 infection and provides persistent protection from infection by a direct and specific interaction with CXCR4. These results are being used to guide studies designed to reveal the specific mechanism of action by which NB325 inhibits HIV-1 infection through the co-receptor molecule CCR5.

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From β-Amino-γ-Sultone to New Bicyclic Pyridine and Pyrazine Heterocyclic Systems: Discovery of a Novel Class of HIV-1 Non-nucleoside Inhibitors

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Sultones are versatile heterocyclic intermediates whose chemistry, industrial applications and biological properties continue to be of interest. Even though recent chemical literature on saturated γ-sultones has been described (Enders and Iffland, 2007), research on the chemistry of α,β-unsaturated-γ-sultones (Braverman et al., 2007), and particularly of β-amino-substituted representatives of this family, is much less intensive. We recently described (de Castro et al., 2008) the reactivity of the 4-amino-5*H*-1,2-oxathiole-2,2-dioxide (β-amino-γ-sultone) heterocyclic system towards electrophiles and amines on readily available model substrates. One of the interesting features of the system is their ambident nucleophilicity; nucleophilic reaction can take place either at the site of the enaminic carbon (C-3) or at the primary amino nitrogen depending on the nature of the electrophile and the reaction conditions (de Castro et al., 2008). We now explore the synthetic usefulness of this ambident nucleophile for the preparation of unusual fused nitrogen heterocyclic systems containing a γ-sultone moiety. In spite of the poor nucleophilicity of the amino group, which is considered to have an "amide like" character, the β-amino-γ-sultone system reacts with a variety of bis-electrophilic reagents to give previously unknown pyridine- and pyrazine-based bicyclic heterocyclic systems. The synthetic approaches for the preparation of these novel heterocycles and their biological evaluation against a broad panel of viruses in cell culture will be described. Interestingly, some substituted pyrido fused compounds showed a significant activity against HIV-1 infection, being inactive against a variety of other DNA and RNA viruses. The new "hit" compounds are an excellent starting point for the exploration of a novel class of HIV-1 non-nucleoside inhibitors.

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A Small Llama Antibody Fragment Efficiently Inhibits the HIV Rev Multimerization *In Vitro*

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The human immunodeficiency virus type 1 Rev protein is essential for the expression of single spliced and unspliced HIV mRNA, encoding for the structural proteins of the virus. In order to do so, Rev binds as a multimeric complex to the Rev responsive element (RRE)-containing mRNA and transports it from the nucleus to the cytoplasm exploiting the CRM1-mediated cellular machinery. An important aspect of the Rev function is its requirement for multimerization. We have used a unique strategy to identify a multimerization inhibitor of Rev based on the isolation of llama single-domain antibodies. The *Camelidae*, besides containing conventional antibodies consisting of heterodimers of a heavy and a light-chain, also contain heavy-chain antibodies that are homodimers of heavy-chain only. Therefore, single-domain antigen-binding fragments (VHHs) can be easily generated from the variable domain of these heavy-chain antibodies. These VHHs, also called nanobodies, are minimally sized, highly soluble entities that bind the antigen with nanomolar affinity. Our strategy consisted of producing single-domain nanobodies against HIV Rev by immunizing a llama with recombinant Rev protein. Using a FRET-based multimerization assay we discovered a nanobody that efficiently inhibits the Rev oligomerization *in vitro*. Our results suggest that the oligomeric assembly of Rev may represent a new approach to the chemotherapy of HIV.

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Study of the Retention of Metabolites of 4′-ED4T, A Novel Anti-HIV-1 Thymidine Analog, in Cells

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2′,3′-Didehydro-3′-deoxy-4′-ethynylthymidine (4′-Ed4T), a novel thymidine analog, has potent anti-human immunodeficiency virus type 1 (HIV-1) activity than its progenitor stavudine (D4T). The profile of intracellular 4′-Ed4T metabolites was qualitatively similar to that of zidovudine (AZT) but not to that of D4T, while it showed more persistent anti-HIV activity after drug removal than AZT or D4T in cell culture. When the CEM T cells were exposed to higher concentrations (2, 5 and 10 μM) of 4′-Ed4T, the amounts of major metabolite 4′-Ed4TMP increased proportionately. Furthermore, the higher amount of intracellular metabolites, especially 4′-Ed4TMP, brought about much longer retention of 4′-Ed4TTP after drug removal. We further investigated the efflux profiles of 4′-Ed4T in the comparison with AZT in CEM cells. After drug removal, both 4′-Ed4T and AZT were efflux from the cells in a time and temperature-dependent fashion. 4′-Ed4T was efflux from cells in its nucleoside form, while AZT was efflux from cells in its nucleoside and monophosphate (MP) form. The efflux of 4′-Ed4T from cells was much less efficient than that of AZT and kept higher amount of intracellular 4′-Ed4TMP than AZTMP. Dipyridamole could inhibit the efflux of AZT but not 4′-Ed4T in a dose dependent manner. The mechanism study showed that dipyridamole-dependent efflux of AZT nucleoside might due to an unknown transporter which was not related to the equilibrative nucleoside transporters. The effect of dipyridamole on AZTMP efflux might come from the inhibition of multidrug resistance protein 4 (MRP4). Those results demonstrated that less efficient efflux of 4′-Ed4T might be one of the biochemical determinants for its persistent antiviral activity in the cell culture.

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A High-content Screening Approach to Identify Compounds that Interfere with the Formation of the Hepatitis C Virus Replication Complex

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The hepatitis C virus (HCV) subgenomic replicon is routinely used in large screening campaigns to identify compounds that inhibit HCV RNA replication. Commonly used subgenomic replicons contain the HCV non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B (NS3–NS5B) that assemble into membrane-associated replication complexes. These complexes are represented by "dot-like" structures when standard fluorescence microscopy techniques are applied. To screen compound libraries for inhibitors that interfere with formation of the HCV replication complex independent of replicon replication and cell cycle, we developed a high-content based assay utilizing inducible expression of the HCV non-structural proteins. A stable cell line was generated in which a fluorescent protein (GFP) was fused to NS5A for detection of replication complexes in fluorescence microscopy. HCV polyprotein expression was well regulated with doxycycline, and polyprotein processing appeared unaffected by the GFP insertion within NS5A. The morphology of the replication complexes was "dot-like" in appearance and comparable to what has been observed in replicon cells. Draq5 staining of nuclei and cytoplasm prior to assay readout allowed development of image analysis tools that simultaneously detected nuclei, cytoplasm and replication complexes as well as parameters that could indicate compound toxicity. As expected, replication complex formation was not affected in the presence of a polymerase inhibitor, whereas incubation in the presence of a protease inhibitor induced a dose dependent reduction of "dot-like" structures. Image analysis of the effect of a selection of replicon hits and kinase inhibitors on replication complex formation in this assay is currently ongoing. In conclusion, we have designed a high-content based assay to identify and characterize compounds that