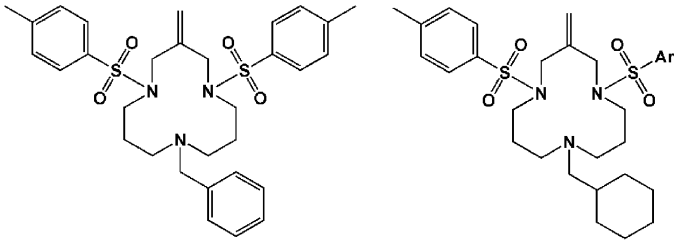
ies and on the potencies of two known unsymmetrical CADA analogs, decreased symmetry may likely lead to improved activity of the compounds. To fully explore the potential of the unsymmetrical analogs as antiviral agents, a new synthetic route was developed towards their production. One of the synthetic 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

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

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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water-soluble negatively charged derivative of the antifungal antibiotic PRM-A in which the terminal xylose moiety has been replaced by 3-sulfated glucose.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.

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

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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.

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

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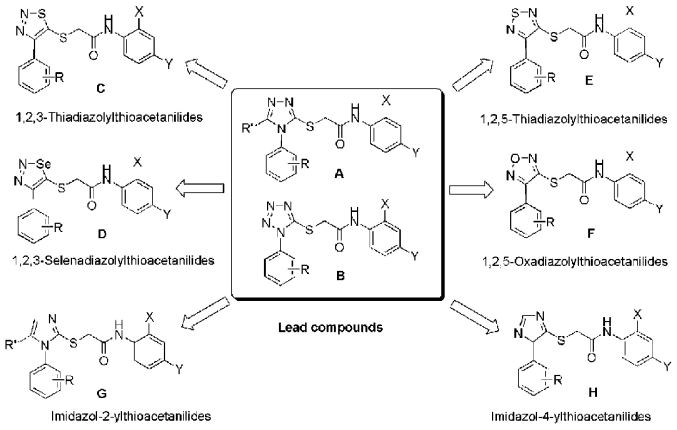
Despite the demonstrated clinical efficacy of combination antiviral regimens using HIV-1 NNRTIs, the emergence of clinical resistance has become a key issue for this class of compounds and has become a major cause of treatment failure. Therefore, to search for the novel NNRTIs with potent and broad spectrum 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

structure from the other HIV-1 NNRTIs (Fig. 1). Extensive structural modification and bioactivity research demonstrated that most derivatives showed submicromolar activity in cell assay and significant against the WT and mutant strains of HIV-1 RT. In order to further confirm the importance of the five-membered heterocycle, several novel series of novel arylazolylthioacetanilides were designed and synthesized based on the general principle of bioisosterism in medicinal chemistry. In the new analogues, other azoles rings, such as 1,2,3-thiadiazole (C), 1,2,3-selenadiazole (D), 1,2,5-thiadiazole (E), 1,2,5-oxadiazolyle (F) and imidazole (G and H), were substituted for the triazole or tetrazole moiety in the corresponding parent leads (Fig. 1), the other fragments which were considered to be necessary for conserving anti-HIV-1 activity, such as the "SCH_2CONH" linker and the 2-substituted anilides, were left unchanged. The newly synthesized arylazolylthioacetanilides (C and D) were evaluated for anti-HIV activities in MT-4 cells. Most derivatives proved to be highly effective in inhibiting HIV-1 replication at nanomolar ranges. The activity evaluation against NNRTIs resistant strains of selected (C and D) active derivatives, and the activity and cytotoxicity screening of other newly synthesized arylazolylthioacetanilides (E–H) are in progress and will be reported.



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ZNRD1 as a Host Cellular Factor Influencing HIV-1 Replication

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HIV takes advantage of multiple host proteins to support its ownreplication. *ZNRD1* gene has been identified as such a factor, influencing HIV disease progression in a whole-genome association study [Fellay et al., 2007. Science], but also as a required factor for HIV replication in an *in vitro* large-scale siRNA screening [Brass et al., 2008. Science]. In the present work, we evaluated in more detail the effect of *ZNRD1* in HIV-1 infection by means of genetic and *in vitro* functional studies.

siRNA and shRNA specifically targeting *ZNRD1* were used totransiently or stably downregulate ZNRD1 expression in lymphoid and non-lymphoid cells. Cells were infected using X4-tropic NL4-3 HIV strain. *ZNRD1*downregulation either by siRNA or shRNA impaired HIV-1 replication in lymphoid and non-lymphoid cells without affecting cell viability. To determine themechanism of action of ZNRD1 on HIV replication cycle, proviral and integrated DNA was quantified by qPCR, detecting no differences between wt or ZNRD1-inhibited cells. These results are consistent with ZNRD1 affecting HIV-1 replication at a post-integration step.

In addition, *ZNRD1* gene genotyping of 214 HIV+ subjects (122 LTNPand 92 normal progressors) was carried out by direct sequencing and association study was performed. Significant association with HIV progression after correction for multiple testing was found for 4 SNPs (p-value < 0.005). Haplotypes were estimated and association was also tested, resulting in the identification of an haplotype associated to LTNP (p-value < 0.005).

Our data confirm ZNRD1 as a host cellular factor influencing HIV-1 infection, both *in vitro* and *in vivo*. *In vitro* studies pointed to ZNRD1 affecting HIV replication at a post-integration step. Moreover, genetic association in HIV+ subjects progressing differently identified a haplotype in *ZNRD1* gene associated to slow disease progression.

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CXCR4 Chemokine Receptor Antagonists from Ultra-rigid Metal Complexes Profoundly Inhibit HIV-1 Replication, and also AMD3100-resistant Strains

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We have investigated the anti-HIV potential of new cross-bridge derivatives of bis-aza-macrocyclic compounds and compared their biological properties with the best known inhibitor of this type, being the CXCR4-antagonist bicyclam AMD3100 (also known as Mozobil or Plerixafor). The design of typical bicyclams incorporates two rings which allow binding interactions with multiple amino acid residues across different transmembrane helices. From a series of cross-bridge bis-aza-macrocyclic compounds we selected the Cu-complex, SJA-5 that inhibits HIV-1 replication in the nanomolar range in various cell lines and PBMCs. In order to elucidate the mode of action of this molecule, we have performed a complete mechanistic study on SJA-5. Based on the use of VSV-G pseudotyped virus, the study of the SDF-1-induced calcium signaling in U87.CD4.CXCR4 cells and SUPT1 cells, virus binding assays, time-of-addition experiments, resistance selection in the presence of increasing concentrations of SJA-5 and genotypic analysis of the selected resistant strains, we can conclude that akin to AMD3100, SJA-5 inhibits HIV-replication by a specific CXCR4-antagonist mode of action. Surprisingly, although being structurally related to AMD3100, SJA-5 only slightly lost activity against AMD3100-resistant strains. Additional experiments were performed to explain the limited loss of susceptibility of an AMD3100-resistant strain to the anti-HIV activity of SJA-5 in cell culture. Our observations suggest that SJA-5 binds to the CXCR4 receptor at a place nearby, but distinct of the interaction site of AMD3100. Metal complexes of cross-bridge bis-aza-macrocyclic compounds could open perspectives for the development of new HIV-1 co-receptor antagonists as antiretrovirals and serve as tools for optimizing metallodrugs.

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