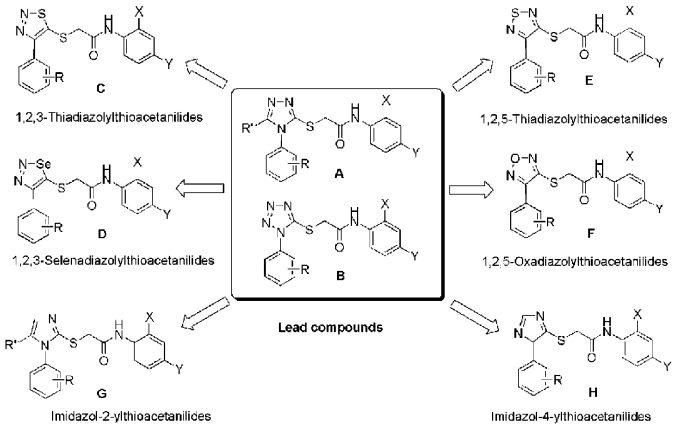
structure from the other HIV-1 NNRTIs (Fig. 1). Extensive structural modification and bioactivity research demonstrated that most derivatives showed submicromolar activity in cell assay and significant against the WT and mutant strains of HIV-1 RT. In order to further confirm the importance of the five-membered 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.



doi:10.1016/j.antiviral.2009.02.098

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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.

doi:10.1016/j.antiviral.2009.02.099

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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.

doi:10.1016/j.antiviral.2009.02.100