Summary

Due to the ongoing occurrence of drug-resistant virus variants the development of novel inhibitors and therapeutic strategies is a major challenge in current biomedical research. This is particularly true in case of rapidly mutating pathogens, like the human immunodeficiency virus (HIV). One of the most common treatment strategies aims at the direct inhibition of viral enzymes lacking a cellular homologue, which allows highly specific suppression of virus replication. However, single point mutations are sufficient to confer drug-resistance to HIV. A new innovative approach relies on the pharmacological interference with cellular proteins that are essential for virus replication, since such an intervention strategy may be more difficult to be overcome by viruses. The particular challenge herein lies in the achievement of an inhibitory effect in absence of cellular toxicities. Such new therapeutic approaches, which are based on cellular target structures, are the central subject of this thesis. Thus, potential inhibitors for the activation of the cellular HIV-1 cofactor eIF-5A were analyzed and new synthetic peptides were identified as potential inhibitors of HIV-1 and herpes simplex virus (HSV). Furthermore, their antiviral mechanism of action was characterized in detail.

The activated eukaryotic initiation factor 5A (eIF-5A) is a cellular protein that is essential for HIV-1 replication. This cellular factor serves as a cofactor of the viral Rev-protein that mediates the nucleocytoplasmic translocation of intron-containing viral mRNA. In its active form eIF-5A is the only cellular protein containing the unusual amino acid hypusine, a modification of a specific lysine residue that is catalyzed in a highly specific manner by two succeeding enzymatic reactions. It has been shown that the inhibition of either of the two responsible enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), leads to an efficient block in HIV-1 replication. However, the DOHH inhibitors described so far demonstrated significant cell-toxic effects. Furthermore, in these former studies DOHH could only be inhibited by unspecific iron-chelators, whereupon no evidence for an influence of Rev-mediated mRNA transport was demonstrated. In contrast, DHS-inhibitors have been reported to prevent HIV Rev-mediated mRNA transport via the CRM1 nuclear export pathway. This observation renders DHS an attractive target for a new class of antiviral inhibitors.

Hence, in this thesis work the antiviral activity of seven new potential anti-DOHH compounds was examined. One of these compounds, JK8-2, indeed inhibited HIV-1 replication by more than 70%. However, subsequent functional studies showed no influence of JK8-2 on Rev-function, as well as no specificity for DOHH. The examination of its mode of action revealed an activity during or before chromosomal integration of the HIV provirus.

Subsequently, RNA interference (RNAi) technology was applied to clarify whether or not the DOHH-catalyzed step in hypusine modification of eIF-5A is essential for Rev activity. By means of various RNAi methods stable cell lines were established in which the endogenous DOHH level was knocked down by 70%. However, subsequent functional analyses revealed that neither an influence on Rev-activity, nor on HIV-1 replication was detectable in these cells. Therefore, the DOHH-reaction is either not essential for HIV-replication or the remaining protein level after DOHH silencing was still sufficient for Rev-function.

In search of new DHS inhibitors six compounds have been analyzed, featuring structural similarity with the natural DHS substrate spermidine. Of these one compound, the DNA intercalating fluorescence dye DAPI exhibited a pronounced antiviral effect in HIV-1 infected cell cultures, without negatively affecting cell metabolism, cell cycle progression or apoptosis. However, when compared to the established DHS inhibitor CNI-1493, its specificity towards DHS was rather low and an impact on Rev-activity was not detectable.

Globally, HIV is mostly transmitted during unprotected sexual intercourse. Over time, the infection weakens the immune system leading to AIDS, which is followed by death due to various opportunistic infections. Those opportunistic pathogens include the wide spread herpes simplex viruses (HSV), which can also be transmitted sexually.

In the second part of this work it was demonstrated that synthetic anti-lipopolysaccharide peptides (SALP), initially described as inhibitors of bacterial sepsis, are efficient inhibitors of HIV-1 and HSV without causing any detectable cellular toxicities. Furthermore it was shown that treatment of cells with these cationic peptides prevents the binding of viral particles to target cells. By employing confocal and electron microscopy, as well as isothermal titration calorimetry measurements, it was demonstrated that SALP bind to heparan sulfate, which is ubiquitously expressed on cell surfaces. Importantly, heparan sulfate is utilized as a primary cellular attachment site by many pathogens, including HIV and HSV. Therefore, SALP may be exploited as valuable components of topically applied broad-spectrum microbicides for prevention of sexually transmitted virus infections.