acids from the HR1 central trimeric coiled-coil of the 6HB. To obtain more information on the precise binding mode and mechanism of inhibition of the potent RSV inhibitor TMC353121 (Bonfanti and Roymans, 2009), we determined the high resolution crystal structure of the compound bound at a hydrophobic pocket of the 6HB (Roymans et al., 2009). In contrast to what is generally believed, the binding site of TMC353121 is formed by amino acids from both HR1 and HR2. Binding of TMC353121 stabilizes the interaction of HR1 and HR2 in an alternate conformation of the 6HB, in which direct binding interactions are formed between TMC353121 and both HR1 and HR2. Rather than completely preventing 6HB formation, our data indicate that TMC353121 inhibits fusion by causing a local disturbance of the natural 6HB conformation. If binding with both HR1 and HR2 is a general requirement for the inhibition of 6HB formation by small-molecules, these results may fuel the structure-based discovery of other fusion inhibitors targeting viruses that use class 1 fusion proteins.

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Enterovirus 3C Proteases: Structure-based Discovery of Inhibitors

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Crystal structures have been determined for the 3C proteases of coxsackievirus B3 and enteroviruses 68 and 71 in our laboratory, and for poliovirus, rhinovirus, and hepatitis A virus in other institutions. We are using these structural data for virtual screening and for fragment-based design of non-peptidic inhibitors. Fragment-screening using saturation-difference-transfer (STD) NMR spectroscopy turns out to be particularly successful in identifying small molecules (<300 kDa) that bind to the target. These binding events are confirmed by surface plasmon resonance and X-ray crystallography. Fragments are subsequently linked by medicinal chemistry. An interesting approach to self-ligation of fragments has been developed. An overview of 3C(pro) inhibition, with the latest results included, will be provided.

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A Novel 9-Arylpurine Acts as a Selective Inhibitor of *In Vitro* Enterovirus Replication Possibly by Targeting Virus Encapsidation

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We identified a class of 9-arylpurines as selective inhibitors of the replication of various enteroviruses; analogue TP219 [9-(3-acetylphenyl)-6-chloropurine], was selected for further studies. The antiviral activity was assessed by means of CPE and virus yield reduction assays, q-RT-PCR, bioluminescence and antigen detection. TP219 did not inhibit early steps (entry/uptake) nor did it affect polyprotein synthesis/processing or the synthesis of viral RNA, thus suggesting that the drug interacts at a late stage in the replication cycle. Drug-resistant variants were selected that are not cross-resistant to other classes of enterovirus inhibitors (including 3A, 2C and a 3D inhibitor); and they were found to carry several mutations in VP1 and VP3. These mutations were reintroduced in the wild-type genome to confirm their role in the drug-resistant phenotype. The above described experiments revealed that TP219 probably prevents the correct encapsidation of the virion. Mammalian two-hybrid studies are used to explore whether TP219 hinders VP1/VP3 interactions. To study whether the drug prevents virion assembly, we optimized and implemented the nanoLC–MS/MS based SILAC-assays. This allows quantification of capsids and precursors in the infected cells. Since (inhibition of) assembly as such is not a critical step in virus induced cell lysis, probably other (cellular) mechanisms are involved. To study whether specific conformational changes in the capsid inhibit a particular 'death signal' eventually leading to inhibition of virus induced cell death, the effect of TP219 on different apoptosis pathways (in infected and uninfected cell cultures) is being studied. Together, these studies may provide exciting insights in an entirely novel strategy to inhibit picornavirus replication.

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Is the Large T Antigen a Target for the Inhibition of SV40 Replication?

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Background: Simian virus (SV40) belongs to the polyomaviruses, a small DNA virus family that causes severe diseases in immunocompromised patients. The large T antigen (LTag) encoded by SV40 is involved in viral replication and in transformation. The in vitro activity of acyclic nucleoside phosphonates against SV40, especially cidofovir (CDV, HPMPC, Vistide®), has already been shown. But the mechanism of action has not been elucidated yet. Polyomaviruses do not express their own DNA polymerase and require the cellular DNA replication machinery for replication of their DNA.

Methods: CDV resistant SV40 clones were selected for their ability to grow in presence of the drug and their genome sequenced to identify mutations in the LTag gene. In addition, phenotyping of