Recent Advances in Sialidase Inhibitors for the Treatment of Influenza

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Influenza is one of the best-known and most-common diseases, but an effective therapy has still to be found. Therapeutic drugs have included both *Amantadine* and *Rimantidine*, which are active only against influenza A virus (they act by blocking the ion-channel function of the virus protein M2, which is not found in influenza B virus). Both drugs are characterized by side-effects at therapeutic doses and rapid development of resistance during treatment. Vaccines, on the other side, as a preventive approach, provide only a temporary solution, because viruses rapidly change their surface antigens.

The discovery of two influenza-virus surface proteins, haemagglutinin and sialidase, provided new targets. The haemagglutinin receptor recognizes host-cell adhesion molecules and thus helps virus penetration. Sialidase is an α-glycohydrolase which cleaves α-ketosidically-linked sialic acids from glycoproteins, glycolipids, and oligosaccarides and helps the spread of newly synthesized virions from infected cells and their movements within the respiratory tract.

Based on the transition state of sialic acid in the active site during the sialidase-catalyzed hydrolysis, the unsaturated sialic acid analogue Neu5Ac2en (DANA) and some derivatives have been synthesized. These early inhibitors (transition-state analogues) needed to be improved due to their disadvantages (non-selectivity, virus aggregation at host-cell surface, virus-spread inhibition in cell culture, no efficacy in animal models. and rapid renal clearance).

The X-ray crystal structure of sialic acid with influenza virus sialidase and GRID calculations made it possible to rationally design 4-guanidino-Neu5Ac2en (*Zanamivir*, GG167). The 4-guanidino group of *Zanamivir* increases the overall binding of the molecule by forming a salt bridge with the side-chain carboxy group of Glu 119 in the active site. This compound (which is at present in phase-III clinical trials) inhibits both influenza A and B virus sialidases and is selective for viral *vs.* bacterial and mammalian enzymes. It is effective when intranasally administered, but its bioavailability is very low when systemically administered. Structure-activity relationship (SAR) analysis of *Zanamivir* showed that removal of each of the four groups linked to the dihydropyran ring resulted in a dramatic loss of activity. Moving from the initial notion that the glycerol pocket could accomodate novel substituents and that the replacement of the glycerol side chain would result in more straightforward structures, 4-guanidino- and 4-amino-4*H*-pyran-6-carboxamides have been synthesized. SARs of this new set of compounds have revealed that amides with lipophilic sidechains are the most active, whereas secondary amides are weak inhibitors of both influenza A and B viral sialidases. In contrast, tertiary amides, which contain one or more small alkyl groups, are much more active. In particular, *cis*-amides are selective towards the influenza A virus enzyme. Crystallographic and molecular dynamic studies performed on a number of protein-ligand complexes showed that tertiary amides bind to both enzymes forming a planar salt bridge between the side chains of Glu276 and Arg224. Sialidase of influenza B has to undergo a conformational change in order to accommodate the *cis* substituent of the carboxamide. This energetically unfavourable distortion of the protein was suggested to cause selectivity of *cis*-amides of influenza A virus enzyme.

In order to further increase the selectivity towards influenza A virus sialidase, compound GS4071, a cyclohexyl analogue of sialic acid bearing an ether group instead of a carboxamide, and its ethyl ester prodrug GS4104 have been modified. Synthesis of 6-ether (acetal), 6-ketone and reverse-pyrane analogues has been performed in order to investigate the effects of cyclohexyl and pyran rings. Biological studies showed no increase in selectivity of new analogues, but rather a marked reduction in the activity of ethers and reversed-pyrans. The fact that ethers adopt the B-conformer in solution and that the aminomethyl group in reverse pyrans does not fit the basic pocket were suggested as explanation for the reduced activity. Based on these results, it was concluded that incorporation of a more flexible side chain into pyrans did not improve fluA/B selectivity and that subtle differences in pyran/cyclohexene ring geometry enabled GS4104 to achieve good binding *vs.* both fluA and -B sialidases.

Helicase-Primase Inhibitors as Novel Anti-HSV Agents

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Epidemiology of Herpes Simplex Virus Infections. HSV Type-1 is responsible for labial herpes, whereof one out of every two individuals worldwide is affected, and herpes-induced keratitis which is the leading cause of infectious blindness with 300'000 new cases per year. HSV Type-2 causes genital herpes, a disease with 500'000 new cases per year. Both types are responsible for neonatal encephalitis with a mortality of 50%. Most significant is their capability for latent infection.

Current Therapies for HSV Infections. The nucleoside-based *Aciclovir* (*Valaciclovir*, ACV) and *Penciclovir* (*Famciclovir*) are currently used as therapeutics. Major indications of ACV are primary and recurrent genital herpes, herpes labialis, keratitis, and encephalitis. Its most significant deficits are the dosing frequency, rate of recurrence after primary infection, the slow onset of action and resistance in immunocompromised patients. Therefore, the antiviral research focuses on improvement of oral bioavailability (dosing) and different mechanisms of action (resistance).

HSV-1 Proteins Essential for DNA Replication. Seven proteins essential for DNA replication in HSV-1 have so far been identified and might be valuable targets for new antiviral drugs. Among those,