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Evaluation Of The Antiviral Activity And Cytotoxicity Of Peptidic Inhibitors Of Human Rhinovirus 3C Protease, A Novel Target For Antiviral Intervention.

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Human rhinoviruses (HRV) are the most important etiological agents of the common cold. Currently, no specific antivirals are available for the treatment of rhinovirus infection. The rhinovirus 3C protease is responsible for the cleavage of viral precursor polyproteins into structural and enzymatic proteins which are essential for viral replication. We evaluated the antiviral activity and cytotoxicity of a series of tripeptide aldehydes derived from the sequence of a natural rhinovirus 3C cleavage site, Leu-Phe-Gln. AG6084, a representative peptide aldehyde, demonstrated potent activity against the enzyme with a K_i of 6 nM. In HeLa cell protection assays, AG6084 effectively inhibited the replication of many diverse HRV serotypes with ED_{50}s ranging from 2.1 to 6.3 μM. The 50% cytotoxic dose was 316 μM yielding therapeutic indices of 50 to 151. AG6084 was also effective in inhibiting HRV-10 replication in primary human bronchial epithelial cells. In a single cycle, time-of-addition assay, AG6084 could be added late in the viral life cycle and still reduce levels of infectious virus. In contrast, compounds targeting viral attachment or uncoating were ineffective when added only one hour after infection. In a time-of-addition assay performed with a low m.o.i., the addition of AG6084 could be delayed until 50 hours after infection and still inhibit >50% virus-induced cytopathic effect. Direct inhibition of 3C proteolytic activity in infected cells treated with AG6084 was demonstrated by SDS-PAGE analysis of radiolabeled proteins which showed a dose-dependent accumulation of viral precursor polyproteins and reduction of processed protein products. The broad spectrum of antiviral activity against diverse rhinovirus serotypes for this compound, combined with its efficacy even when added late in the virus life cycle, highlight the advantages of 3C protease as a target and suggest that 3C protease inhibitors will be promising clinical candidates.

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Antirhinoviral Vinyl Acetylene Benzimidazoles

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The common cold is quite often referred to as the most common malady of mankind. Despite the number of large and intensive research programs which have focused on this problem over time, there are currently no marketed drugs which actually treat the virus and not just symptoms. Research on antirhinovirals at Lilly began over 20 years ago, and the first major effort in this area culminated in two clinical candidates: LY122772 (enviroxime) and LY127123 (enviradene). These compounds suffered from poor pharmacokinetics in humans and some undesirable toxicology which led to their abandonment. Recently we have refocused our efforts in this area in order to try and build off of the information from the past. In particular, we have concentrated on a new series of compounds containing a vinyl acetylene moiety. These compounds possess both good activity (enviroxime-like, ~0.05 ug/mL) and promising bioavailability in animals.

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PEPTIDE ANALOGS OF THE HUMAN PARAINFLUENZA VIRUS FUSION PROTEIN INHIBIT VIRAL FUSION AND VIRUS INFECTION

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We have studied synthetic peptides containing the heptad repeat regions derived from the fusion (F) proteins of human parainfluenza virus type 2 (PI2) and type 3 (PI3) for their function as potential inhibitors of virus-induced cell fusion as well as their effects on spread of viral infection. Peptides containing sequences of heptad repeat B and extending to the transmembrane domain of the F protein showed complete inhibition of cell fusion induced by the respective virus as well as by the vaccinia-expressed F and hemagglutinin-neuraminidase (HN) proteins. The 50% effective concentration to inhibit virus-induced cell fusion was 2.1 μM for PI2 and 1.2 μM for PI3. Moreover, the inhibitory effects of each peptide on virus-induced cell fusion were found to be virus type specific. These peptides were found to inhibit viral entry when mixed with the virus inoculum in plaque reduction assay. Furthermore, the peptides caused a 15 fold reduction in virus yield when added to culture medium in a multiple cycle infection. These peptides were also found to cause reduction of plaque size when added to the agar overlay. These results indicate that peptides containing the heptad repeat B sequence have the potential to specifically inhibit virus-induced cell fusion, virus entry and spread of virus infection. Such peptides could be used as a potential approach to control paramyxovirus induced respiratory diseases.

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Discovery of Peptide and Small Molecule Inhibitors of Paramyxovirus Fusion. J. Antczak, P. Sista, G. Merutka, M. Anwer, M.K. Lawless, M.C. Kang, J. Erickson, M. Ross Johnson, and D.M. Lambert. Trimeris, Inc., PO Box 13963, Research Triangle Park, NC 27709

Synthetic peptides derived from the heptad repeat 2 (HR2) domains within the F proteins of paramyxoviruses are potent and selective inhibitors of virus infection and fusion (*Lambert et al., 1996, PNAS 93, 2186-2191*). Antiviral peptides (EC_{50} values = 15 to 250 nM) were identified for three representative paramyxoviruses, respiratory syncytial (RS) virus, and human parainfluenza virus type 3 (HPIV-3). A lead RS virus peptide (T-786), optimized for solubility, blocked RS virus-mediated syncytia formation and infection *in vitro*. Furthermore, this peptide exhibited efficacy in the RS virus infected cotton rat model, reducing virus titers by as much as 1.5 log_{10} (compared with 0.7 log_{10} reduction with ribavirin) providing *in vivo* proof of concept for this target. An additional value of these peptides is that they are ligands for unique anti-viral screening targets useful for identification of small molecule inhibitors. High throughput screens were developed and used to screen compounds from structurally diverse libraries. Several potent (~100 nM) paramyxovirus-specific lead compounds have been identified. Data demonstrating the antiviral properties of these small molecule leads will be presented. Thus antiviral peptides which block fusion provide a novel approach to the development of targeted therapies for paramyxovirus infections. The peptides themselves may be potential drug candidates and screens derived from understanding their mechanism of action can be used to identify small molecule inhibitors of viral fusion.