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A Compound Derived from Podocarpic Acid Inhibits Influenza A Virus Replication In Vitro. K.A. Staschke, S.D. Hatch, J.C. Tang, W.J. Hornback, S. Mauldin, J.E. Munroe, J.M. Colacino*, and M.A. Muesing*. Lilly Research Laboratories, Indianapolis, IN USA.

A random antiviral screening effort has led to the identification of a group of compounds related to podocarpic acid which possesses potent in vitro antiviral activity against influenza A/Kawasaki/86 (H1N1). Time-of-drug-addition studies indicated that the mechanism of antiviral activity is mediated late in the influenza replication cycle since these compounds retained full antiviral activity even when added up to 8 hours post-infection. A representative of this class of compounds, designated LY180299, was chosen for further studies. This compound displayed potent activity against influenza A/Kawasaki/86 (IC_{50}=0.03 μg/ml), A/Fukushima/88 (H1N1; IC_{50}=0.10 μg/ml), and A/Ann Arbor/60 (H2N2; IC_{50}=0.05 μg/ml) but only marginal activity against A/WSN/33 (H1N1), strains of H3N2 viruses, or strains of influenza B. Analysis of reassortants between the sensitive A/Kawasaki and the resistant A/WSN revealed that the hemagglutinin (HA) of A/WSN was necessary and sufficient to confer the drug-resistant phenotype. Eleven independent isolates of A/Kawasaki were selected for growth in LY180299 (5 μg/ml). The HA gene segment of each of these viruses was sequenced and in each case the mutant RNA segment contained at least one sequence alteration. The location of these mutations, many of which clustered near the fusion domain of HA, suggests a novel mechanism of action in which the fusogenic function of HA is abrogated.

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Inhibitors of Influenza Virus Neuraminidase: Their Interaction with Enzymes from Sensitive and Resistant Viruses.

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We have recently demonstrated that analogues of 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (Neu5Ac2en) with basic substituents at the 4-position are potent and selective inhibitors of the influenza virus neuraminidase.[1] These compounds have been shown to possess antiviral activity both *in vitro* and *in vivo*, and one such inhibitor, 4-guanidino-Neu5Ac2en (GG167) is presently in phase 2 clinical trials for the prophylaxis and therapy of influenza virus infection. By the analysis of progress plots, we have demonstrated that GG167 is a slow-binding inhibitor of neuraminidases from a number of different influenza A and influenza B viruses. The data are consistent with the slow association of the inhibitor and protein via a single-step mechanism, with association rate constants of $10^5 M^{-1}sec^{-1}$ and dissociation rate constants of $10^{-4}sec^{-1}$. In an attempt to characterize the phenotype of influenza viruses resistant to neuraminidase inhibitors, a number of strains have been multiply passaged *in vitro* in the presence of GG167. A number of viruses have been isolated which have a much reduced sensitivity to GG167 *in vitro*, although as yet none have been demonstrated to have reduced sensitivity *in vivo*. The interaction of GG167 and other neuraminidase inhibitors with the enzymes from these resistant viruses will be described, as will the behaviour of these viruses *in vitro* and *in vivo*.

1. M von Itzstein *et al.* Nature, **363**, 418-423 (1993)

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Inhibition Of Influenza A Virus Replication By Compounds Interfering With The Fusogenic Function Of The Viral Hemagglutinin. S. Plotch, B. O'Hara, J. Morin, O. Palant, J. LaRocque, J. Bloom, K. Curran, A. Ross, M. Bradley, and Y. Gluzman. Wyeth-Ayerst Research, Pearl River, NY 10965 USA.

Two unrelated synthetic chemicals (Flul-1A and -2) were discovered as inhibitors of influenza virus A. Each inhibited viral protein synthesis when added before or immediately after infection but were ineffective when added 30 minutes later, indicating they blocked fusion or uncoating. Virus resistant to either compound was obtained and was cross-resistant to the other but not to amantadine. Analysis of reassortants between Flul-1A-resistant virus and wild type indicated the hemagglutinin (HA) protein was the target of the compound. Sequencing of HA genes of Flul-1A- and Flul-2-resistant mutants revealed single amino acid changes clustered in the stem of the HA trimer in and near the HA2 fusion peptide. Flul-1A could be docked in a pocket in this region by computer-assisted molecular modeling. The compound blocked the fusogenic activity of HA as evidenced by inhibition of low pH-induced cell-cell fusion in infected cell monolayers, but had no effect on adsorption, as evidenced by hemagglutination. An analog (Flul-1B) was synthesized which was several times more effective than Flul-1A in inhibiting virus replication, low pH-induced viral hemolysis of red blood cells, and low pH-induced virus inactivation. These results demonstrate that Flul-1A, its analogs, and Flul-2 inhibit the fusion process. The finding that unrelated chemicals discovered in screening inhibit the same process suggests the process is particularly sensitive to inhibition. However, the frequency of resistance and variation in strain sensitivity suggest such inhibitors are unlikely to be clinically useful.

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Characterization Of A New Class Of Respiratory Syncytial Virus Agents Inhibiting The Fusion Process. B. O'Hara[1], G. Ellestad[1], J. Morin[1], A. Gazumyan[1], J. LaRocque[1], B. Mitsner[1], U. Raifeld[1], A. Nikitenko[1], W.-D. Ding[1], P. Wyde[2], B. Gilbert[2], and Y. Gluzman[1] [1] Wyeth-Ayerst Research, Pearl River NY 10965, [2] Baylor College of Medicine, Houston TX 77030 USA.

A synthetic chemical, RSVI-1, was found to be a potent inhibitor of respiratory syncytial virus. Only one member of a series of chemically synthesized analogs (RSVI-2) was more potent than the parent. These two compounds display uniform potency in inhibiting each of 7 strains of RSV, from serotypes A and B, in a variety of cell lines (IC50 = 50-200 nM). Their action is specific, in that little or no activity is seen against parainfluenza virus type 3, measles virus, influenza type A, or herpesviruses. Time-of-addition experiments indicated that RSVI-1 must be present early in infection in order to inhibit, making it likely that the target is either the fusion process or a step immediately thereafter. However, both compounds inhibit syncytium formation when added several hours after infection of cell monolayers at a multiplicity of infection of 3. The compounds therefore inhibit the fusion process. RSVI-2 was capable of inhibiting growth of RSV in cotton rats when delivered by small particle aerosol or by intranasal dosing before or after the start of infection.