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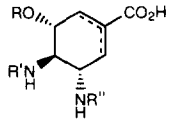
A New Class of Carbocyclic Influenza Neuraminidase Inhibitors: Structure-Activity Relationships. M.A. Williams[1], C.U. Kim[1], W. Lew[1], H. Liu[1], M.S. Chen[1], D. Mendel[1], W. Li[1], L. Tai[1], P. Escarpe[1],S. Swaminathan[1], N. Bischofberger[1], R.C. Stevens[2], W. G. Laver[3]. [1]Gilead Sciences Inc., 353 Lakeside Dr., Foster City, CA 94404; [2]Dept. of Chem., Univ. of California, Berkeley, CA 94720; [3]John Curtin School of Med. Res., Australian Nat'l Univ., Canberra 260, Australia.

The structure-activity relationships (SAR) of a new class of influenza neuraminidase inhibitors were established. These transition state analogues were based on a cyclohexene scaffold and synthesized from shikimic or quinic acid in a stereo- and regiospecific manner. Following determination of the optimum position for the double bond in the cyclohexene ring, systematic manipulation of R, R', and R" was performed to increase binding affinity. A new induced hydrophobic pocket in the neuraminidase active site was exploited by modification at the lipophilic group (R) position. A series of lipophilic linear alkyl, branched alkyl, cyclic alkyl and aryl analogues were synthesized. Hydrophobic interactions which appeared to be optimal for the 3-pentyl (R) group were confirmed by X-ray crystallographic analysis. At the amide functional group (R') position, substitutions were made to probe the steric limitations of this region of the active site. Amino and guanidino analogues were prepared at the basic functional group (R") position.



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GS 4104: A Highly Potent Orally Bioavailable Influenza Neuraminidase Inhibitor. M.A. Williams[1], C. U. Kim[1], W. Lew[1], L. Zhang[1], S. Swaminathan[1], N. Bischofberger[1], M.S. Chen[1], D. Mendel[1], W. Li[1], L. Tai[1], P. Escarpe[1], K.C. Cundy[1], E.J. Eisenberg[1], S. Lacy[1], R. W. Sidwell[2]; R.C. Stevens[3], W. G. Laver[4]. [1]Gilead Sciences Inc., 353 Lakeside Dr., Foster City, CA 94404; [2]Inst. for Antiviral Res., College of ADVS, Utah State Univ., Logan, UT 84322; [3]Dept. of Chem., Univ. of California, Berkeley, CA 94720; [4]John Curtin School of Med. Res., Australian Nat'l Univ., Canberra 260, Australia.

A series of transition state analogues based on a carbocyclic scaffold was synthesized as inhibitors of influenza neuraminidase. A novel hydrophobic binding mode for these compounds was discovered in the region of the neuraminidase active site which binds the glycerol side-chain of sialic acid. Structure-activity relationships and X-ray crystallography established the importance of these hydrophobic interactions. A lead compound GS 4071 was identified as a potent inhibitor (K_i < 1nM) and was shown to be active in an *in vitro* plaque reduction assay against influenza A and B. Administration of GS 4071 intranasally demonstrated efficacy in a mouse model at a dose of 0.01 mg/kg/day. Conversion of GS 4071 to the ethyl ester GS 4104 afforded a prodrug with oral efficacy in animal models of both influenza A and B virus infection. The oral bioavailability of GS 4104 was demonstrated in several animal species, with 100% absorption found in dogs. Radiolabeled GS 4104 was widely distributed in rats with appreciable levels (>30 fold of plasma level) found in lung tissue after 24 hr. Following oral administration of GS 4104 to rats at a dose of 30 mg/kg, the concentration of GS 4071 in the lung epithelial lining fluid after 2 hr was equal to the plasma level and >1000 times the IC_{50} value for influenza A neuraminidase (H1N1). A 14 day toxicity study in rats at an oral dose of 800 mg/kg/day gave no observable toxicity. (Supported in part by contract NO1 AI-65291 from the Virology Branch, NIAID, NIH)

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In Vitro Activity of the Neuraminidase Inhibitor GS4071 Against Influenza Viruses. FG Hayden and BS Rollins, University of Virginia, Charlottesville, Virginia, USA

The carbocyclic sialic acid analog GS4071 is a selective inhibitor of influenza virus neuraminidases (NA) and is active in experimental murine influenza after oral administration. We assessed the in vitro activity of GS4071 in comparison to the NA inhibitor GG167 for a range of clinical isolates and laboratory strains of influenza A and B viruses by plaque inhibition assay in Madin Darby canine kidney (MDCK) cell monolayers. We also determined effects on virus yield in explants of human adenoid and in the transformed human bronchial epithelial cell line BEAS-2B. By plaque inhibition assay in MDCK, the 50% inhibitory concentrations (EC_{50}) for GS4071 and GG167 were generally comparable. The median EC_{50} values for both drugs were <0.1 μg/ml for 18 influenza A/H3N2, >10 μg/ml for 8 A/H1N1, and 0.1 μg/ml for 10 B clinical isolates recovered in cell culture between 1983-96. For egg-grown strains of A/Texas/91(H1N1) and B/Yamagata/88, the EC_{50}s were <0.1 μg/ml for both. For both drugs the EC_{90} values (≥1 log_{10} reduced yield at 48 hrs) were <0.1 μg/ml for an A/Virginia/95(H3N2) and <0.01 μg/ml for A/Texas/36/91(H1N1) in adenoid explants. The drugs were somewhat less active against the A/Texas strain in BEAS-2B monolayers (EC_{90}, 0.02 μg/ml for GS4071, 0.2 μg/ml for GG167). An A/Singapore/1/57(H2N2) virus selected for resistance in the basis of a hemagglutinin mutation was not inhibited by either drug (EC_{50}>10 μg/ml). In contrast, a reassortant A/NWS-G70C virus resistant to GG167 on the basis of a neuraminidase mutation had EC_{50} values of >10 μg/ml for GG167 but 0.2 μg/ml for GS4071. GS4071 has potency and antiviral spectrum at least comparable to that of GG167 in vitro and is also active against certain viruses with reduced susceptibility to GG167 on the basis of a neuraminidase mutation.

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Characterization of influenza A viruses that are resistant to a fusion inhibitor related to podocarpic acid. K. A. Staschke, S. D. Hatch, J. C. Tang, W. J. Hornback, J. E. Munroe, J. M. Colacino, M. A. Muesing. Lilly Research Laboratories, Indianapolis, IN 46285 USA

Entry of influenza virus into the host cell is dependent upon the fusion of the viral envelope with the endosomal membrane and is mediated by a low-pH-induced change of the viral hemagglutinin (HA) to a conformation that is fusogenic. A compound related to podocarpic acid (180299) was identified which inhibits the replication of influenza A/Kawasaki (H1N1) virus in culture (IC_{50} = 0.01 μg/ml). Pre-treatment of MDCK cells with 180299 at 1 hr prior to infection with this virus (MOI of 10) resulted in the inhibition of viral protein synthesis (IC_{50} = 5.5 μg/ml). Addition of 180299 (20 μg/ml) 1 hr post-infection had no effect, indicating that the mechanism of action for 180299 affects an early step of the viral replication cycle. Genetic analysis of reassortants between sensitive and resistant viruses demonstrated that HA conferred the 180299' phenotype. Twelve independent isolates of influenza A/Kawasaki were selected for resistance to 180299. Sequence analysis revealed that each of these viruses contained amino acid substitutions in the HA. These mutations are dispersed throughout the HA primary amino acid sequence and cluster in one of two regions: (1) the interface between HA1 and HA2; and (2) in a region near the fusion domain of HA2. When compared to the parent virus, the pH of virus inactivation of the resistant mutants was increased by 0.3 to 0.6 pH units. This suggests that the mutations found in the resistant HAs result in the elevation of the pH at which the conformational change of HA occurs. Fusion of huRBCs to MDCK cells infected with parent influenza A/Kawasaki was inhibited by 180299 in a concentration-dependent manner (0.1 to 10 μg/ml). However, fusion of huRBCs to MDCK cells infected with 180299' mutants was not affected. These results suggest that 180299 interacts with the neutral pH conformation of influenza A HA and prevents the low-pH-induced change of HA to its fusogenic conformation.