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Design, Synthesis and Activity of Substituted Pyrrolidine Influenza Neuraminidase Inhibitors

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Influenza virus infection causes substantial morbidity and mortality worldwide but safe and effective treatments have been lacking. Recently, an increased understanding of the influenza virus replication cycle has led to the identification of several targets for therapeutic intervention, of which influenza neuraminidase has proven to be a highly attractive target for drug design. We have discovered a novel series of influenza neuraminidase inhibitors that utilize a previously unrecognized hydrophobic inhibitor-enzyme interaction directed at the "amine binding" subsite. Prior optimization strategies for this subsite have focused on positively charged groups such as primary amines and guanidines. A variety of substituents (Y) ranging from amines, heterocycles, esters, substituted alkenes and substituted alkyl groups was surveyed for several series of pyrrolidine inhibitors. Using an iterative structure-based approach, the activity of analogs containing hydrophobic groups in this site was optimized to give a 200-fold improvement in activity over analogs containing the primary amine.

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Novel Amino Acid Inhibitors of Influenza Neuraminidase

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In an effort to discover novel, non-carbohydrate inhibitors of influenza neuraminidase, we tested nearly 300 α and β amino acids from Abbott's chemical repository for inhibitory activity. We reasoned that the positively charged amino group might be a good structural mimic for the departing partial positively charged glycosidic oxygen which occurs in the transition state during substrate turnover. Two lead compounds were discovered, a phenyglycine and a pyrrolidine, which exhibited Ki values in the 40 to 60 μM range against an A/N2 strain neuraminidase. X-ray structural studies of these compounds bound to N9 neuraminidase showed that the amino groups were hydrogen bonded to Asp 152 as expected. The hydroxy analogs were bound two to three orders of magnitude weaker than their amine containing counterparts which underscores the importance of the electrostatics and hydrogen bonds in this region of the active site. The structural studies also showed that the Glu-278 side chain of neuraminidase was induced into a new conformation in order to accommodate the hydrophobic regions of these compounds although slow binding kinetics were not observed. Both series of compounds exhibited reduced binding affinity for B strain neuraminidase, presumably because the Glu 278 induced conformation is relatively unfavorable in B strain enzymes. This presentation will also summarize efforts to improve the activity of these compounds to the submicromolar Ki value range. These studies serve as the foundation for Abbott's anti-influenza drug discovery program.

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The Neuraminidase Inhibitor Oseltamivir is Efficacious Against A/Hong Kong/156/97 (H5N1) and H9N2 Influenza Viruses.

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Since 1997, two avian influenza viruses transmitted directly from birds to humans. H5N1 virus killed 6 of 18 people in Hong Kong. Five cases of H9N2 influenza have been reported from China and two have been confirmed in Hong Kong. When new viruses enter human populations, vaccines are not available and antiviral drugs are critical for prophylaxis and therapy until vaccines can be prepared. Here we demonstrated the efficacy of the neuraminidase inhibitor GS 4104 that can be taken orally against H5N1 and H9N2 viruses. GS 4071 inhibited the replication of H5N1 and H9N2 influenza viruses in MDCK cells with IC_{50} values ranging from 7.5 to 10μM and it inhibited enzyme activity of the neuraminidases of these viruses, with IC_{50} values ranging from 7.0 to 15uM. GS 4104, administered orally was efficacious against A/Hong Kong/156/97 (H5N1) and A/Qa/Hong Kong/G1/97 (H9N2) viruses reducing lung and brain titers of the virus in infected mice. Oral administration of GS 4104 at doses 1 and 10 mg/kg/day exerted protective effect against A/Hong Kong/156/97 (H5N1), A/Qa/Hong Kong/G1/97 mouse adapted (H9N2) and A/Hong Kong/1/74/99 human virus with prevention against death. When the time of therapy was delayed to 36 hours after exposure with A/Hong Kong/156/97 (H5N1) virus, GS 4104 was still effective. Oral administration of GS 4104 in combination with rimantadine reduced mortality from high challenge with A/Qa/Hong Kong/G1/97 (H9N2) virus. Combination of low doses of GS 4104 and Rimantadine (GS 4104 0.1 mg/kg/daily, rimantadine 1 mg/kg/daily) fully protected mice from death at low virus challenge. Thus GS 4104 is efficacious in the treatment of emerging influenza H5N1 and H9N2 viruses including systemic transmission to the brain.

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A New Class of Cyclopentane Derivatives with Potent Inhibitory Activity Against Influenza A and B Viruses in Cell Culture.

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A novel series of cyclopentane derivatives has been found to exhibit strong and selective inhibitory effects on influenza virus neuraminidase. These compounds, designated as RWJ-270201, RWJ-270204, RWJ-302410 and RWJ-303408, were tested in parallel with zanamivir and GS4071 against a spectrum of influenza A (H1N1, H3N2, and H5N1) and influenza B viruses in Madin Darby canine kidney (MDCK) cells. Inhibition of viral cytopathic effect ascertained visually and by neutral red uptake was used, with 50% virus–inhibitory concentrations (EC_{50} values) determined. Against the H1N1 viruses A/Texas/36/91, A/Bayern/07/95 and A/PR/8/34, EC_{50} values (determined visually) for the compounds were less than 1 μM, whereas against the A/NWS/33 strain 50% inhibition was seen at 7-20 μM. Six strains of H3N2 and two strains of H5N1 viruses were inhibited at <0.1 μM. The cyclopentane derivatives were active against influenza B with higher EC_{50} values compared to influenza A. Naturally-occurring and cell culture-prepared strains of influenza virus have also been identified that are resistant to all of these compounds *in vitro*. Their molecular characterization is underway. The novel inhibitors were comparable in potency to zanamivir and GS4071, and no cytotoxicity was seen with concentrations as high as 1 mM. Antiviral activity decreased with increasing multiplicity of virus infection. Time of addition studies indicated treatments needed to begin between 0 and 12 hours after virus exposure. RWJ-270201, which has been chosen for further preclinical development, was also investigated in virus yield assays. Treatment of infected cells with this compound caused the virus to remain cell-associated, with extracellular virus decreased in a concentration-dependent manner. This is consistent with its effect as a neuraminidase inhibitor. The cyclopentane derivatives hold great promise for the treatment of influenza virus infections in humans. [Supported by Contract NOI-AI-85348 from the Virology Branch, NIAID, NIH]