

# Evaluation of Drug-resistance and Development of the Next Generation Anti-influenza

## (약제내성 인플루엔자 발생과 차세대 항인플루엔자제 연구동향)

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The membrane of the influenza virus contains two immunodominant glycoproteins, hemagglutinin (HA) and neuraminidase (NA), that play key roles in viral infection and spread. HA effects attachment of the virus to the host cell via its interaction with surface sialic acids, thereby initiating entry. Once the virus has replicated, the NA cleaves sialic acids from the viral and cell surfaces, allowing the virus to spread to uninfected cells. The specific antigenic properties of the different HA and NAs are used to classify influenza type A viruses into subtypes (H1-17 and N1-9). Based on the notion that potent and specific viral NA inhibitors should function to reduce viral spread, structure-based inhibitor design programs have produced two widely used anti-influenza drugs, zanamivir (Relenza) and oseltamivir (Tamiflu). Initial design of these drugs was based upon the mimicry of the flattened transition state conformation of the sugar through incorporation of an endocyclic alkene within a carbocycle (oseltamivir) or a pyranose ring (zanamivir). Specificity for the influenza enzyme, along with additional affinity, was provided by incorporation of a guanidinium or ammonium substituent at the position corresponding to C-4 of the natural substrate to interact with a highly conserved anionic pocket at that location in the active site.

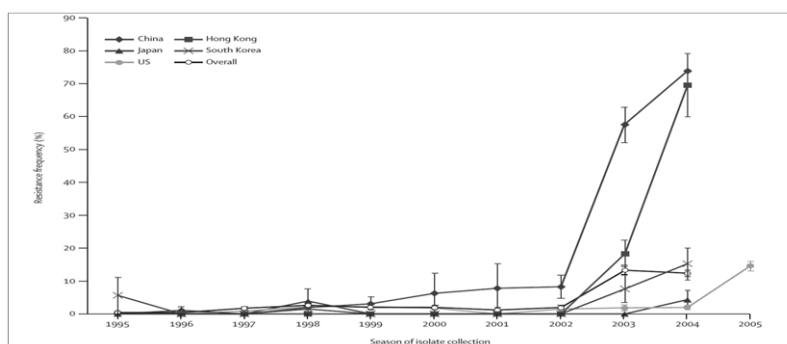
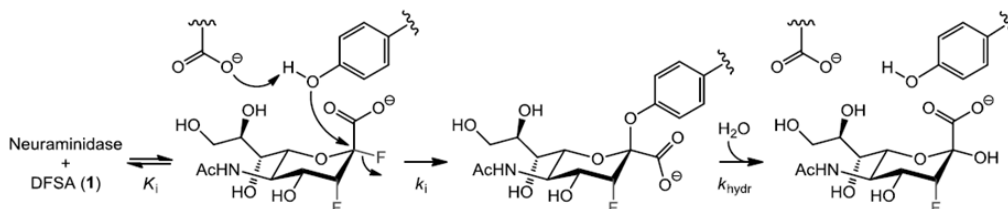


Figure: Trend of adamantane-resistant H3N2 viruses, 1994-2005  
Each point represents the percentage of resistant viruses of the total tested. Error bars represent the 95% CI for the proportion of resistant viruses out of the total tested.

We are now seeing the emergence of drug-resistant strains, particularly against the more widely used and structurally divergent drug oseltamivir. Mutations can be both drug-and

influenza subtype-specific. The most commonly seen mutation in viruses with the N1 subtype is H275Y which interferes with binding of the isopentyl side chain of oseltamivir, but still permits binding of zanamivir and the natural substrate. Mutations most commonly detected in clinical isolates with the N2 subtype include Arg292Lys and E119V. Like the H275Y, the Arg292Lys precludes full rotation of the E276 necessary to create the hydrophobic pocket for oseltamivir to bind. In contrast, E119V confers oseltamivir specific resistance due to altered interactions with the 4-amino group. E119A,D,G mutations seen *in vitro* affect binding of oseltamivir and/or zanamivir, demonstrating the critical nature of the interactions of C-4 amino or guanidino group for high affinity binding. Some of the recent mutations seen in pandemic H1N1 viruses, including I223R, confer reduced sensitivity to both inhibitors. The emergence of these mutant strains highlights an urgent need for new classes of NA inhibitors that differ minimally in structure from the parent sialic acid, since the development of resistance to structurally conservative, mechanism-based inhibitors should be a much less probable event.



We report here a completely new class of specific, mechanism-based anti-influenza drugs that function via the formation of a highly stabilized covalent intermediate in the neuraminidase enzyme, and confirm this mode of action via structural and mechanistic studies. These compounds function not only in cell-based assays, but also in animal models, with efficacies comparable to that of zanamivir and with broad spectrum activity against resistant strains. Their novel mode of action and their structurally conserved and mechanism-based design provide a class of drug that should be less prone to resistance development. They therefore represent possible solutions to a major public health problem.

