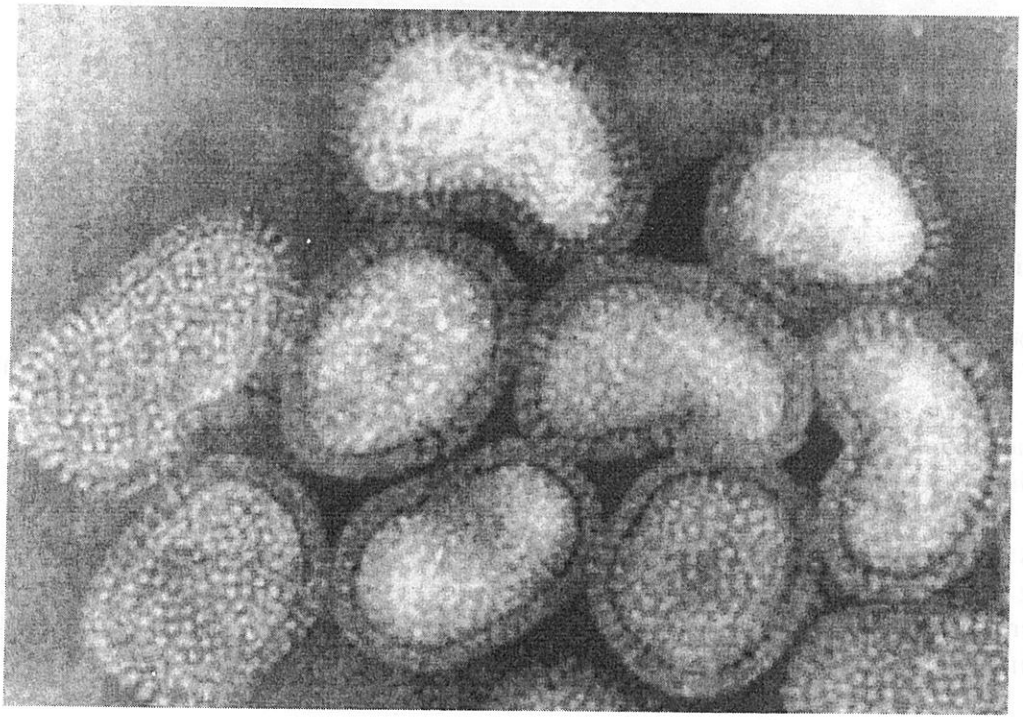
literature seminar No.2
Yao Du(M2)

Influenza and Anti-influenza Drugs



~The greatest medical holocaust in history~

In 1918, Spanish flu pandemic (type A influenza, H1N1 subtype) outbroke.
It lasted from 1918 to 1919, killed 20~50 million people, causing the death of more people than did World War I. It is estimated that 2.5% to 5% of the world's population was killed.

~How severe?~

Mortality rate was 2~20 %.(usual rate 0.1 %)
99 % of deaths occured in people under 65.(normal flu is deadly to under 2 and above 70.)
25 million may have been killed in the first 25 weeks.(HIV has killed 25 million in its first 25 years.)

~Contents~

I Influenza

Influenza or **flu virus** is an infectious disease of birds and mammals caused by RNA viruses of the **family Orthomyxoviridae**. It is an enveloped RNA virus consisting of an **internal nucleocapsid** and an **envelop** made up of an **inner matrix** protein, a **lipid bilayer**, and **external glycoroteins**.

Figure 1. Filamentous particles of influenza A

Characteristics:

1. The shape is not uniform: round particles of 100 nm diameter, sometimes elongated filamentous particles of the same diameter.

2. The surface glycoprotein spikes represent two different shapes and chemical types which are called **hemagglutinin(HA)** and **neuraminidase(NA)**.

3. The RNA genome is divided. There are **eight different molecules of RNA**(seven for type C), each representing the gene for a protein, except that the two smallest RNAs carry two overlapping genes read in differnt phases and responsible in each case for two proteins. Each molecule of RNA contains nucleoprotein and RNA polymerase(PA, PB1, PB2).

4. The flu virus changes frequently and at times dramatically, as a consequence of the multiplicity of its genome components. **Antigenic shift** represents the abrupt appearance of new serotype due to reassortment of RNA genome components in cells infected with two differnt strains. Spontaneous point mutations can in turn lead to minor differences, termed **antigenic drift**.

5. The RNA molecules are weakly encapsidated and thus sensitive to ribonulease.

6. The transcription, particularly rich in U, occurs in the nucleus.

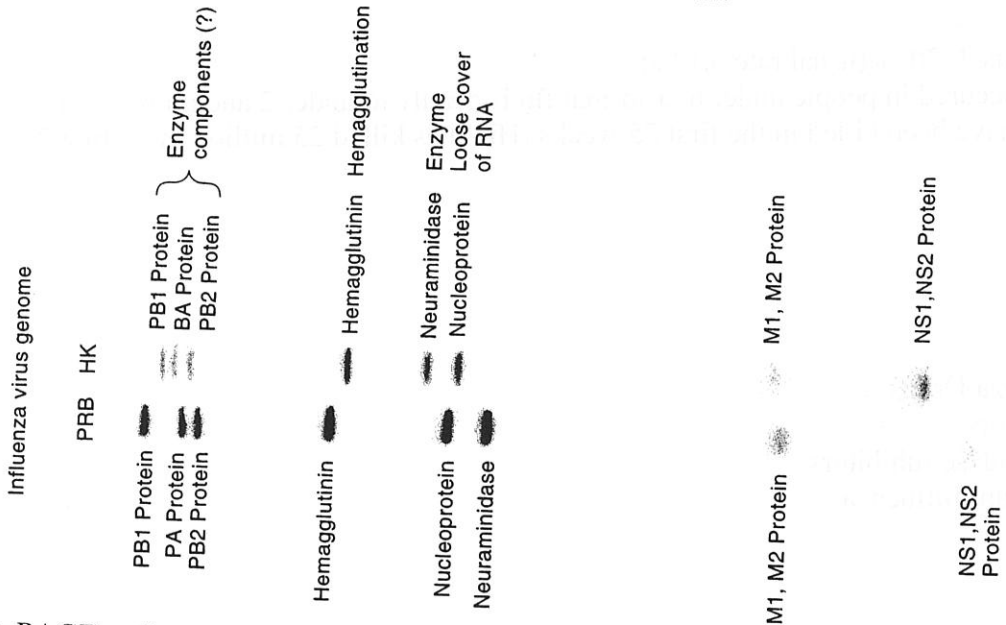


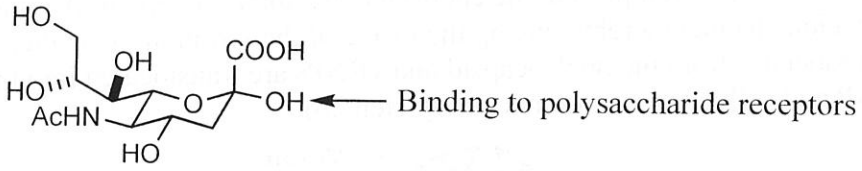
Figure 2. PAGE radioautogram of the influenza virus genome. PRB=a type A flu. HK=Hong Kong flu

Envelope:

1. The main protein(M1) of the flu virus forms a shell around the nucleocapsid on which rests the envelope. There are several channels made by M2 protein which is a tetramer.

2. The hemagglutinin(HA) is made up of rod-shaped protein molecules of 14 nm. Three molecules make up one spike. It is the major viral antigen against which neutralizing antibodies are formed.

3. The neuraminidaseNA) spikes appear as thin fibers 10 nm long with bigger structures of different sizes at the ends. Each spike is a tetramer of identical subunits. It breaks the bond holding N-acetylneuraminic or sialic acid to the end of many polysaccharide receptors on cell surfaces.



Sialic acid

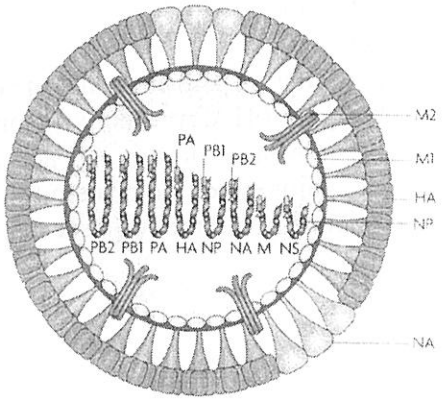


Figure 3. Influenza A virus

Classification:(Classified by HA and NA)

Type A
Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics.

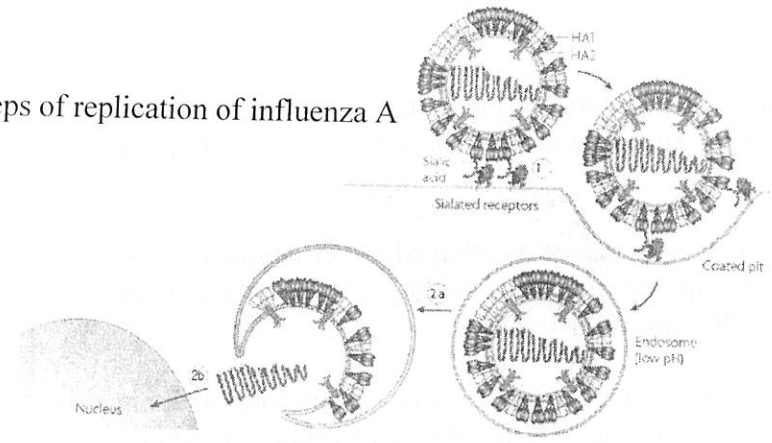
Subtype of type A
For birds, there are 16 kinds of HAs and 9 kinds of NAs.==>16 x 9=144
For human, only 10 reported. For example, H1N1, caused Spanish flu in 1918
H2N2, caused Asian Flu in 1957
H3N2, caused Hong Kong Flu in 1968
H5N1, a worldwide pandemic threat in 2007~08

Type B
Almost exclusively infects humans and is less common than influenza A. Low rate of antigenic change, combined with its limited host range (inhibiting cross species antigenic shift), ensures that pandemics of influenza B do not occur.

Type C
Infects humans and pigs, is less common than the other types and usually seems to cause mild disease in children

Replication:

Figure 4. First steps of replication of influenza A



Step 1

A influenza virion binds the host cell membrane via HA to terminal sialic acids present on glycoproteins or on glycolipids and enters the cytoplasm by receptor-mediated endocytosis, thereby forming an endosome(low pH).

Step 2

A cellular trypsin-like enzyme cleaves HA into products HA1 and HA2 (not shown). Conformational changes of HA2 promote fusion of the virus envelope and the endosome membranes(step 2a).A minor virus envelope protein M2 acts as a ion channel thereby making the inside of the virion more acidic. The major envelope protein M1 dissociates from the nucleocapsid and vRNPs are translocated into the nucleus via interaction between NP and cellular transport machinery(step 2b).

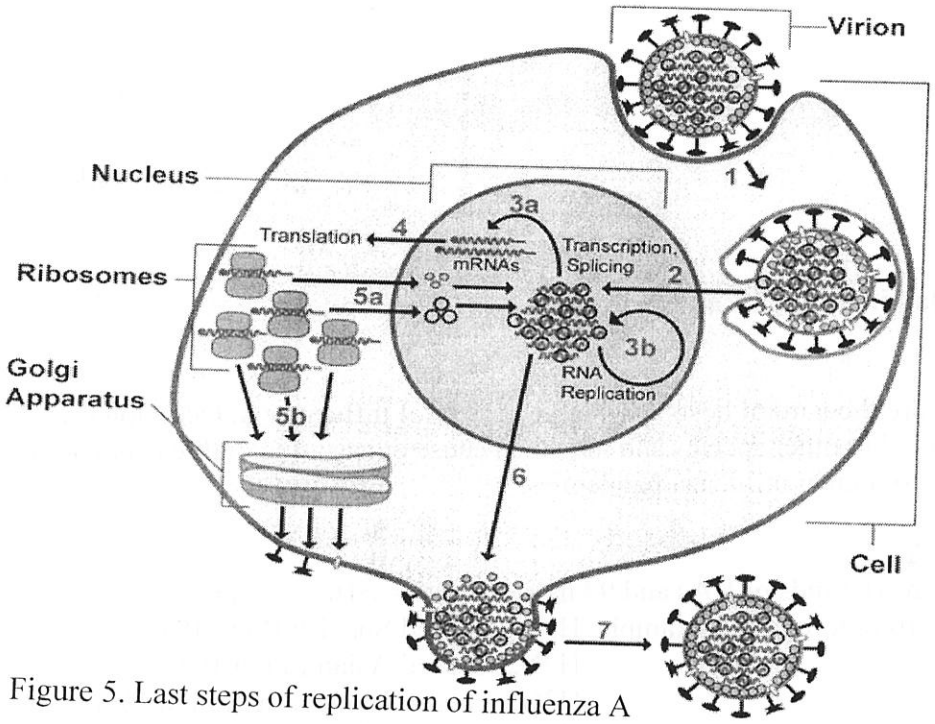


Figure 5. Last steps of replication of influenza A

Step 3~4

In the nucleus, the viral polymerase complexes transcribe (Step3a) and replicate (Step 3b) the vRNAs. Newly synthesized mRNAs migrate to cytoplasm (Step 4) where they are translated.

Step 5

Posttranslational processing of HA, NA, and M2 includes transportation via Golgi apparatus to the cell membrane (Step 5b). NP, M1, NS1 (nonstructural regulatory protein - not shown) and NEP (nuclear export protein, a minor virion component - not shown) move to the nucleus (Step 5a) where bind freshly synthesized copies of vRNAs.

Step 6~7

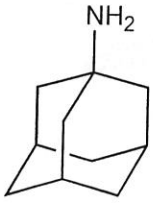
The newly formed nucleocapsids migrate into the cytoplasm in a NEP-dependent process and eventually interact via M1 with a region of the cell membrane where HA, NA and M2 have been inserted (Step6). Then the newly synthesized virions bud from infected cell (Step 7). NA destroys the sialic acid moiety of cellular receptors, thereby releasing the progeny virions.

II Anti-influenza Drugs

The two classes of anti-virals are neuraminidase inhibitors and M2 inhibitors (adamantane derivatives). Neuraminidase inhibitors are currently preferred for flu virus infections.

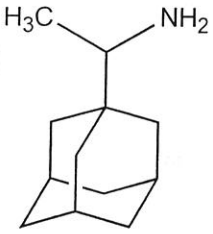
II-i M2 Inhibitors

The antiviral drugs amantadine and rimantadine are designed to block a viral ion channel (M2 protein, influenza B does not possess this protein), which is required for the viral particle to become uncoated once it is taken inside the cell by endocytosis, and prevent the virus from infecting cells.



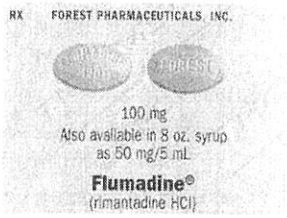
amantadine
(1-aminoadamantane)

used as a prophylaxis and treatment of influenza A infection;
sold under the name "Symmetrel";
approved in 1966 by the U.S. Food and Drug Administration;
in 1969 discovered by accident to help reduce symptoms of Parkinson's disease;
side effects: nervousness, anxiety, agitation, insomnia, difficulty in concentrating...;
declining effectiveness: resistance rate 92 % in H3N2, 25 % in H1N1 (some Asian contry, 100 %).



rimantadine

used to treat, and in rare cases prevent, influenzavirus A infection;
sold under the trade name "Flumadine";
approved by the Food and Drug Administration (FDA) in 1994;
side effects: nausea, upset stomach, nervousness, tiredness....

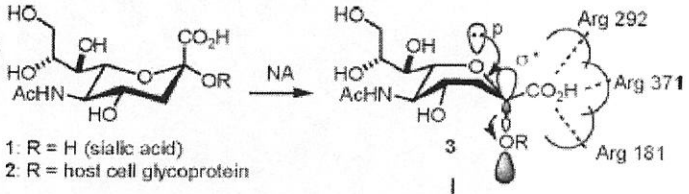


II-ii Neuraminidase Inhibitors

Neuraminidase inhibitors are designed using knowledge of the enzyme structure to halt the spread of the virus in the body by cleaving the bond holding N-acetylneutaminic or sialic acid to the end of glycoproteins and glycolipids on cell surfaces. The sturcture of neuraminidase was determined in 1983.

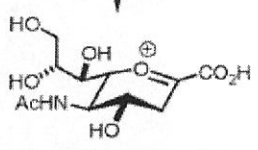
Masakatsu Shibasaki and Motomu Kanai, *Eur. J. Org. Chem.* **2008**, 1839-1850

Scheme 1. A closer look at the hydrolysis step of silaic acid by NA and design of NA inhibitors.



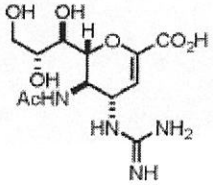
1: R = H (sialic acid)
2: R = host cell glycoprotein

3

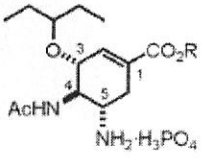


4: oxonium intermediate

mimic



5: zanamivir



6: oseltamivir phosphate (R = Et)
7: Ro64-0802 (R = H)

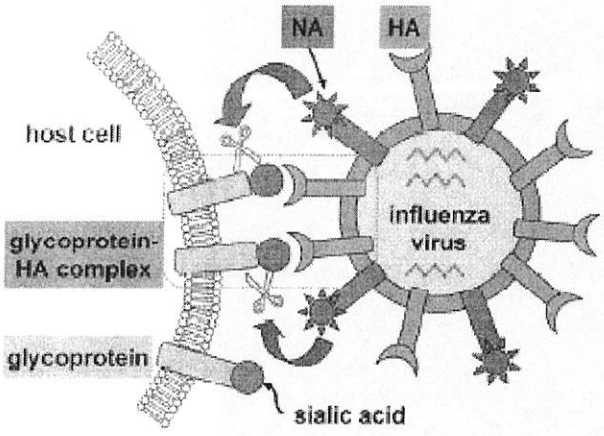
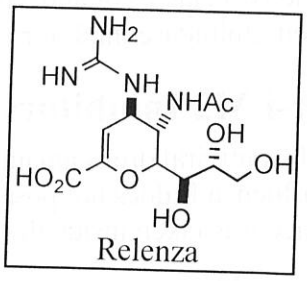


Figure 6. Schematic representation of an influenza virion budding from a host cell.

Zanamivir(trade name Relenza)

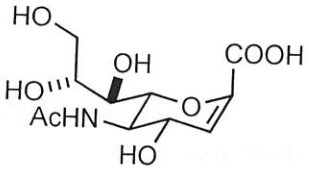
Relenza, discovered in 1989, was the first neuraminidase inhibitor commercially developed. It was developed by a team of scientists, led by Mark von Itzstein, at the Victorian College of Pharmacy at Monash University, as a part of the Australian biotechnology company Biota's project to develop antiviral agents via rational drug design. It is currently marketed by GlaxoSmithKline.



Relenza

Mark von Itzstein. et al. *Nature* **1993**, *363*, 418-423.

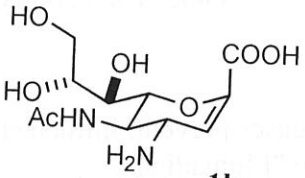
Computer-asisted drug design



1a

2-deoxy-2,3-di-dehydro-D-N-acetylneuraminic acid (Neu5Ac2en)

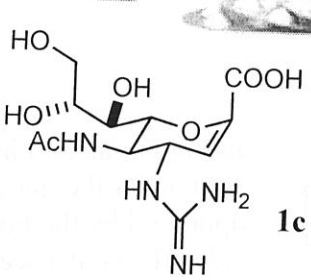
no inhibitor activity



1b

4-amino-Neu5Ac2en

inhibitor activity



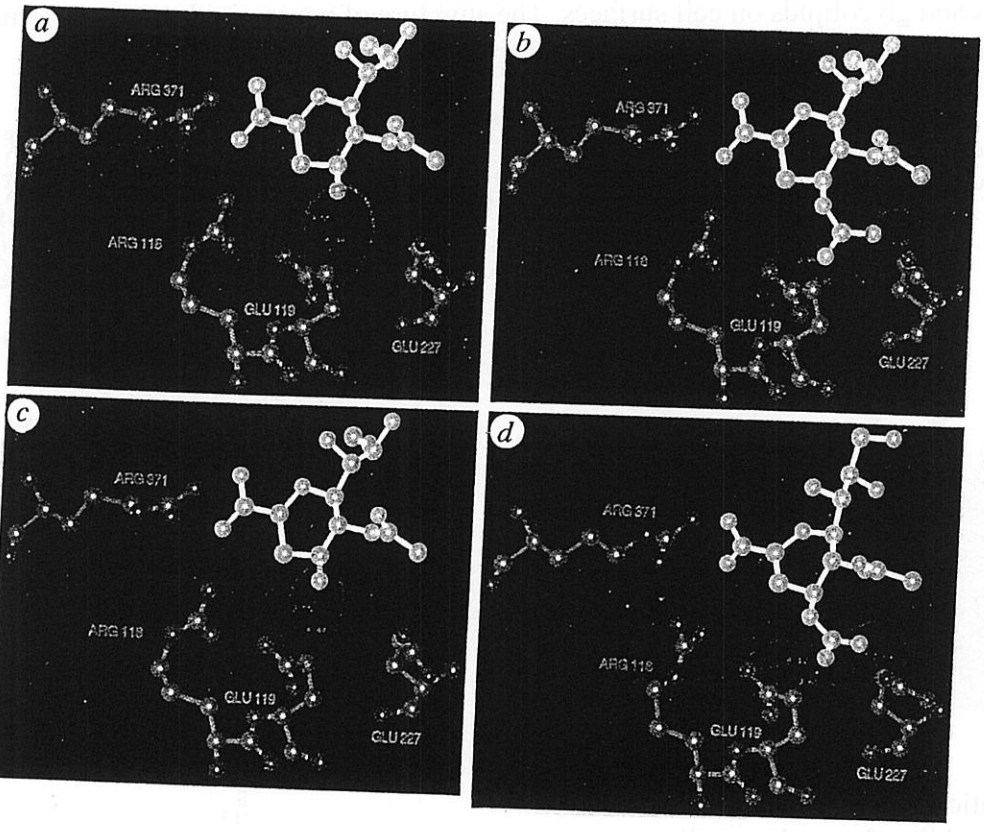
1c

4-guanidino-Neu5Ac2en

inhibitor activity

The active site of neuraminidase: a deep cavity on the protein surface and is lined entriely by amino acids that are invariant in influenza A and B. Strain-variable amino acids are found next to the active site.

predicted 1b



1c predicted

observed 1b

1c observed

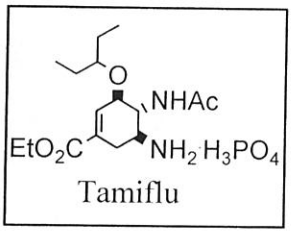
Figure 7. A comparison of the predicted and observed interations of two inhibitors with neuraminidaase.

Oseltamivir(trade name Tamiflu)

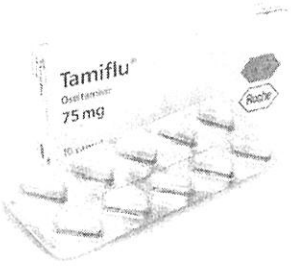
Oseltamivir, used in the treatment and prophylaxis of both Influenzavirus A and B, was the first orally active neuraminidase inhibitor commercially developed. It was developed by Gilead Sciences and is currently marketed by Hoffmann-La Roche (Roche) under the trade name Tamiflu. In Japan, it is marketed by Chugai Pharmaceutical Co., which is more than 50% owned by Roche.

It is estimated that 50 million people have been treated with oseltamivir. The majority of these have been in Japan, where an estimated 35 million have been treated.

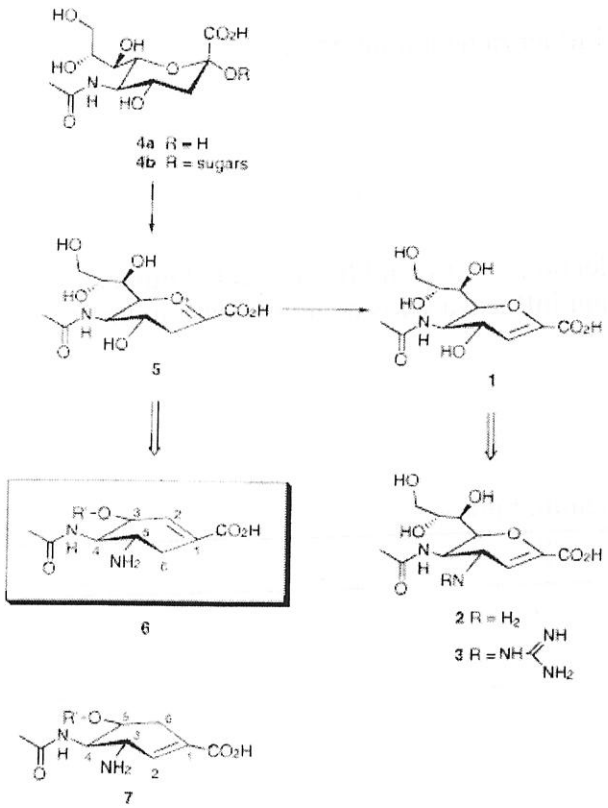
Choung U. Kim. et al. *J. Am. Chem. Soc.* **1997**, *119*, 681-690.



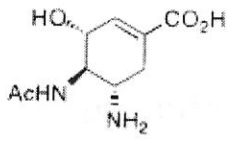
Tamiflu



Scheme 2. Rational Design of Carbocyclic Transition-State Analogues



Scheme 3.Importance of the Double Bond Position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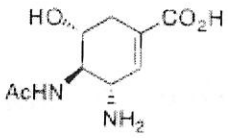
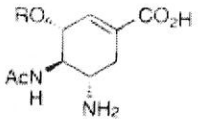

9

The inhibitory activity of 8 and 9: determined in a NA enzymatic assay. 8 proved to be a potent NA inhibitor.

Table 1. Influenza Neuraminidase Inhibition and Plaque Reduction by Carbocylic Analogues


6

R	compd	enzyme[a] IC_{50} (nM)	plaque[b] EC_{50} (nM)
H	8	6300	ND[c]
CH_3	6a	3700	ND
CH_3CH_2	6b	2000	
$CH_3CH_2CH_2$	6c	180	ND
$CH_3CH_2CH_2CH_2$	6d	300	ND
$(CH_3)_2CHCH_2$	6e	200	ND
$CH_3CH_2(CH_3)CH$*	6f	10	80
	(*R*)-isomer		
	6g	9	135
	(*S*)-isomer		
$(CH_3CH_2)_2CH$	6h	1	16
$(CH_3CH_2CH_2)_2CH$	6i	16	ND
	2	150	2500
	3	1	15

[a] NA. [b] H1N1, A/ws. [c] ND = not determined.

IC_{50}: It is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}).

EC_{50}: It refers to the concentration of a drug or antibody which induces a response halfway between the baseline and maximum(hslf maximal effective concentration(EC_{50})).

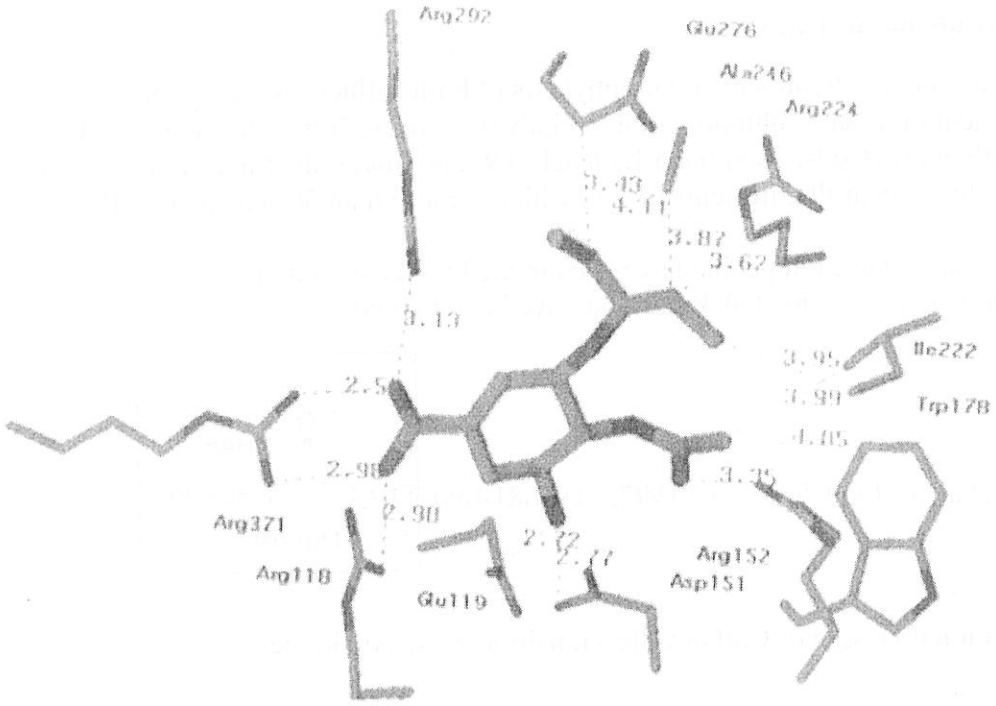


Figure 8. X-ray structure of 6h bound to influenza neuraminidase.

III Drug-resistant Influenza

There have been reports of drug-resistant mutant selection in vitro and from infected humans. Recently, the crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants were reported.

Patrick J. Collins. et al. *Nature* **2008**, *453*, 1258.

Table 2. Activity, binding and kinetic parameters for N1 neuraminidases

NA type	V_m relative to wild type	K_m (μM)	Oseltamivir relative K_i*	Zanamivir relative K_i†	k_{on} ($\mu M^{-1}s^{-1}$) oseltamivir	k_{off} (s^{-1}) oseltamivir ($\times 10^4$)	k_{on} ($\mu M^{-1}s^{-1}$) zanamivir	k_{off} (s^{-1}) zanamivir ($\times 10^4$)
Wild type	1.0	6.3	1.0	1.0	2.52 (0.21)	8.1 (1.2)	0.95 (0.08)	0.95 (0.13)
His274Tyr	0.8	27.0	265	1.9	0.24 (0.06)	180 (30)‡	0.35 (0.02)	0.67 (0.08)
Asn294Ser	1.15	53.0	81	7.2	1.1 (0.18)	235 (40)‡	0.52 (0.04)	3.7 (0.6)
Tyr252His	0.94	7.5	0.1	1.2	3.9 (0.15)	1.25 (0.13)	1.38 (0.15)	1.66 (0.33)

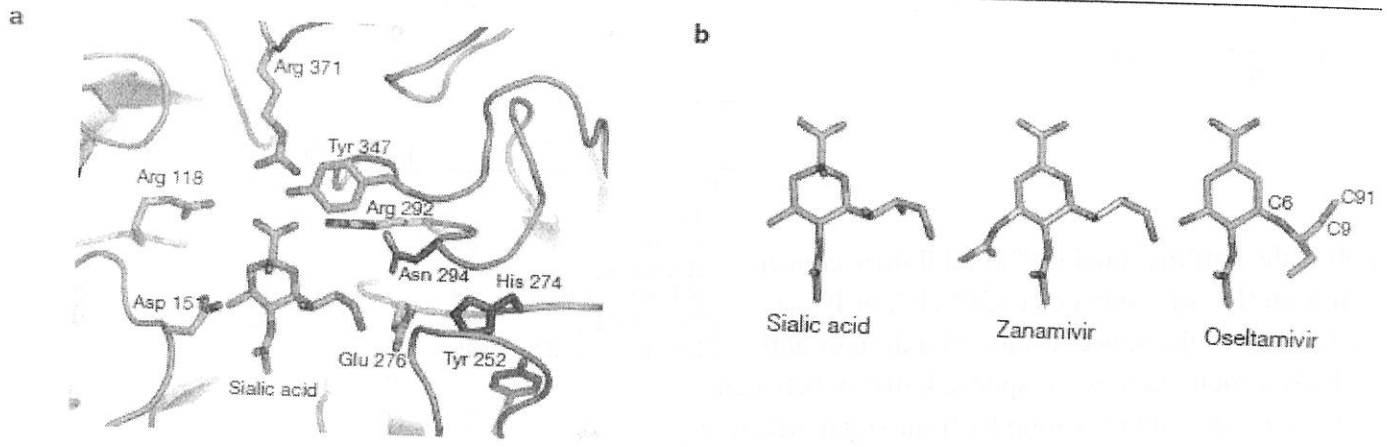
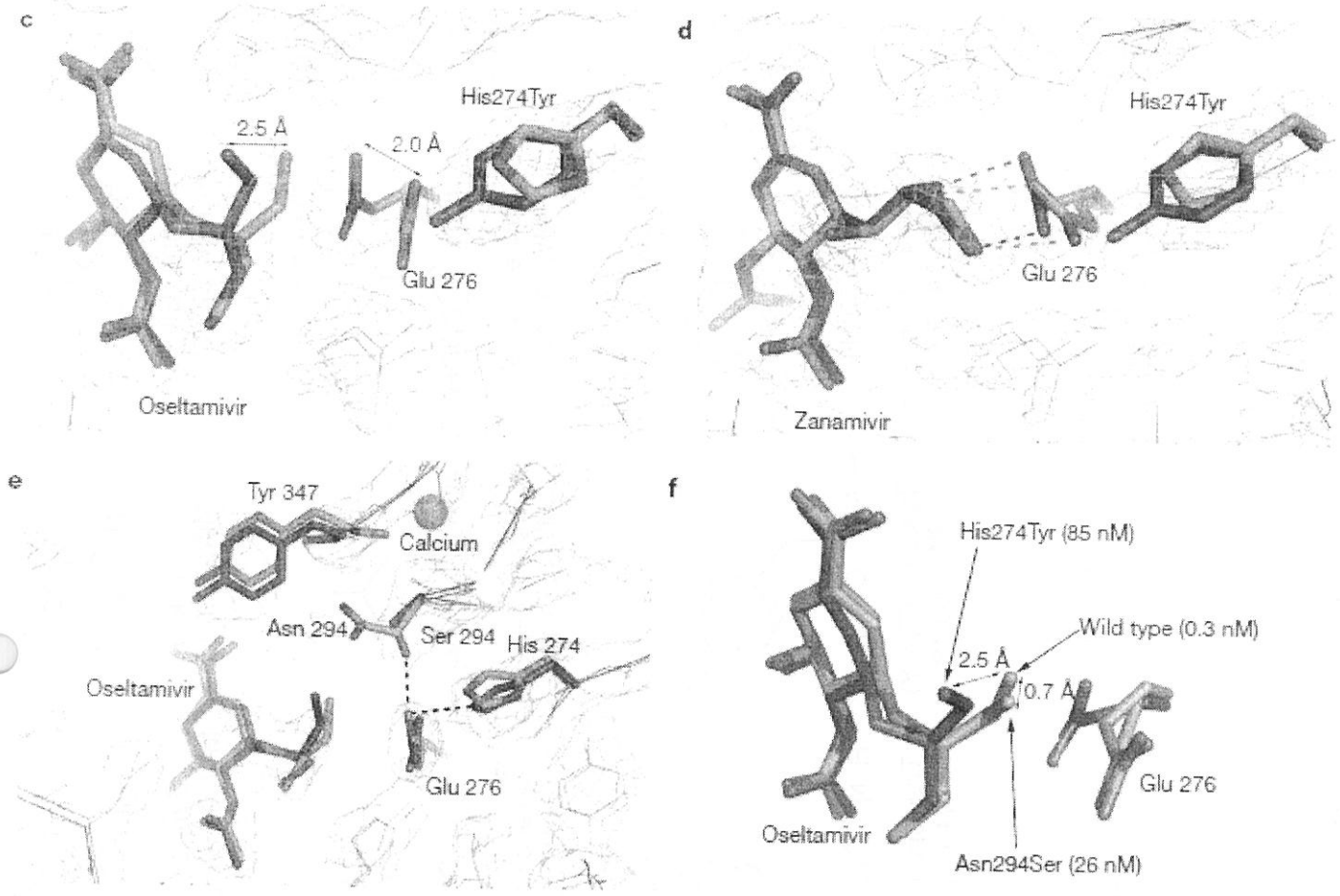


Figure 9. Structure of N1 neuraminidase complexes.

a, Sialic acid docked into the active site of wild-type N1 NA from superposition of the sialic acid complex of N2.

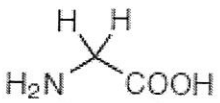
b, The structures of sialic acid, zanamivir and oseltamivir are shown in similar orientations with selected carbon atoms numbered.



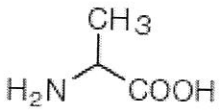
c, His274Tyr in complex with oseltamivir.
d, His274Tyr in complex with zanamivir.
e, Asn294Ser in complex with oseltamivir.
f, The conformation of oseltamivir and Glu 276 from three complexes is shown after superposition using protein atoms only.

Since Tamiflu is not omnipotent, what kind of anti-influenza drug can take the place? If there was one, what kind of structure should it have in your imagination.....

Small

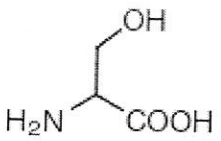


Glycine (Gly, G)
MW: 57.05

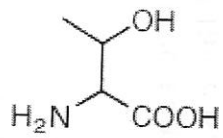


Alanine (Ala, A)
MW: 71.09

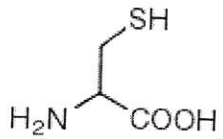
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

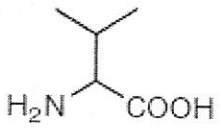


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

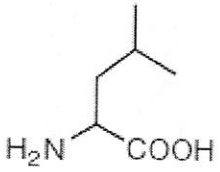


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

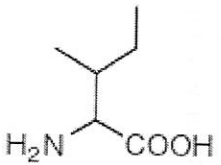
Hydrophobic



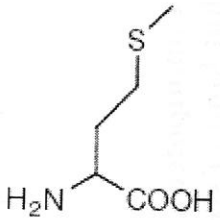
Valine (Val, V)
MW: 99.14



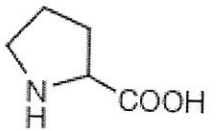
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

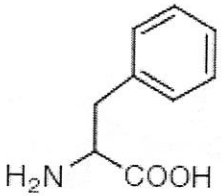


Methionine (Met, M)
MW: 131.19

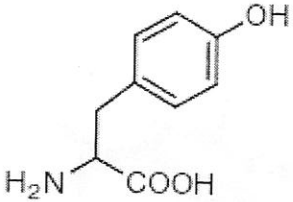


Proline (Pro, P)
MW: 97.12

Aromatic



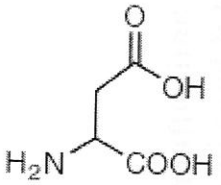
Phenylalanine (Phe, F)
MW: 147.18



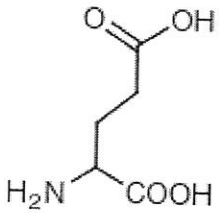
Tyrosine (Tyr, Y)
MW: 163.18

Tryptophan (Trp, W)
MW: 186.21

Acidic

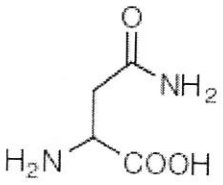


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

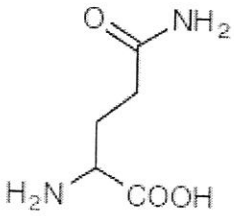


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

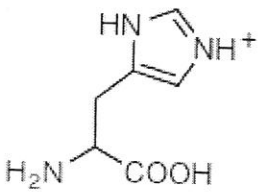


Asparagine (Asn, N)
MW: 114.11

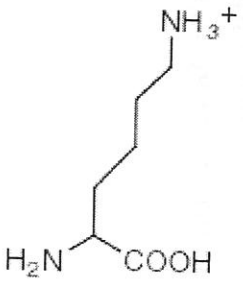


Glutamine (Gln, Q)
MW: 128.14

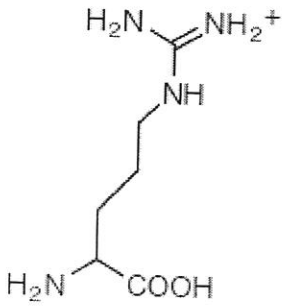
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48