Discipline

GENERAL PHARMACEUTICAL CHEMISTRY

SEMESTER VI
LESSON 12

INFLUENZA VIRUS ANTIVIRAL DRUGS AGAINST COLDS AND FLU

QUESTIONS FOR THE LESSON

1. The flu virus. Characteristics of the virus.
2. Classification of influenza viruses. Their designation.
3. The structure of the influenza virus particle.
4. The life cycle of the influenza virus.
5. The mechanism of escape of the influenza virus from the immune system.
6. Antiviral drugs – inhibitors of the M2 ion channel. Amantadine, remantadine.
7. Antiviral drugs – neuraminidase inhibitors. Oseltamivir, zanamivir. Favipiravir.

1. THE FLU VIRUS. CHARACTERISTICS OF THE VIRUS

The flu - (French. grippe, gripper - to grasp, grab, scratch; Ital. influenza – the influence of cold) is an acute respiratory viral human disease characterized by damage to the respiratory tract, fever, general intoxication, impaired activity of the cardiovascular and nervous systems. Periodically, usually during the cold season, the flu virus spreads in the form of epidemics and pandemics. Annual flu epidemics affect up to 10-20% of the human population. According to WHO estimates, from 250 to 500 thousand people die every year during epidemics in the world. At the same time, most of the deceased are over 65 years old.

Influenza is associated with high mortality during pandemics, epidemics, and sporadic outbreaks. Flu pandemics occur approximately every 50 years, while epidemics occur more frequently. Outbreaks of seasonal flu occur almost all over the world every year.

There are cases in history when the flu has become widespread. The most widespread of the known is the Spanish flu (H1N1) pandemic of 1918-1919, when about 500 million people were infected, which at that time accounted for about 30% of the world's population. At the same time, the death toll is estimated at 20-100 million people.

Special attention should be paid to the "bird flu". This disease has low virulence, but an extremely high level of pathogenicity. WHO believes that avian influenza poses a potential threat to humans because it can develop into a worldwide pandemic if the virus undergoes gene recombination with other types of influenza virus, such as, for example, the swine flu virus, a disease with low pathogenicity but high virulence.

Influenza has been studied quite well and has been known for a long time, but

due to the fact that influenza viruses mutate rapidly, there are still no effective anti-influenza drugs.

CLASSIFICATION OF INFLUENZA VIRUSES

Influenza viruses:

- The first influenza virus was isolated from birds (chickens) in 1901 in Italy, but was identified as the causative agent of "bird plague" or "chicken plague" (50 years after that, it was established that the avian plague virus is one of the avian influenza "A" viruses).
- Then the influenza A virus was isolated from pigs by the American scientist Richard Shoup in 1931.
- The human influenza virus was isolated in 1933 in England at the National Institute of Medical Research by virologists Wilson Smith, Christopher Andrews and Patrick Laidlaw.
- In 1940, the influenza B virus was isolated. In 1951, the influenza virus "C" was isolated using the technology of virus cultivation "on chicken embryos". In 2003, after four years of work in laboratories, the virus that caused the 1918 pandemic was recovered and studied.

Contrary to popular belief, the influenza virus causes a person to have a strong immune system. The recurrent incidence of influenza is the result of the variability of the influenza virus (antigenic drift and antigenic shifts).

Influenza viruses (Latin Influenzavirus) are four monotypic genera of viruses from the family of orthomyxoviruses (Orthomyxoviridae), whose representatives cause diseases.

CLASSIFICATION

The same type of influenza virus genera are named (October 2018 was called October Influenzavirus, B, D, c. year):

- Alphainfluenzavirus,
- Betainfluenzavirus,
- Gammainfluenzavirus,
- Deltainfluenzavirus

They belong to the family Ortomyxoviridae, which in addition to these four genera includes other viruses, thoroviruses and quaranfilviruses. Since 1980, on the recommendation of the WHO, the designation of influenza virus strains includes:

- type;
- place of isolation (geographical origin of the strain);
- the index assigned in the laboratory (the serial number of the strain);
- year of allocation;
- the name of the animal (for animal viruses only) that is the natural host of the virus (from which the virus was isolated).
- the index of surface proteins, placed last and enclosed in parentheses, makes

sense only for type "A" virus;

Alphainfluenzavirus

Monotypic genus, Influenzavirus A, Influenza virus type "A". It causes flu outbreaks every year, often epidemics, and occasionally pandemics. This is due to the high degree of variability of the virus: type "A" virus is susceptible to both antigenic shift and antigenic drift.

In 2018, influenza viruses of subtypes are circulating among humans A(H1N1) and A(H3N2).

The natural reservoir of the influenza A virus is waterfowl. Sometimes it is transmitted to other birds, and as a result, it can infect domestic birds, pets from them, and then humans, leading to epidemics and pandemics. In birds, the virus infects epithelial cells in the digestive tract, in humans – epithelial cells of the respiratory tract.

Within the species Influenza A virus, several serotypes have been identified (observed in nature):

– H1N1, it caused pandemics of Spanish flu in 1918 and swine flu in 2009 (according to the old classification, there are three seropodotypes in it: Hsw1N1, H0N1 и H1N1[8]);
– H1N2 Endemic to humans, pigs, and birds;
– H2N2, which caused the Asian flu pandemic in 1957;
– H3N2, caused the Hong Kong flu pandemic in 1968;
– H5N1, who caused the avian flu pandemic in 2004;
– H6N1, It was detected in a single patient and was cured.;
– H7N2
– H7N3
– H7N7, It is associated with human conjunctivitis and has a high potential for epizootics;
– H7N9, Responsible for six epidemics in China, it now has a high pandemic potential among other influenza A serotypes;
– H9N2
– H10N7
– H17N10
– H18N11

Betainfluenzavirus

Monotypic genus, former name: Influenzavirus B. Influenza virus type "B". It is not subdivided into subtypes, but it can be subdivided into lines. The natural reservoir of Influenza virus B is humans. The virus affects the upper and lower respiratory tract, and the symptoms are similar to those caused by the type A virus. It has a limited number of lines, which is probably why most people acquire immunity to Influenzavirus B at an early age.

The influenza B virus causes epidemics, but rarely enough, once every 4-6 years they develop slowly compared to those caused by the A virus and, as a rule, cover 8-10% of the population. There are two known epidemics in the USSR with peaks in the spring of 1963 and the spring of 1974. In addition, the "B" virus was

present in many epidemics together with the "A" virus. The type B influenza virus is similar to the type A virus, they are difficult to distinguish under an electron microscope. The virus genome consists of eight RNA fragments.

Gammainfluenzavirus

Monotypic genus, former name: Influenzavirus C. Influenza virus type "C". Influenza virus "C" is detected in patients less frequently than "B" and "A", it usually leads to mild infections, is not dangerous for humans and does not pose a problem for public health. The natural reservoir of Influenzavirus C is humans, it also infects pigs and can be transmitted between pigs in experiments. Affects the upper respiratory tract, mainly in children, with mild clinical symptoms. Serological studies have revealed a global

the prevalence of the type "C" virus. Most people become immune to it at an early age. The influenza C virus causes multiple diseases and almost never causes epidemic outbreaks. It contains 7 fragments of the genome. It has only one envelope (penetrating through the wall of the victim cell) glycoprotein HEF (English hemagglutinin esterase fusion - fusion of hemagglutinin and esterase), which plays the role of both glycoproteins (HA and NA) of viruses of types "A" and "B". It is not divided into subtypes. 6 genome lines have been identified, but due to the frequent recombination of different lines, new variants have recently emerged that pose an epidemic threat.

Deltainfluenzavirus

Influenzavirus D (Influenza virus type "D") - mainly infects cattle. According to reports, they do not infect people and do not cause them diseases. There are signs of transmission of the type "D" virus from cows to humans – people in contact with cows have antibodies to it, but no infected people have been identified. Structurally similar to the type C virus. Influenzavirus D contains 7 fragments of single-stranded RNA, at least 50% of the amino acids match the type C virus. This virus has been isolated into a separate species because when its genetic material is mixed with the "C" virus, they do not produce viable offspring.

2. THE STRUCTURE OF THE INFLUENZA VIRUS PARTICLE.

The influenza virus is an RNA-containing virus and belongs to the Orthomyxoviridae family. This family is divided into six genera: Influenza virus types A, B and C, Thogotovirus, Isavirus and Quaranjavirus. The greatest danger to humans are viruses belonging to the genus Influenzavirus type A. Viruses of this genus infect humans, mammals, birds, and it is viruses of this genus that cause epidemics and pandemics of influenza. Viruses belonging to the genera Influenzavirus types B and C are also capable of infecting humans, but they do not pose a serious threat to life. The genus Isavirus includes viruses that infect fish. The genus Thogotovirus includes viruses that infect rodents. Viruses belonging to the genus Quaranjavirus are capable of infecting both arthropods and vertebrates. These viruses can infect humans, but infection is usually asymptomatic.

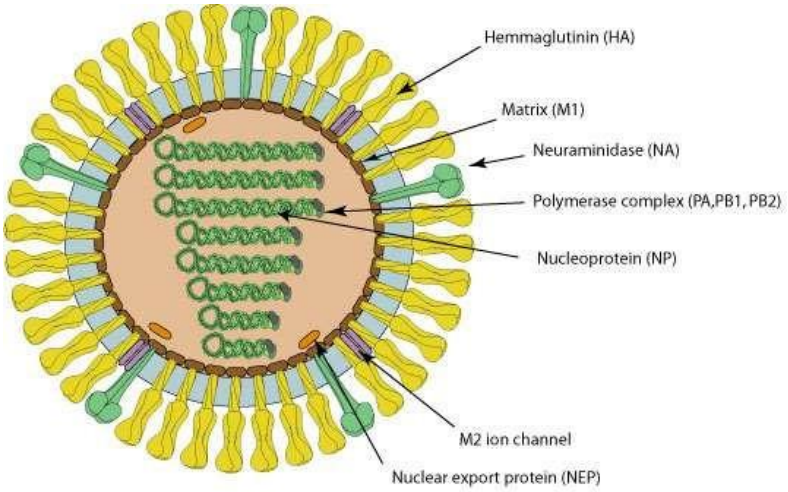


Figure 1. Structure of the influenza virion

➢ The virion (infectious particle) of influenza has the shape of a sphere or approaching spherical, its diameter is 80 – 120 nm.

➢ The influenza virus is an enveloped virus: the outer layer is a lipid membrane into which "spikes" are inserted: glycoproteins and matrix protein M2, which forms ion channels.

➢ Viral glycoproteins, hemagglutinin and neuraminidase, are located on the surface of the virion. They are denoted by Latin letters.

➢ "H" and "N", respectively. Glycoproteins hemagglutinin and neuraminidase are key proteins for the reproduction of virus types "A" and "B". Hemagglutinin is used to enter the cell, and neuraminidase is used to exit it.

➢ In addition to hemagglutinin and neuraminidase, the M2 protein is included in the lipoprotein envelope of the virion, which is an ion channel that plays an important role in the process of virus entry into the cell. The M1 matrix protein is located under the lipid membrane, it forms the inner layer of the virus shell, gives stability and rigidity to the outer lipid shell.

➢ The M1 matrix protein connects the lipid envelope (inside the virion) with eight ribonucleoproteins, complexes of viral RNA with RNA polymerase (the genome of the virus, which carries genetic information about the envelope and internal proteins of the virus). The genome is represented as a ribonucleoprotein complex, which contains fragments of segmented single-stranded RNA attached to the protein nucleoprotein (NP), and three polymerase complex proteins: PB1, PB2, and PA.

3. THE LIFE CYCLE OF THE INFLUENZA VIRUS

The flu virus does not destroy every cell. Typically, the following happens: the virus enters the cell, multiplies and exits it in an organized manner – the cell remains intact and sometimes alive. At the same time, the virus is able to exploit the cell several times.

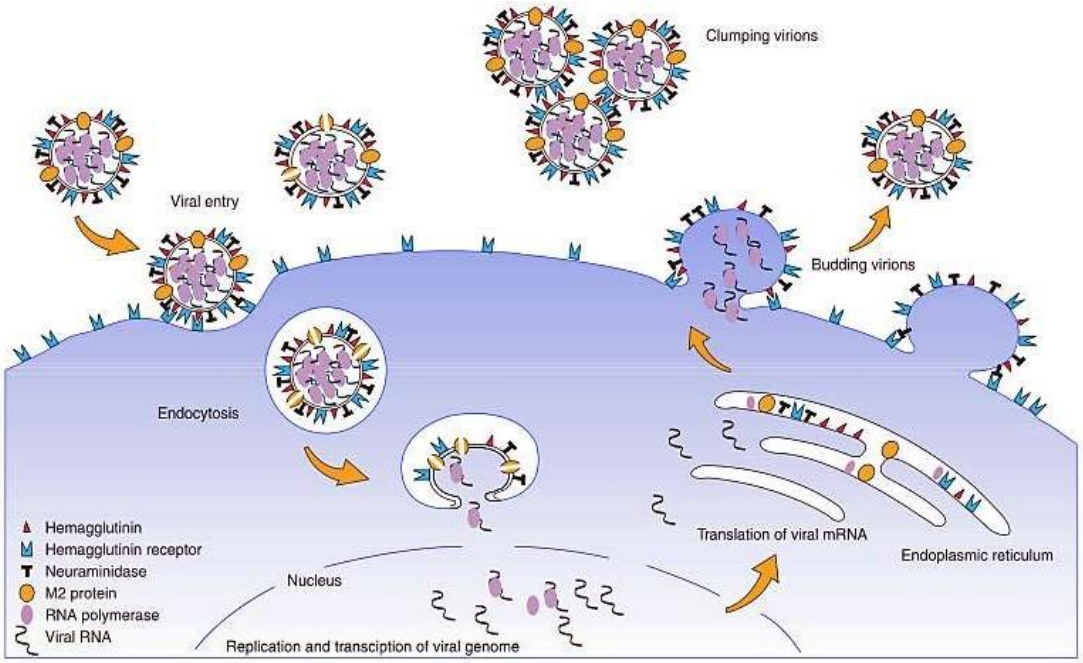


Figure 2. Scheme of the influenza virus life cycle

– Virus replication is initiated by the interaction of hemagglutinin with specific receptors on the surface of epithelial cells of the respiratory tract.

– The virion enters the cell by endocytosis. In the acidic environment of the endosome, the M2 ion channel opens, protons penetrate into the virion.

– This leads to its undressing and dissociation of M1-RNP complexes.

– The viral genome is integrated into the nuclear apparatus of the cell.

– After replication, the genomic and mRNA migrate into the cytoplasm, associate with viral proteins and the cell membrane.

– After budding, the daughter virions remain bound to the outer cell membrane and stick together due to the interaction of viral hemagglutinin with glycoproteins captured along with the membrane of the host cell. The release of virions becomes possible after the cleavage of sialic acid residues from cellular glycoproteins under the action of neuraminidase.

In addition to replicating viral RNA, viral particles in the cell synthesize proteins, one of which, PB1-F2, is released from the cell, enters the lungs through the bronchi in humans and destroys macrophages of lung tissue, thus provoking lung infections, in particular, pneumonia.

4. THE MECHANISM OF FLU VIRUS ESCAPE FROM THE IMMUNE SYSTEM

The mechanism of escape of the influenza virus from the immune system is associated with a mutation of genes encoding surface antigens – hemagglutinin and neuraminidase. The influenza A virus is characterized by high variability, which is due to two features of the genome.

- The first property, the fragmentation of the virus genome, makes it possible for two viruses of the same type to exchange genes if they both infect the same cells. In this case, two sets of identical genes of two different viruses are synthesized in the cell, and viruses with different combinations of the same genes and with a different set of surface antigens appear in the offspring. Such viruses are called recombinants or reassortants (viruses with rearranged genes), and the phenomenon is an antigenic shift.
- The second property of influenza viruses is the variability of their glycoproteins as a result of mutations, the antigenic differences are small at first, but gradually increase.

Influenza viruses survive in the air for up to 4 hours, while viruses of type "A" are more resistant than "B". In dried and settled aerosol droplets, the virus persists on bed linen for up to 2 weeks, and in indoor dust for up to 5 weeks. Wet cleaning with disinfectants completely disinfects the room.

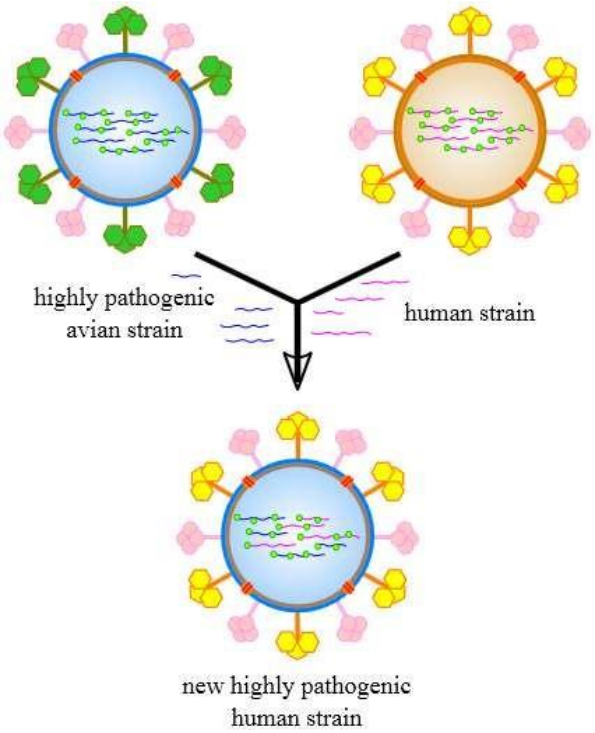


Figure 3. Scheme of occurrence of the antigenic shift of the influenza virus

4. ANTIVIRAL DRUGS – NEURAMINIDASE INHIBITORS AND ION CHANNEL INHIBITORS – M2

Due to the prevalence of the virus in winter, WHO monitors it from May to August in the southern hemisphere, and from November to March in the northern hemisphere, and makes forecasts for the spread of the virus in the next half of the

year. It is assumed that antiviral drugs acting on a particular phase of the development of a viral infection in vitro can also be effective in vivo, especially as a preventive measure. In general, treatment with antiviral drugs should be started even before the onset of clinical manifestations of influenza, a later start of their administration is practically ineffective..

There are two classes of drugs:

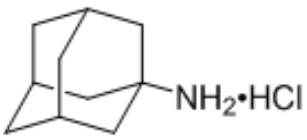
- M2 ion channel inhibitors
- Neuraminidase inhibitors.

Interferon preparations, which have antiviral, anti-inflammatory and immunomodulatory effects, represent a separate group.

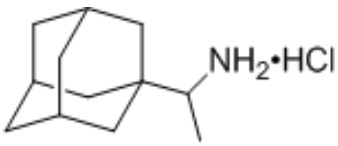
6. ANTIVIRAL DRUGS ION CHANNEL INHIBITORS M2. AMANTADINE, REMANTADINE

The transmembrane domain of the M2 ion channel is a target for adamantane-derived antiviral drugs. Amantadine (Symmetrel) was the first influenza-specific antiviral drug and has been licensed in the United States since 1966 for the treatment and prevention of influenza A. Rimantadine was licensed in 1993. Adamantylamines allosterically block the M2 ion channel, which leads to inhibition of virion stripping, RNA release, and blocking of viral replication. These drugs are effective only against influenza type A, since the influenza type B virus contains an ion channel type NB.

Rimantadine is 4-10 times more active than amantadine. Both drugs are bioavailable by oral and intranasal administration. Both are equally effective if treatment is started within 48 hours after the onset of symptoms. Most studies show a decrease in fever symptoms within 1-2 days, as well as a decrease in virus release. They are also useful as a preventive measure.



Amantadine



Rimantadine

AMANTADINE

The method of obtaining amantadine was patented in 1967. It consists in obtaining 1-bromodamantane, which, when reacted with acetonitrile in sulfuric acid, gives 1-acetylaminoadamantane, hydrolyzable to 1-adamantylamine– the base of amantadine. In 1976, it was found that 1-adamantol nitrate can be used instead of 1-bromodamantane..

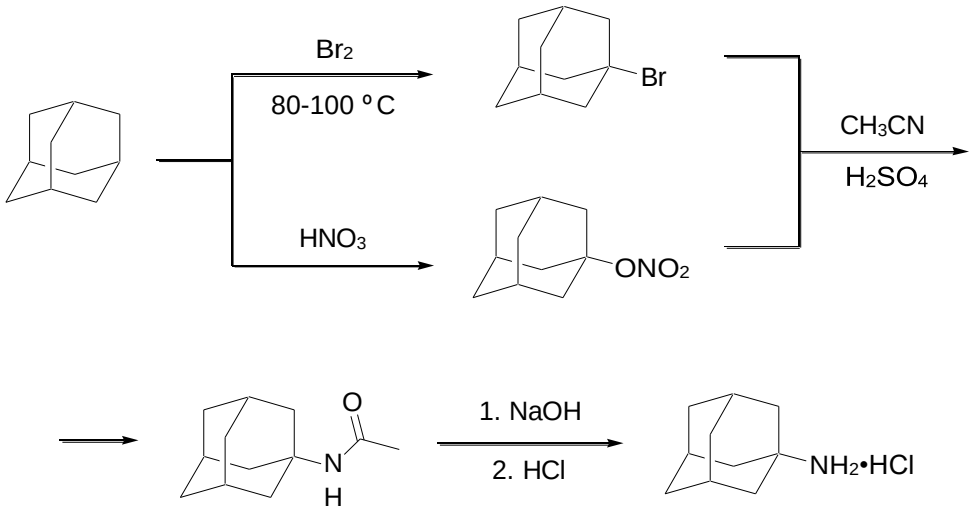


Figure 4. Amantadine synthesis scheme

REMANTADINE

The synthesis of rimantadine was patented by du Pont in 1965. Currently, its modified version in 1984 is in use. In the first stage, adamantane is carboxylated with formic acid in carbon tetrachloride. The process is initiated by the formation of the adamantinium cation under the action of tert-butanol and sulfuric acid. Then, by treatment with thionyl chloride, adamantoyl chloride is obtained, which reacts with ethoxymagnesium diethyl malonate to form adamantylmethyl ketone. The latter is converted to ketoxime and reduced with hydrogen on platinum in an acidic medium to rimantadine

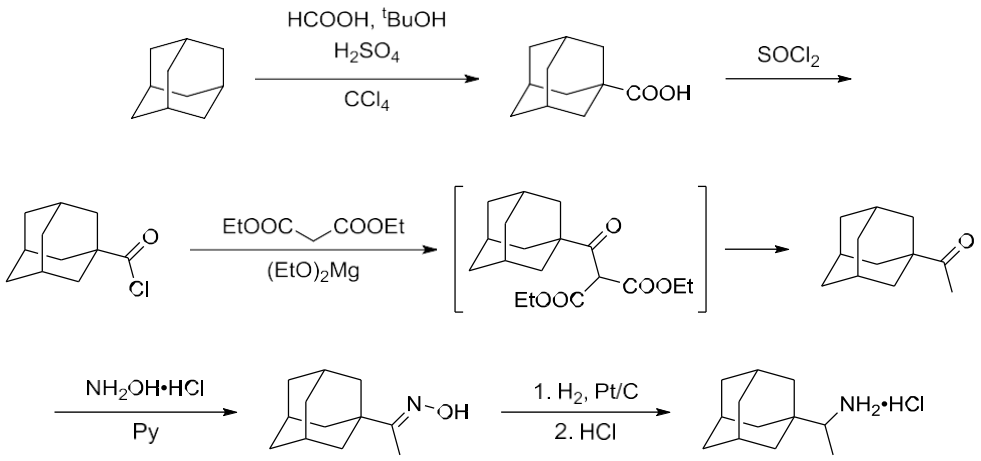


Figure 5. Rimantadine synthesis scheme

The use of adamantane derivatives is limited by side effects from the central nervous system and gastrointestinal tract, as well as the development of drug resistance. Although side effects are less common with rimantadine, use in elderly patients is still limited to lower doses. Resistance to amantadine and rimantadine may occur within the first 3-5 days in 50% of children, the elderly, and immunocompromised patients. The mechanism of resistance is apparently related to point mutations of amino acids of the M2 protein. The rapid and widespread development of resistance significantly limits the use of these drugs as therapeutic and preventive regimens, especially in environments with close contacts. Viruses are cross-resistant to both drugs. According to the US Centers for Disease Control and Prevention, 100% of seasonal H3N2 influenza samples in 2009 proved to be resistant to rimantadine. Thus, it is no longer recommended for the treatment of influenza.

6. ANTIVIRAL DRUGS NEURAMINIDASE INHIBITORS OSELTAMIVIR, ZANAMIVIR. FAVIPIRAVIR.

If the discovery of the antiviral properties of amantadines was accidental, the development of sialidase inhibitors is an example of the successful implementation of a rational structure-oriented approach. The structure of influenza type A and B neuraminidase was deciphered using X-ray crystallography in the early 1990s. Neuraminase is a homo-tetrameric glycosylated protein. Four identical subunits are connected in the form of a square and are connected to the shell of the virion by a long protein leg. The active center is located in a recess on the upper surface of each subunit. Despite the high variability of neuraminase, the structure of the active center remains highly conserved not only for influenza A strains, but also for influenza type B. This makes this enzyme an extremely attractive target for antiviral agents.

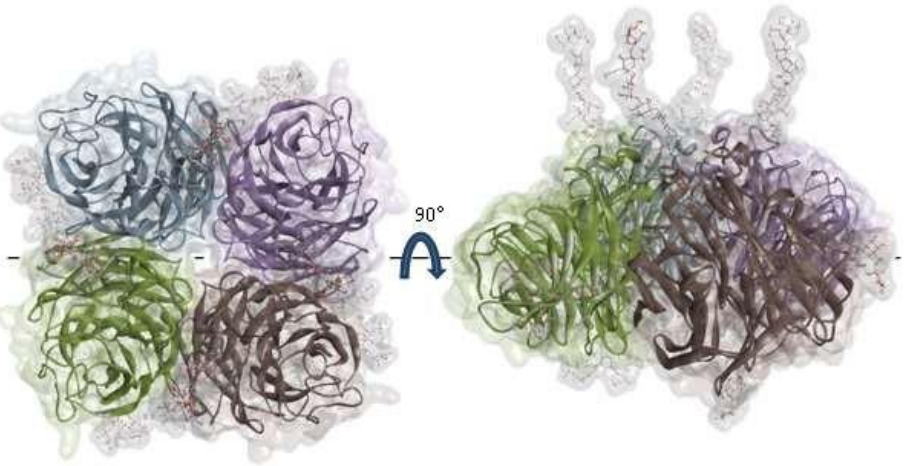
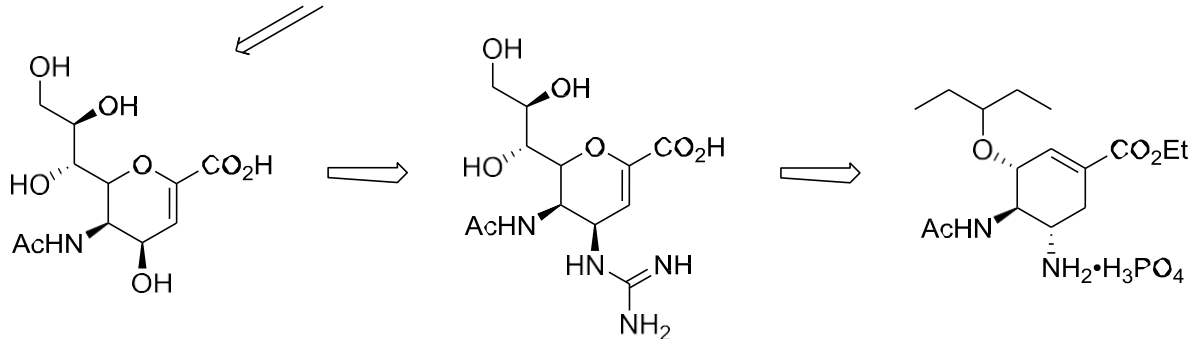


Figure 6. Three-dimensional model of influenza virus neuraminidase

As a result of attempts to create the enzyme, a non–selective enzyme inhibitor, an unsaturated derivative of sialic acid, was discovered Neu5Ac2en.

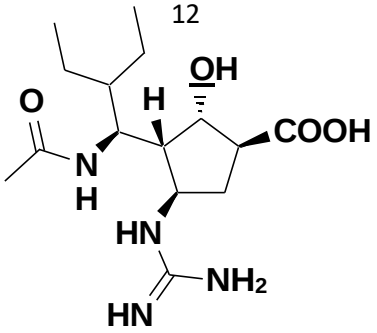


Neu5Ac2en Zanamivir Osteltamivir

Computer modeling methods made it possible to further obtain more active and highly selective inhibitors, of which two are currently approved for clinical use: oseltamivir (Relenza) and zanamivir (Tamiflu).

Both drugs have low bioavailability: about 2% when taken orally and 10-20% when inhaled. Medications are contraindicated in children under 5 years of age. There is no reliable information on whether taking oseltamivir affects the incidence of complications, such as the risk of hospitalization or pneumonia. In addition, influenza A viruses H5N1 ("bird flu") and H1N1 ("swine flu") were found to be insensitive to these drugs.

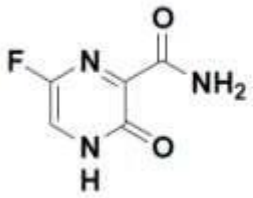
For this reason, efforts have been made to create an effective antiviral drug. In December 2014, a new drug, Peramivir, was approved, which proved effective against a wide range of influenza A and B virus variants, including H5N1 avian influenza and H1N1 swine flu..



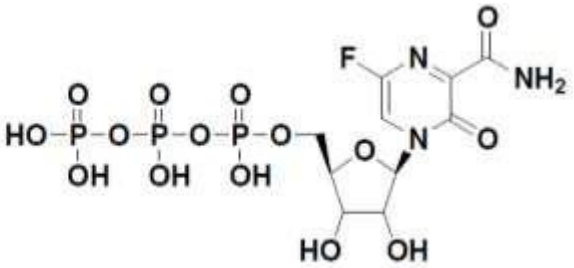
Peramivir

Inhibitors of RNA-dependent RNA polymerase

The new antiviral drug Favipiravir from the company BioDefense Therapeutics has successfully passed the second phase of clinical trials. The third phase is currently underway. It turned out that the remedy quickly relieves the six main symptoms of the flu, is well tolerated by patients and does not cause serious side effects. Favipiravir has a wide range of antiviral effects. It is active against all variants of the influenza virus..



Favipiravir



Ribofuranosyl-5’-triphosphate naphtha form

The mechanism of action of favipiravir is as follows. Once in the cell, favipiravir is converted to the ribofuranosyl-5’-triphosphate form, which is a substrate of RNA-dependent RNA polymerase and which competitively inhibits the incorporation of 5’-adenosine triphosphate and guanosine into viral RNA and thereby blocks its elongation and/or induces fatal mutations for the virus in the viral genome.

However, the use of favipiravir as a medicinal product remains questionable, as the compound has been found to have a teratogenic effect.