

Common Antiviral Agents

Structure, mechanism and spectrum of activity

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THE PHYSICIAN'S DESK REFERENCE lists more than 60 antiviral drugs presented either as oral, parenteral or topical dosage forms, singly or as combinations. Many are based on the theories of the *antimetabolite-sulfonamide* era (1935 to 1960), others are unique and developed since the outbreaks of herpes and AIDS (1981 to present). None can cure a viral disease, and drug resistance develops in every case. The great majority of these are used for HIV infections with marvelous outcomes in the prolongation of life, especially when used in combinations. Only about 10 are used for the herpes and influenza infections that most physicians see; fewer are available for blood-borne viruses such as hepatitis B and C and the hemorrhagic fatalities. None are useful in other viral diseases of great frequency such as those that attack either the upper respiratory tract (the common cold), the gastrointestinal tract (enteroviruses and noroviruses) or multiple organ sites (adenovirus).

It is the purpose of this paper to review, for specialist and non-specialist alike, the status of antiviral drug development in the pharmaceutical industry. To this end, we will present a compilation of current antiviral agents, a summary of major virus targets and a discussion of structure-activity relationships.

The Major Drugs

Table 1 lists and describes some of the standard drugs used for many years for herpes and influenza infections. The majority of the compounds are given orally and are in three categories:

- a) nucleoside antimetabolites,
- b) enzyme inactivators or
- c) entry inhibitors.

These design modalities still remain in the forefront of antiviral drug discovery. The cell culture assay remains the primary test for activity.

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Table I: Some Common Antiviral Agents (Non-HIV). The mechanisms (column D) outline virus life cycles that suggest targets for antiviral interventions. (Legend on next page.)

Drug (A)	Route (B)	Structure (C)	Mechanism (D)	Indication (E)
Acyclovir (Zovirax) – Nobel Prize (1988)	Oral, topical, intravenous	Synthetic guanine nucleoside (novel open “sugar”)	Purine antagonist preferentially blocks viral DNA synthesis. Well tolerated (a selective DNA inhibitor)	DNA viruses HSV-1, 2, VZV, suppression of genital herpes and cold sores, less effective EBV and CMV
IUDR (1968); Pioneer Antimetabolite nucleoside (Stoxil) (first FDA approved)	Topical ophthalmic	5-iodo-2deoxyuridine	Original fraudulent thymine nucleoside, inhibits DNA synthesis both viral and cellular (a non-selective DNA inhibitor)	HSV (herpes keratitis) (host toxicity, accompanies the antiviral effect)
Trifluridine (1980)	Topical, ophthalmic	Tri-fluorinated uracil nucleoside	Inhibits DNA base pair bonding (partially selective DNA inhibitor)	HSV and HSV-2 (keratitis) (minimal cross resistance to IUDR)
Vidarabine (1976)	Oral	Unusual arabinose purine	Blocks DNA, less virus resistance than IUDR.	Herpes, VZV, Vaccinia
Foscarnet (1991)	IV, intravitreal, Specialty drug (significant toxicity)	An organic analog of inorganic pyrophosphate (simplest organic structure)	Blocks pyrophosphate reactions required for DNA synthetase reactivity	CMV Retinitis, resistant herpes infections, EBV, VZV, severe Roseola
Ribavirin (Virazole) (1972 – FDA 1980)	Oral, inhalant for RSV in children with caution	Synthetic nucleoside with triazole ring	Guanosine antagonist, blocks DNA and RNA synthesis	RNA viruses and DNA especially RSV, HCV with IF, broad spectrum, others, mutagen, toxic
Amantadine (Symmetrel) (FDA 1966)	Oral	1-adamantane-HCl (high drug resistance) (first approved influenza drug)	Blocks M2 protein site; affects attachment/entry and uncoating of virus	Influenza A such as H1N1, H2N2 (Asian), H3N2 (HK) (Inactive influenza B)
Rimantadine (Flumadine) (FDA 1994)	Oral	Ethyl amino derivative of Amantadine (high drug resistance)	Blocks M2 protein site; affects attachment/entry and uncoating of virus	Influenza A such as H1N1, H2N2 (Asian), H3N2 (HK) (Inactive influenza B)
Oseltamivir (Tamiflu) (FDA 1999)	Oral (capsules, suspension)	Cyclohexene carboxylic ester acetyl amino PO ₄ (see later)	Inhibits the surface viral enzyme Neuraminidase, blocks release of virus (see later)	Influenza A and B. Various A subtypes (H1, H2, H3), H1N1, H2N2 and H3N2.
Fomivirsen (1998)	Intravenous injection	Oligonucleotide antisense molecule. Complimentary to virus gene sequence.	Binds to and mimics viral RNA causing translation errors	HCV, Dengue fever, Corona, West Nile, CMV Retinitis, others
Zanamivir (Relenza) (FDA 1999)	Inhalant	Dehydro form of sialic acid- ([mimics NANA] dehydro, N-acetyl neuraminic acid) (DNANA) (see later)	Inhibits the surface viral enzyme Neuraminidase, blocks release of virus (see later)	Influenza A and B. Various A subtypes (H1, H2, H3), H1N1, H2N2, H3N2 (HK); minimal drug resistance
Enfuvirtide (2003)	Subcutaneous	Fusion inhibitor	Prevents fusion to CD4 receptor; blocks entry	HIV
Topical Antiseptics (FDA) (CFR 21, OTC Monograph Jan. 1994) “Hand Sanitizers”	Skin	Ethanol, isopropanol, quaternary ammonium compounds. Iodine, H ₂ O ₂ , phenolics	Act at surface of virus (denature protein, dissolve surface lipids, oxidize enzyme or receptors). FDA and WHO recommended or surveyed	Health care personnel, pre-surgical and general hand hygiene. Disinfectants (EPA) (CFR 113) public health destruction of pathogens in the environment (FIFRA)

Legend

- VZV – Varicella zoster virus (chicken pox, shingles (DNA))
- EBV – Epstein Barr Virus (infectious mononucleosis (DNA))
- CMV – Cytomegalo virus (DNA) – frequent complication in AIDS patients (retinitis)
- RSV – Respiratory Syncytial Virus (RNA)
- IF – Interferon
- HCV – Hepatitis C Virus (RNA)
- HSV – herpes Simplex Virus (DNA)
- H1N1 – General subtype designation for various pandemics (Spanish Flu 1918, Puerto Rico 1934, Swine Flu 2009) and routine seasonal flu (Brisbane, Solomon Islands, etc.) (RNA) (very common)
- H2N2 – Asian Flu 1957, pandemic (RNA)
- H3N2 – 1968 “Hong Kong Flu,” pandemic (RNA)
- HK – Hong Kong
- M2 Protein Proton (H⁺) entry site that channels influenza A into the cell, but not influenza B (see later)

Theory of Some Important Antiviral Categories

1. Modified Nucleosides (HSV) Group

These compounds inhibit the synthesis of DNA and are used primarily for the herpes virus group. The chemist takes three approaches to the synthesis of these compounds: altering the nitrogenous base (purine or pyrimidine), altering the sugar (ribose or deoxyribose, cyclic or acyclic) or altering both. Examples are given later. When the inhibition of host DNA is equal to or greater than the viral DNA, the compound is toxic (5-Iodo-deoxyuridine). The chemist strives to perfect a molecule that preferentially inhibits the viral DNA and not the cellular DNA and thus is less toxic (e.g. Acyclovir).

2. Reverse Transcriptase Inhibition RTI's (HIV)

Chemotherapy is more complicated for HIV, but the target is obvious. It is a unique enzyme called Reverse *Transcriptase*. This enzyme is responsible for making viral DNA from viral RNA which is the exact opposite of normal nucleic acid metabolism. The HIV armamentarium as shown later includes a battery of *nucleoside reverse transcriptase inhibitors* (NRTI's), a series of *non-nucleoside reverse transcriptase inhibitors* (NNRTI's) and *nucleotide analogue inhibitors* (NARTI's) used singly or in combination. The NNRTI's bind directly to the enzyme and thus are not strictly antimetabolites.

3. Other Enzyme Inhibitors (Influenza and HIV)

More recent than the nucleoside and RTI compounds are the antivirals that effect either release of virus (neuraminidase inhibitors for influenza) or maturation of virus (protease and integrase inhibitors for HIV). These compounds react with cat-

alytic sites on their respective enzyme and block formation of the enzyme-substrate complex.

a. Neuraminidase Inhibitors (Influenza)

These are synthetic organic chemicals which bind to the active catalytic site buried within the enzyme. In neuraminidase, this site is reserved for the destruction of sialic acid (NANA) and is divided into three “pockets.” The inhibitors (e.g. Tamiflu, Relenza) are structurally related to the sialic acid only in terms of the manner in which sialic acid affects hydrogen bonding to the three pockets. The three bonding sites in the catalytic site are chemically as follows: pocket 1 – glucose, arginine and aspartic acid; pocket 2 – isoleucine, arginine and alanine; pocket 3 (which is the largest) - glucose, aspartic acid, arginine, tryptophane, serine and isoleucine. An effective anti-influenza drug must fit by charge, hydrogen bonding and lipophilicity into the pocket.

b. Protease Inhibitors (PI's) (HIV) – Powerful advances for HIV treatment.

Certain proteolytic enzymes are required for maturation of the HIV particle. The immature HIV protein has to be split into fragments so that the virus can incorporate RNA and be released. This occurs as the virus buds from the cell. Examples of these inhibitors, which occupy and block the catalytic site, are Tipranavir, Amprenavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir and Saquinavir. This therapy has provided major gains in the treatment of AIDS. Some protease inhibitors can also be used with HBV and HCV infections. Protease inhibitors (developed in the 1990's) have had an enormous impact on survival.

c. Integrase Inhibitors (HIV)

Integrase is a viral enzyme that integrates (inserts) the HIV genetic material into human DNA to promote synthesis of the intact virus. Like PI's, integrase is a later development in AIDS therapy. Compounds such as these do not interfere strongly with host metabolism and tend to be less toxic. The enzyme is inhibited by drugs such as Raltegravir and Elvitegravir. This HIV enzyme attaches to and enters host DNA. Once inside the genome, it prepares the DNA to receive a strand of virus RNA. This strand enters and is modified to produce a viral DNA. This then produces mRNA encoded for the viral proteins at the ribosomes of the host cell.

4. M2 Protein Target (Inhibition of Uncoating) (Influenza)

The M2 protein is the third surface protein on the surface of influenza A (it is absent on influenza B). The M2 protein, a complex tetramer polypeptide, is a proton-selective ion channel buried in the envelope of influenza A. It enables hydrogen ions to enter the virion. When the internal portion of the virus achieves low pH, a dissociation (or “uncoating”) occurs. Drugs such as Amantadine and Rimantadine fit inside the channel-like configuration of the M2 protein. This blocks the acidification of the virus, uncoating is prevented and the eight RNA genes never enter the cell.

5. Fusion Inhibitors (FIs)

Fusion inhibitors, which work outside the cell, are useful when virus has become resistant to the drugs that work inside the cell (the RTI's and protease and integrase inhibitors). These inhibitors attach to the proteins on the surface of CD4 cells or proteins on the surface of HIV and prevent entry of the virus into the cell. Approved agents are Enfuvirtide (2003) and Maraviroc (2007). Others are now in clinical trial. The drugs are given by injection.

In summary, the protease, integrase and fusion inhibitors, mentioned above, are the more recent types of anti-HIV drugs. They are used in conjunction with standard NRTI's, especially when drug resistance is a problem. They are not anti-metabolite based.

Some Major Virus Groups

The following virus groups are causes of common diseases in man. They are the subject of antiviral research world-wide as scientists search for agents that may provide more satisfactory results, especially regarding spectrum and resistance. Most of the drugs discussed in this report are indicated for herpes, influenza and AIDS, the groups for which drug discovery has been successful.

1. Human herpes Virus (HHV 1 to HHV 8) (Mucocutaneous pathogens) (DNA)

There are at least six approved anti-DNA drugs for treatment of herpes infections, especially herpes Simplex Virus-1 (HHV-1) and herpes Simplex Virus-2 (HHV-2), the causes of mucocutaneous, oral, eye and genital infections. Infections go through a symptomatic stage and then the virus retreats to a latent phase remaining in regional neurons. This is how they survive. The main drug is *Acyclovir* (for which there are several derivatives). These drugs ameliorate symptoms and reduce shedding, but never produce a cure. Genital herpes is a major sexually transmitted disease (STD). It enters through breaks in the skin, wreaking mental as well as physical distress to those it infects.

2. Influenza Viruses (Members of the orthomyxovirus group) (Type A – Human, pigs, birds, other animals) (Type B – Humans and seals) (Type C – Humans, dogs, pigs) (RNA)

There are four approved drugs and they are discussed later in terms of two surface molecules, H and N. There are no drugs to interfere with metabolism as with herpes and AIDS. The Italian literature of the 15th and 16th centuries speaks of a recurring illness associated with cold weather which was called "influenza di freddo." Despite that chilly lineage, the virus of influenza was first isolated from a patient in Puerto Rico (1933, PR-8). The RNA genome consists of one chromosome with eight genes with either a spherical or filamentous structure up to 300 nM long. There are about 500 H and N protein spikes on surface with an H-N ratio of 4-5 to 1. The eight genes (which come from birds, pigs and humans) can move like joints or slipped discs and cross or assort inside of the infected cell with genes from another animal. The combinations of gene mutation (genetic

drift) and gene re-assortment (genetic shift) provide the background for influenza pandemics (which seem to occur every 10 to 40 years). Examples of the four most deadly gene shifts of the last 100 years are as follows: 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and Swine 2009 (H1N1). Although H1N1-1918 and H1N1-2009 contained the same gene sources, H1N1-2009 did not match the at least 20 million deaths of 1918. The distribution of human, bird and pig RNA was the same, but the differences per gene (e.g. different G:C and A:U ratios), were obviously different. The 1918 WWI tragedy remains unparalleled.

Schoolyard Rhyme – 1918:

"I had a little bird,
Its name was Enza.
I opened the window,
And in-flu-Enza."

(Although figures vary, it is believed that more people died from influenza in the years following WWI than in 35 years of AIDS.)

a. Discussion of H (attachment and initiation of infection) – No drugs available to inhibit this gene product.

The H or hemagglutinin molecule derives from gene H4 and is involved with both immunity and pathology. H is a complex glycoprotein trimer whose specific amino acid composition (epitope) recognizes the receptor site on the cell (sialic acid or N-acetyl-neuraminic acid, NANA). An antibody will block the epitope. Without immunity, epitope binds with receptor and the virus will subsequently enter the cell. The 16 different H structures all have a similar polypeptide composition, but differ in amino acid composition at certain sites. Only molecules H1, H2 and H3 will fit into the respiratory tract of humans; birds accept all 16. Initiation of infection is always determined by this gene. There are no drugs to inactivate hemagglutinin; only an antibody accomplishes this.

b. Discussion of N (release and contiguous spread) – Two drugs are available to inhibit this gene product.

The newly formed influenza particle tries to leave the cell but it is held back by its sialic acid bond. The surface enzyme, neuraminidase, destroys the sialic acid as the newly-formed influenza particle leaves the cell. To date, there are nine different N structures all with a similar polypeptide composition but differing in amino acid content or sequence at certain critical sites. Humans seem to accept and synthesize only N1 and N2. Birds favor N2, N3, N5, N7 and N9.

c. The H-N Combinations (total pathology)

Thus, as relates to the above, there can be different H1N1 viruses (e.g., H1N1 swine, H1N1 human, H1N1 avian, H1N1 equine), all with similar, but not identical H1 molecule composition, with different degrees of avidity and pathogenicity. And so, while genes H and N control the entry and exit of a viral particle, it is the concerted activity of the other six genes (and which animal and what ratio they came from) that control structure, maturation and eventual pathogenicity.

3. Adenoviruses (DNA)

There are no approved drugs even though the virus is similar physiochemically to herpes as to size, DNA, symmetry and shape (icosahedron). This is a large assemblage of more than 50 viruses producing highly infectious — some may be oncogenic — but non-fatal illnesses of adults and children, including croup, bronchitis, pneumonia, gastrointestinal and ophthalmic infections, tonsillitis, pneumonia, pharyngitis, hand to body fecal contamination and occasional water-borne water transmission (conjunctivitis). Respiratory and conjunctival epidemics are known to occur in the military. The virus (65 to 80 nM in diameter) contains a linear double-strand of DNA. The virus does not contain a lipid envelope and thus is resistant to common chemical germicides.

4. Enteroviruses (EV's) (RNA)

There are no approved drugs. This group is responsible for 10 to 20 million symptomatic infections per year, especially among children. The major EV's, are Coxsackie A, Coxsackie B, the ECHO viruses and Polio types 1, 2 and 3. There are 60 to 70 non-polio enteroviruses that cause infections in man. These are small naked icosahedrons (20 to 30 nM in diameter) RNA viruses resistant to certain chemical germicides. These pathogens are harbored in the large intestine infecting the following organs: brain (encephalitis), lungs (pleurodynia), GI tract (diarrhea), heart (myocarditis), meninges (aseptic meningitis) and pancreas to produce a variety of diseases from severe septicemia to mild cases of diarrhea, rash and fever, as well as a disease called acute hemorrhagic conjunctivitis (AHC) caused by EV 70. With the exception of Polio and Hepatitis A, there are no vaccines and, despite many leads, no approved drugs. Of recent interest is a more serious virus, EV71, which causes Hand, Foot and Mouth Disease (HFMD) especially in children, to epidemic proportions. Resistant to common germicides. The Norwalk agents are similar.

5. Rhinoviruses (RNA)

There are no approved drugs. There are small non-enveloped icosahedra resistant to certain chemical germicides similar to the enteroviruses. They produce the common cold. There are more than 100 serologically distinct strains. Neither vaccines nor antiviral drugs are available. The disease is mild and self-limiting. The virus only multiplies in the nose and succumbs at body temperature.

6. Retroviruses (HIV and AIDS) (RNA)

There are more than 30 approved drugs or drug combinations for this unique group of viruses. The genome consists of an inverted dimer of linear RNA, but is dependent on cellular DNA for replication. A lipoprotein envelope is on the outside of the virion, with a diameter 80 to 120 nM. Reverse transcription occurs as the infected cell converts viral RNA to DNA. The virus is sensitive to numerous drugs singly or in combination and is sensitive to common chemical germicides.

The complex series of reactions for the synthesis of HIV has offered the chemist several targets for chemotherapy, as follows:

- Attachment and fusion to CD4+ receptor
- Inhibition of enzyme reverse transcriptase to block RNA \longrightarrow DNA.
- Inhibition of enzyme integrase to block insertion of virus into host cell DNA.
- Inhibition of enzyme protease which organizes virus into mature form as it leaves the cell.

The control of these reactions requires multiple drug treatment (cocktails) also referred to as, "Highly Active Anti Retroviral Therapy," or HAART. HAART aims to suppress viral replication totally.

7. Hepatitis (RNA or DNA)

Hepatitis A (RNA, enteric), B (DNA) and C (RNA), both blood-borne, are produced by three major groups and are characterized as follows: HBV and HCV are both oncogenic; HCV is the most pathogenic. Therapy is available only for HCV (Ribavirin + IF) and HBV. Several drugs are under investigation. The bovine diarrhea virus (BDV) is a tissue culture surrogate for HCV, which is not propagated in vitro.

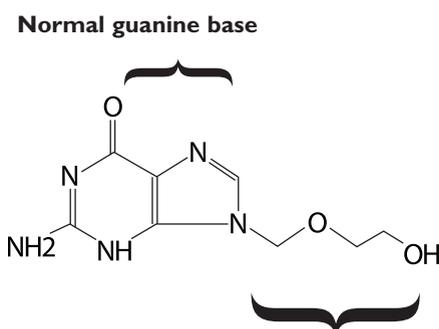
Anti-Viral Structure-Activity Relationships (Mini-Monographs)

The chemical structure of an antimicrobial drug may or may not provide hints as to its spectrum and/or mechanism of action. A partial assembly is provided. The principle of molecular modification in drug development is demonstrated.

Herpes and Related DNA Viruses

1. Acyclovir (an acyclic nucleoside)

The chemical name of acyclovir is 2-amino-1, 9-dihydro-9-[(2-hydroxyethoxy) methyl]-6H-purin-6-one. It has the below structural formula. There are several derivatives of Acyclovir, all the same mechanisms of action. Cross resistance occurs with all derivatives. This drug, like Penicillin, Isoniazid and Aureomycin before it, counts as one of the major chemotherapeutic advances of the 20th century. It was developed by Nobel Prize winners (1988) George Hitchings and Gertrude Elion, of Burroughs-Wellcome Inc., the first pharmaceutical company to mount comprehensive anti-leukemia, anti-tumor and anti-viral chemotherapy program.



Note: Open moiety replaces sugar (induces less toxicity)

Notes

Spectrum

Narrow X
Broad

Dosage

Oral X
Topical X
Intravenous X

Category

Antimetabolite X
Entry inhibitor
Enzyme inactivator

Viruses

Herpes group
HSV-1 (oral)
HSV-2 (genital)
CMV, VZV (less effective)
EBV

Drug Resistance

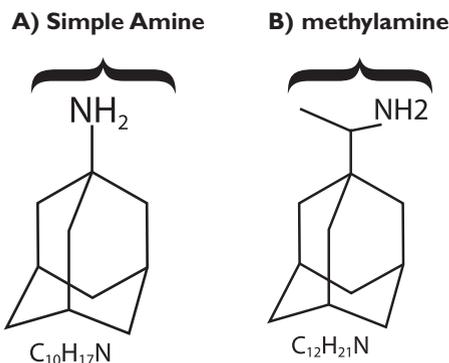
High
Low to moderate X

Note: Contains pyrimidine and sugar moiety and is a member of the halogenated pyrimidine group of chemotherapeutics, which include the well known anti-tumor and anti-psoriasis drug 5-fluorouracil, the first drug effective against solid tumors (1960).

Influenza

3. Amantadine (A) (Symmetrel) and Rimantadine (B) (Flumadine)

Amantadine hydrochloride is designated chemically as 1-adamantanamine hydrochloride. Amantadine and Rimantadine are both tricyclic amines with the basic cuboidal bonding configuration of carbon atoms as present in the diamond structure (thus the name). Amantadine and Rimantadine are oral drugs that block the M2 channel. They have the following structural formulas:

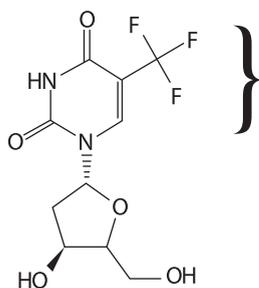


The purine portion is recognized preferentially by the viral DNA polymerase, less so by the host cell enzyme. This is the opposite of the pioneer drugs of the 1960's and 70's.

Derivatives that are converted to the parent compound are Valganciclovir, Valcyclovir, Penciclovir, Famcyclovir and Ganciclovir. They differ as to bioavailability, cost and regimen.

2. Trifluridine (Viroptic)

Trifluridine is an anti-herpes drug, used primarily in the eye for herpetic keratitis as a 1% aqueous ophthalmic solution (epithelial keratitis caused by HSV) (other ocular anti-herpes drugs are the pyrimidine analogues Brivudine and Idoxuridine).



Tri-fluoro group is the basis of activity (modified thymine at C5) as it replaces methyl group

Notes

Spectrum

Narrow X
Broad

Dosage

Oral X

Category

Antimetabolite
Entry inhibitor X
Enzyme inactivator

Virus

Influenza A only

Drug Resistance

High XX
Low to moderate

Note: Drugs afford temporary relief in Parkinson's Disease, a post-market finding.

4. Oseltamivir (Tamiflu)

Oseltamivir phosphate is a white crystalline solid with the chemical name (3R, 4R, 5S)-4-acetylamino-5-amino-3(1-ethyl-propoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1). The chemical formula is $C_{16}H_{28}N_2O_4$ (free base). The molecular weight is 312.4 for oseltamivir free base and 410.4 for oseltamivir phosphate salt. It is an oral drug for influenza A and B. The structural formula is as follows:

Notes

Spectrum

Narrow X
Broad

Dosage

Topical (eye) X

Category

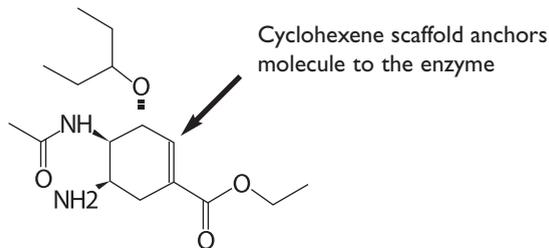
Antimetabolite X
Entry inhibitor
Enzyme inactivator

Viruses

Herpes group
(HSV-1, HSV-2)
Also vaccinia, some strains of adenovirus

Drug Resistance

High X
Moderate to low



Notes

Spectrum
Narrow X
Broad

Viruses
Influenza A and B

Dosage
Oral X

Drug Resistance
High X
Moderate
Low

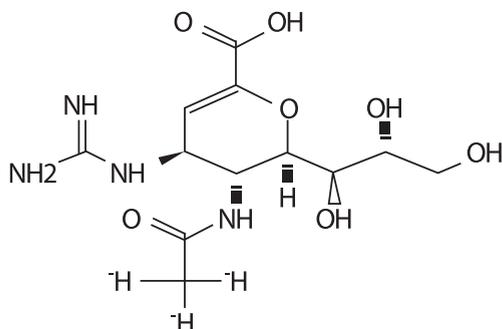
Category
(mimics sialic acid)
Antimetabolite
Entry inhibitor
Enzyme inactivator X
(blocks viral release)

Note: Polarity and hydrophobic-hydrophilic balance allows bonding to polypeptide site in the enzyme pockets.

Action: Drug inhibits the enzyme neuraminidase by occupying the site reserved for sialic acid (N-acetyl neuraminic acid, NANA). The sialic acid site is rich in the amino acid arginine and in addition contains alanine, tryptophane, aspartic acid residues and glucose.

5. Zanamivir (Relenza)

Zanamivir is a white, crystalline, solid with the chemical name 5-(acetylamino-4-[(aminoiminomethyl)-amino]-2,6-oxo-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid. The molecular weight is 332.3 and the structural formula is:



Polarity and hydrophobic-hydrophilic balance allows bonding to polypeptide site in the enzyme pockets.

Notes

Spectrum
Narrow X
Broad

Viruses
Influenza A and B

Dosage
Inhalant X

Drug Resistance
High
Low X

Category
(mimics sialic acid)
Antimetabolite
Entry inhibitor
Enzyme inactivator X
(blocks viral release)

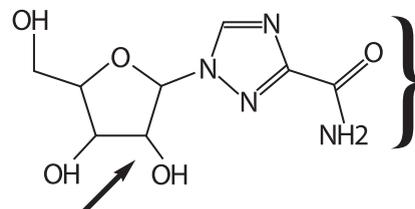
Note: The active enzyme site is more accessible to Zanamivir than Oseltamivir (i.e., mutations less likely to block bonding of drug).

Action: Same as for Oseltamivir, but entry to site is less restricted. (Less drug resistance)

Broad Spectrum

6. Ribavirin (Virazole)

Ribavirin was a very early drug. It produced great hope in the 1970's because of its broad spectrum (DNA and RNA viruses). However, the frequency of toxic reactions became a problem. It is a synthetic, orally administered, toxic nucleoside analog with a molecular weight of 244. The chemical name of Ribavirin is 1-B-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide and has the following structural formula:



Nitrogen base as incomplete purine-like ring

C-2 contains OH group – Sugar (ribose)

Notes

Spectrum
Narrow
Broad X
(limited usefulness)

Viruses
(reported) in vitro and in vivo: Influenza, Polio, HSV, RSV, HCV, Ebola, measles, Small pox, Marburg, Lassa fever, Hantavirus, HCV in combination with Interferon, Korean hemorrhagic fever (KHV).

Dosage
Oral X
Inhalant X
Intravenous X

Category
Antimetabolite X
Entry inhibitor
Enzyme inactivator

Drug Resistance
High to Moderate X
Low

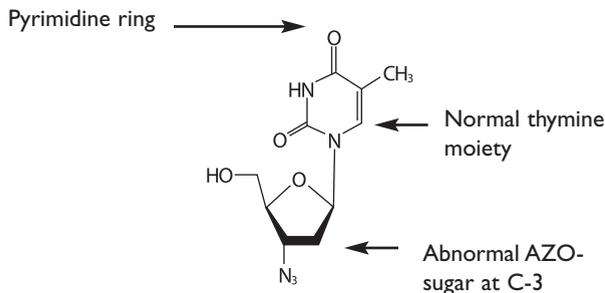
Some HIV Compounds

These examples from many are shown primarily so as to demonstrate the major molecular types: The NRTI and NARTI are competitive inhibitors as opposed to NNRTI which directly binds to the enzyme.

7. Azidothymidine (AZT) (ZDV)

(NRTI – (Nucleoside Reverse Transcriptase Inhibitor))

Zidovudine (INN) or Azidothymidine (AZT) (also called ZDV) is a nucleoside analog of thymidine and is a reverse transcriptase inhibitor (NRTI). AZT was a major breakthrough in AIDS therapy in the 1990's. It significantly altered the course of the illness and helped destroy the notion of the 1980's and early '90's that HIV/AIDS was a death sentence.



Notes

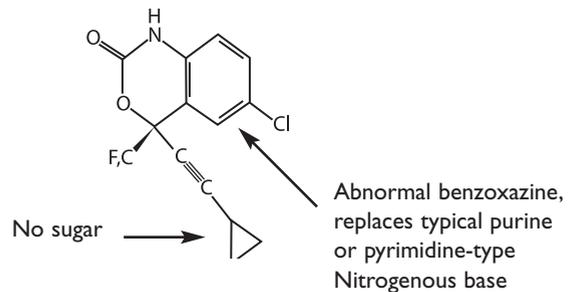
Spectrum Narrow X Broad	Virus HIV
Dosage Oral X (in combinations)	Drug Resistance High to Moderate X Low
Category Antimetabolite X Entry inhibitor Enzyme inactivator	

Note: Insertion of the tri-azo group at C-3 demonstrated, for the first time, the value of sugar modification in converting a normal metabolite to an agent to block this most complex virus. Other NRTIs are Stavudine, Abacavir, Emtricitabine, Zalcitabine, Didanosine and Lamivudine used singly and in various combinations.

8. Efavirenz (an NNRTI – (Non Nucleoside Reverse Transcriptase Inhibitor))

Efavirenz (brand name Sustiva) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy (HAART). It bears no resemblance to the usual purine or pyrimidine analogues used in antiviral therapy. Other NNRTI's are Nevirapine and

Delaviridine. NNRTI's are not active anti-metabolites; they bind directly to the RT enzyme and disrupt the catalytic site.

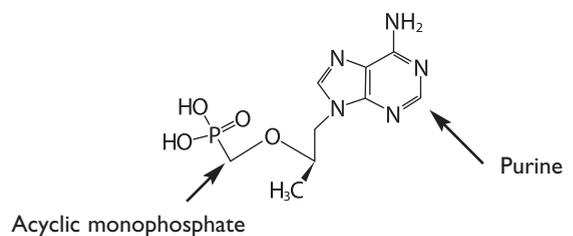


Notes

Spectrum Narrow X Broad	Category RT Blocker X Entry inhibitor
Dosage Oral X (in combinations)	Drug Resistance High X (quickly) Low

Note: No furanose-type sugar ring; a benzoxazine ring structure binds to RT enzyme.

9. Tenofovir (NARTI – (Nucleotide Analogue Reverse Transcriptase)) (an acyclic nucleotide)



Notes

Spectrum Narrow X Broad (some HBV)	Category Antimetabolite X (blocks RTI) Entry inhibitor Enzyme inhibitor
Dosage Oral X (in combinations)	Drug Resistance High to Moderate X Low

Tenofovir is a phosphorylated metabolite that blocks reverse transcriptase. The phosphate group distinguishes a nucleoside

from a nucleotide. Approved for HIV by FDA in 2001 and for chronic HBV in 2008, the chemical formula for this drug is $C_9H_{14}N_5O_4P$. Tenofovir is often prescribed in combination with Emtricitabine (brand name Truvada), a pill-reducing triple regimen.

Brief Synopsis of Laboratory Testing

The FDA, as well as numerous publications and Gibraltar protocols, provide guidance for *in vitro* screening of all of the above types of drugs. These tests use cell cultures of various types and chick embryos. Dose titrations for minimum effective concentration are performed with end-point calculations for activity described in terms of EC₅₀ values (mMoles/ml or ug/ml). Employing a variety of cell cultures (MDCK, Hep-2, Monkey Kidney, H-9, WI-38, etc) screening can be accomplished against influenza, herpes, Vaccinia, HAV, HCV, Polio, Coxsackie, RSV (Hep-2 cells), Adeno, HIV, Norovirus and surrogates. Screening or testing of clinical isolates for resistance is accomplished by challenging cell cultures with calibrated inocula and determining presence or absence of survivors by standard endpoints (cytopathic or erythrocyte response). Other assays are available.

Summary

This review has discussed four types of antiviral agents: metabolic inhibitors, enzyme inactivators, entry inhibitors and anti-sense molecules. The historic background for this unique area of drug discovery is reviewed. Drugs currently used for treating DNA (herpes) and RNA (influenza and HIV) viruses are reviewed as to spectrum, structure and mode of action. Attention is directed to the lack of effective treatment of Adenovirus and Enterovirus infections. An *in vitro* screening model is summarized. ■

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