CHAPTER 14
Biologically Active Nucleosides

INTRODUCTION

Nucleosides and their polymers are naturally occurring molecules with a wide area of functions in cell biology. Probably the best known functions are their roles in storage of information (DNA) and energy (ATP) in cells, in the use of this information via transcription and translation (RNA), as co-factors for enzymatic reactions (NAD) and in signal transduction systems (cAMP, ligands for purinergic receptors). It is not surprising, therefore, that chemists have considered nucleoside analogs as a potentially rich source of biologically active molecules. Three main fields of research that have been explored in the past involve the development of nucleoside analogs as antitumoral and antiviral agents and as ligands for membrane-bound receptors. The antitumoral and antiviral nucleosides are meant to kill cells or viruses, mainly via the interaction of their metabolites with DNA synthesis. Nucleosides developed as ligands for protein receptors are considered as agonists or antagonists for these receptors and may have broad applicability against human diseases. The first series of nucleoside analogs that will be described in this chapter are antiviral nucleosides, because this is the field that covers most of the nucleoside drugs that are on the market.

The first antiviral compounds that were discovered were methisazon and 5-iodo-2′-deoxyuridine. In contrast to evolution in other therapeutic areas, antiviral research has developed rather slowly because the life cycle of viruses is closely associated with cellular metabolism, viruses are localized intracellularly and possess few virally encoded genes, and acute viral infections can be prevented by developing effective vaccines. This has changed since the discovery of a retrovirus as the causative agent of acquired immune deficiency syndrome (AIDS). Most antiviral agents have been discovered using screening programs. Only recently has “rational drug design” entered the field and this is, in most cases, based on a lead compound that was discovered by a trial-and-error approach.

The classical antiviral compounds are nucleoside analogs with structures very similar to those of natural nucleosides: the structures of 5-iodo-2′-deoxyuridine (IDU) and 3′-azido-3′-deoxythymidine (zidovudine) are very similar to that of thymidine (Fig. 14.0.1). IDU is an anti–herpes virus agent (HSV-1, HSV-2) and is used in clinics for the treatment of herpes keratitis. Zidovudine is an anti-HIV nucleoside.

![Structural similarities between two nucleoside analogs and natural thymidine.](image)

**Figure 14.0.1** Structural similarities between two nucleoside analogs and natural thymidine.
Both nucleosides (IDU and zidovudine) have had an important impact on the development of antiviral drugs. IDU has long been the model compound for the development of new anti-herpes agents. The observation that a simple nucleoside (zidovudine) could slow down the evolution of AIDS has given an important stimulus to antiviral research. Since then, specific viral enzymes have been identified and used for the development of new selective antiviral agents. An important milestone in this field is the discovery of phosphate nucleosides for the treatment of cytomegalovirus (CMV), human immunodeficiency virus (HIV), and hepatitis B virus (HBV) infections, to which Dr. Holý has made the most significant contribution. This class of compounds is described in UNIT 14.2. The discovery of phosphate nucleosides as antiviral agents demonstrated that it is possible to circumvent the first step in the metabolic activation of nucleoside analogs (i.e., the conversion of the nucleoside to its monophosphate) and that such charged compounds can be taken up by cells (although not very efficiently).

Antiviral nucleosides can be ranked in several categories. Base-modified pyrimidine nucleosides [IDU, 5-ethyl-2'-deoxyuridine (EDU), 5-(E)-bromovinyl-2'-deoxyuridine (BVDU), and 5-trifluoromethyl-2'-deoxyuridine (TFT); see Fig. 14.0.2] are all anti-herpes agents. They are converted to their triphosphates by viral and cellular kinases. As several herpes viruses code for their own thymidine kinase, which has a broader substrate specificity than the human congener, their nucleosides are preferentially phosphorylated in virus-infected cells. This results in a selective antiviral activity. The triphosphates of the modified nucleosides function as substitutes for polymerases, and the modified nucleosides can be incorporated into DNA and interfere with normal transcription and replication processes. The monophosphate of TFT is also an inhibitor of thymidylate synthetase, which explains its strong antiviral activity. BVDU is active against HSV-1 and varicella-zoster virus (VZV). TFT is used topically for the treatment of herpes keratitis.

9-(β-d-Arabinofuranosyl)adenine (ara-A; Fig. 14.0.3) was the first example of a sugar-modified purine nucleoside that came on the market. It was used against HSV-1 encephalitis and herpes zoster in immunocompromised patients. However, ara-A was soon replaced by another series of sugar-modified purine nucleosides having an acyclic “sugar” chain. In UNIT 14.1, Dr. Holý describes the synthesis of representative acyclic nucleosides. Ara-A is rapidly deaminated in vivo by adenosine deaminase, and the search for deaminase inhibitors has led to the discovery of acyclovir or 9-(2-hydroxyethoxymethyl)guanine. Acyclovir has remained the standard for treatment of HSV and varicella-zoster virus infection. Ganciclovir is being used for CMV infections.

These acyclic nucleosides can be considered analogs of 2'-deoxyguanosine. Acyclovir is phosphorylated in virus-infected cells to its triphosphate. Although this compound

![Figure 14.0.2](image)

**Figure 14.0.2** Base-modified pyrimidine nucleosides (also see IDU in Fig. 14.0.1).
is a purine nucleoside, it is phosphorylated to its monophosphate by the viral-specific thymidine/deoxycytidine kinase. The triphosphate of acyclovir is a competitive inhibitor of dGTP for the viral DNA polymerase. The triphosphate may also function as substrate for the polymerase; upon incorporation of acyclovir into DNA, the triphosphate functions as chain terminator. Ganciclovir is phosphorylated in CMV-infected cells by the virally encoded (UL97) protein kinase. Although this agent has a broad activity spectrum (HSV-1, HSV-2, VZV, CMV, Epstein-Barr virus [EBV]), it is toxic for bone marrow (neutropenia) and its medical use is restricted to the treatment of CMV infections in immunocompromised patients. Penciclovir has the same antiviral spectrum as acyclovir. Its prolonged action is due to the higher intracellular stability of its triphosphate.

Ribavirin (Fig. 14.0.3) is an example of a base-modified purine nucleoside. It has a broad antiviral spectrum against DNA as well as RNA viruses. It is mainly active against influenza virus and respiratory syncytial virus. Therapeutically, it is used for the treatment of respiratory syncytial virus and hepatitis C infection (in combination with interferons).

HIV is a retrovirus, which means that the genomic RNA of the virus is first converted into DNA using reverse transcriptase. This enzyme has a broader substrate specificity than cellular DNA polymerases, and this property has been used as the basis for the development of nucleoside analogs as anti-HIV agents. The first nucleoside analog that came on the market for treatment of HIV was zidovudine (Fig. 14.0.1), and it was followed by didanosine, zalcitabine, stavudine, lamivudine, abacavir, and emtricitabine (Fig. 14.0.4). All these nucleoside analogs interfere with the function of reverse transcriptase once they are converted intracellularly to their triphosphates (in the case of didanosine and abacavir, the base moiety is also modified during intracellular metabolism into an adenine and guanine base, respectively). They all function as chain terminators after incorporation into DNA.

Didanosine, zalcitabine, and stavudine are considered dideoxynucleoside analogs because they lack secondary hydroxyl groups on the sugar moiety. Abacavir is an example of a carbocyclic nucleoside. Many carbocyclic nucleosides were synthesized in the
1980s and 1990s; however, only abacavir has reached the market as an antiviral agent. Lamivudine and emtricitabine are L-nucleosides. These L-nucleosides are less toxic than their D-congeners because they are less effectively transported in mitochondria. Emtricitabine is four- to ten-fold more active than lamivudine in vitro against HIV, although the effect is cell-type dependent. In addition to lamivudine (which is used against HIV and HBV), other L-nucleosides (L-thymidine and L-deoxycytidine) are under development for use against hepatitis B infections.

The in vivo activity of a drug is dependent on the way the drug is administered. The bioavailability of nucleosides is often not very high, and therefore prodrugs are developed. The specific metabolism of nucleosides allows one to make use of enzymes involved in nucleoside metabolism to develop a prodrug. Xanthine oxidase is such a candidate. Guanine nucleosides are not very soluble in water. The 6-deoxy analog of the diacetyl derivative of penciclovir gives blood plasma concentrations that are ten times higher than with penciclovir when given orally. This prodrug (famciclovir; Fig. 14.0.5) is converted to penciclovir in vivo by esterase and xanthine oxidase. Valaciclovir (cfr. supra) is another example of a prodrug of a purine nucleoside. This L-valylester of acyclovir was developed as prodrug of acyclovir. It is absorbed readily in the intestines, making use of a stereospecific transport system, and is rapidly hydrolyzed after absorption. Both
prodrugs (famciclovir and valaciclovir) are used for the treatment of systemic HSV and VZV infections.

Nucleoside phosphonates (Fig. 14.0.6) are composed of a base, a phosphonate group, and a sugar mimic. The compounds that are currently on the market do not have a glycosidic bond and are enzymatically and chemically more stable than regular nucleosides. Nucleoside phosphonates need to be phosphorylated to their diphosphates by cellular enzymes before showing activity against a broad range of viruses. They are not well absorbed when given orally, and they are administered as prodrugs. Cidofovir is active against herpes, adeno, polyoma, papilloma, and pox viruses. It is used for treatment of CMV retinitis in AIDS. Adefovir is active against retroviruses, hepadnaviruses, and herpes viruses. It is used in its prodrug form (adefovir dipivoxil) for the treatment of HBV infections. Tenofovir is active against retroviruses and hepadnaviruses and is used in its prodrug form (tenofovir disoproxil) for treatment of HIV infection. These nucleoside phosphonates have the important characteristic that they have a long action time, which means that they require much less frequent dosing than other nucleosides.

The field of antiviral nucleosides is still expanding and many new compounds are under development and/or in a research phase. Many infectious viruses are still waiting for a series of new nucleoside or nucleotide analogs for treatment. Perhaps the most striking examples are papilloma viruses and hepatitis C viruses. As several papilloma viruses can induce tumors, such nucleoside analogs may also be preventive for these cancers. Hepatitis C infection is a worldwide problem and also the most common cause of hepatocellular carcinoma. Another important direction for investigation is the treatment of pox virus infections (variola virus, the cause of smallpox, could be used as an efficient bioterrorist weapon). Hemorrhagic fever viruses may become a problem with climate changes. Ebola virus and rabies virus are among the most deadly viruses to infect humans. Influenza viruses cause epidemics or pandemics in humans. The SARS-associated coronavirus (severe acute respiratory syndrome) may lead to acute breathing problems and 10% mortality. Many viruses lead to gastrointestinal infections (e.g., reovirus) and cause diarrhea. Chemically, there are many opportunities for developing new antiviral agents in the nucleoside field. Most nucleoside analogs currently on the market have several drawbacks, and there is a lot of room for new and safer compounds. For example,
one might ask how long acyclovir can remain the gold standard for treatment of herpes virus infection when its activity spectrum is not very broad and the compound is not very potent. The mode of action of most antiviral nucleosides is very similar, and new nucleoside analogs will emerge that target other intracellular enzymes to exert an antiviral effect.

Many areas of nucleoside chemistry still need to be evaluated for their potential to generate new antiviral compounds. Although several compounds with a non-nucleosidic structure are being used as antiviral agents, nucleoside analogs will remain an important class of antivirals (as single therapy or in combination therapy) because of the simple reason that the most effective anti-infection agents are those that make and break covalent bonds.

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