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7.10 Viruses and Viral Diseases

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7.10.1 Introduction

There are at present some 40 antiviral drugs that have been formally licensed for clinical use in the treatment of viral infections (Table 1).1 These are mainly used in the treatment of infections caused by human immunodeficiency virus (HIV), hepatitis B virus (HBV), herpes viruses (herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV)), orthomyxoviruses (influenza), paramyxoviruses (respiratory syncytial virus (RSV)), and hepaciviruses (hepatitis C virus (HCV)). As these are the viruses that are most in demand of antiviral therapy, they have prompted the search for new antiviral strategies and drugs directed toward either the same molecular targets as the approved antiviral drugs or to other targets.

Most of the newly described antiviral compounds (that are currently in development) are targeted at HIV, HBV, or HCV. Some are targeted at HSV, VZV, or CMV, but, there are in addition many other important viral pathogens for which medical intervention, either prophylactic or therapeutic, is urgently needed, and, these are, among the DNA viruses, the papillomaviruses (human papilloma virus (HPV)), adenoviruses, poxviruses (variola, vaccinia, monkeypox,
| Virus                  | Compound                                      |
|-----------------------|-----------------------------------------------|
|                       | Approved for medical use | In clinical development | In preclinical evaluation |
| Parvo (B19)           | —                              | —                      | —                        |
| Polyoma (JC, BK)      | Cidofovir (off label)       | —                      | —                        |
| Papillomas (HPV)      | Cidofovir (off label)       | —                      | cPr PMEDAP and other acyclic nucleoside phosphonates Biphenylsulfonacetic acid derivatives |
| Adeno Alpha-herpes    | Cidofovir (off label)       | —                      | HPMPO-DAPy               |
| Alpha-herpes (HSV-1, HSV-2, VZV) | Acyclovir               | H2G prodrug            | A-5021                   |
|                       | Valaciclovir                 |                         | Synguanol                |
|                       | Penciclovir (topical)       |                         | Cyclopropavir (ZSM-I-62) |
|                       | Famiclovir                   |                         | BAY 57-1293              |
|                       | Brivudin                     |                         | BCNA Cf 1743             |
|                       | Idoxuridine (topical)       |                         |                          |
|                       | Trifluridine (topical)      |                         |                          |
| Beta-herpes (CMV, HHV-6, HHV-7) | Ganciclovir          | Maribavir               | CMV 423                  |
|                       | Valganciclovir              |                         |                          |
|                       | Cidofovir                    |                         |                          |
|                       | Foscarnet                   |                         |                          |
|                       | Fomiviren                   |                         |                          |
| Gamma-herpes (EBV, HHV-8) | Cidofovir (off label) | —                      | North-methanocarbathymidine (N-MCT) |
| Pox (variola, vaccinia, monkeypox, molluscum contagiosum, orf, etc.) | Cidofovir (off label) | —                      | HPMPO-DAPy               |
|                       | HDP-CDV, ODE-CDV             |                         |                          |
|                       | CI-1033                      |                         |                          |
| Hepadna (HBV)         | Lamivudine                   | Telbivudine             | 3′-Fluoro-2′,3′-dideoxycytidinosine |
|                       | Adefovir dipivoxil           | Valticicitabine         |                          |
|                       | Entecavir                    | Clevudine               |                          |
| Picorna (entero, rhino) | Peglated interferon-α | Pleconaril             | Pyrrolidin pentenoic acid (ethyl ester) |
|                       |                                |                        | Mycophenolic acid (MPA)  |
|                       |                                |                        | mofetil                  |
|                       |                                |                        | Interferon (inducers)     |
| Flavi (yellow fever, dengue, West Nile, etc.) | —                              | —                      | Interferon (inducers)     |
| Hepaci (HCV)          | Peglated interferon-α combined with ribavirin | BILN 2061 (Ciluprevir) | 2′-C-methylcytidine      |
|                       |                                | VX-950                  | 2′-O-methylcytidine      |
|                       |                                | NM 283 (Valopicitabine) | 2′-C-methyladenosine     |
|                       |                                | Viramidine              | 7-deaza-2′-C-methyladenosine |
|                       |                                | SCH 503034             | 2′-C-methylguanosine     |
|                       |                                |                          | DKA compound 30          |
|                       |                                |                          | Benzimidazole derivative |
|                       |                                |                          | Indole-N-acetamide derivative |
|                       |                                |                          | Benzothiadiazine derivative |
|                       |                                |                          | Phenylalanine derivative |
| Virus                        | Compound | Approved for medical use | In clinical development | In preclinical evaluation |
|-----------------------------|----------|-------------------------|-------------------------|--------------------------|
| Corona (SARS)               | —        | Pegylated interferon-α (off label) | Calpain inhibitors (III, VI) Niclosamide anilide Phe–Phe dipeptide Bananin Valinomycin Glycyrrhizin Chloroquine Niclosamide Nelfinavir |
| Orthomyxo (influenza)       | Amantadine | —                       | RWJ-270201              | A-192558                 |
|                             | Rimantadine | —                       | A-192558                | A-315675                 |
|                             | Zanamivir | —                       | A-192558                | T-705                    |
|                             | Oseltamivir | —                       | A-192558                | Flutimide                |
| Paramyxo (parainfluenza, measles, mumps, RSV, hMPV, etc.) | Ribavirin (approved for RSV only) | —                       | VP-14637                 |
| Arena (Lassa, etc.)         | Ribavirin (off label) | —                       | JNJ-2408068             |
| Bunya (Crimean-Congo, Rift Valley, etc.) | Ribavirin (off label) | —                       | BCX 2798 & BCX-2855     |
| Rhabdo (rabies)             | —        | —                       | BMS-433771              |
| Filo (Ebola, Marburg)       | —        | —                       | 3-Deazaaneplanacn A     |
| Retro (HIV)                 | Zidovudine | BMS-378806              | Cyclotriazadisulfonamide |
|                             | Didanosine | BMS-488043              | Cyanovirin N            |
|                             | Zalcitabine | AMD-3100                | KRH-1636                |
|                             | Stavudine | SCH-C                   | TAK-779                 |
|                             | Lamivudine | Vicriviroc              | TAK-220                 |
|                             | Abacavir | Aplaviroc               | TAK-652                 |
|                             | Emtricitabine | Maraviroc              | MIV-210                 |
|                             | Tenofovir disoproxil fumarate | Racivir | DOT                      |
|                             | Nevirapine | AVX-754                 | 4’-Ed4T                 |
|                             | Delavirdine | Reverset               | PMEO-DAPy               |
|                             | Efavirenz | Elvucitabine            | PMPO-DAPy               |
|                             | Saquinavir | Alovudine               | PMDTA                   |

continued
etc.) and the herpesviruses Epstein–Barr (EBV) and human herpesvirus type 6 (HHV-6), and, among the RNA viruses, enteroviruses (e.g., Coxsackie B and Echo), coronaviruses (e.g., severe acute respiratory syndrome (SARS)-associated coronavirus), flaviviruses (e.g., dengue, yellow fever), and other RNA viruses associated with hemorrhagic fever (arenaviruses (e.g., Lassa fever), bunyaviruses (e.g., Rift Valley fever, Crimean–Congo fever), and filoviruses (e.g., Ebola and Marburg)).

Here I will describe, for each viral family (1) which are the antiviral drugs that have been formally approved, (2) which are the compounds that are under clinical development and thus may be considered as antiviral drug candidates, and (3) which compounds are in the preclinical stage of development and still have a long route ahead before they could qualify as antiviral drugs. The virus families to be addressed are the following: parvo-, polyoma-, papilloma-, adeno-, herpes-, pox-, hepadna-, picorna-, flavi-, hepaci-, corona-, orthomyxo-, paramyxo-, arena-, bunya-, rhabdo-, filo-, reo-, and retroviruses.

7.10.2 Paroviruses

No significant attempts have been made to develop compounds with potential activity against B19, the only parovirus that is pathogenic for humans, and which is responsible for erythema infectiosum, the so-called fifth disease, in children.

7.10.3 Polyomaviruses

No antiviral drugs have been formally approved for the treatment of polyomavirus (JC and BK)-associated diseases such as progressive multifocal leukoencephalopathy (PML) and hemorrhagic cystitis in patients with acquired immune deficiency syndrome (AIDS). There are, however, anecdotal case reports pointing to the efficacy of cidofovir (5')-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC, which has been licensed under the trademark name Vistide for the intravenous treatment of CMV retinitis in AIDS patients, in the treatment of polyoma (JC and BK) virus infections, particularly PML, in AIDS patients.

7.10.4 Papillomaviruses

As for polyomaviruses, no antivirals have been licensed for the treatment of human papillomavirus-associated diseases, including warts, condylomata acuminata, papillomas, and cervical, vulvar, penile, and (peri)anal dysplasia (evolving to carcinoma). Cidofovir has been used ‘off label,’ with success, in the topical and, occasionally, systemic treatment of HPV-associated papillomatous lesions. In many instances, a virtually complete and durable resolution of the lesions was achieved following topical application of cidofovir as a 1% gel or cream. In addition to cidofovir, other acyclic nucleoside phosphonates, such as cPrPMEDAP (N6-cyclopropyl-9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine), are being explored for their potential in the treatment of HPV-associated papillomas and dysplasias. These compounds have been shown to specifically induce apoptosis in HPV-infected cells, which, in turn, may be related to their ability to restore the function of the tumor suppressor proteins p53 and pRb (which are neutralized by the oncoproteins E6 and E7, respectively, in HPV-infected cells).
Recently, biphenylsulfonacetic acid derivatives have been described as inhibitors of HPV E1 helicase-associated ATP hydrolysis.\textsuperscript{4,5} Although these novel ATPase inhibitors can hardly be considered to be good drug candidates, they may serve as leads for further optimization as potential antiviral agents active against multiple HPV types.\textsuperscript{5}

### 7.10.5 Adenoviruses

For the treatment of adenovirus infections, which can be quite severe in immunocompromised patients (e.g., allogeneic hematopoietic stem-cell transplant recipients), no antiviral drugs have been officially approved. Anecdotal reports have pointed to the efficacy of cidofovir against adenovirus infections in such patients.\textsuperscript{2} Among the novel compounds that could be further explored for the treatment of adenovirus infections are (5)-2,4-diamino-6-[3-hydroxy-2-phosphono-methoxy]propoxy]pyrimidine (HPMPO-DAPy),\textsuperscript{3} which akin to some ‘older’ compounds like (5)-9-(3-hydroxy-2-phosphono-methoxypropyl)adenine (HPMPA), the N7-substituted acyclic nucleoside 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine S-2242, the 2',3'-dideoxynucleosides zalcitabine (ddC) and alovudine (FddT, FLT) have been found to inhibit adenovirus replication in vitro.\textsuperscript{6} Also, ether lipid-ester (hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE)) prodrugs of HPMPC and HPMPA have been designed that inhibit adenovirus replication in vitro at significantly lower concentrations than the parent compounds.\textsuperscript{7}

### 7.10.6 Herpesviruses

#### 7.10.6.1 Alpha-Herpesviruses (HSV-1, HSV-2, VZV)

For the treatment of HSV-1, HSV-2, and VZV, a number of compounds have been approved: acyclovir (4) and its oral prodrug valaciclovir (5); penciclovir (6) and its oral prodrug famciclovir (7); idoxuridine (8), trifluridine (9), and brivudin (10). Penciclovir, idoxuridine, and trifluridine are used topically, primarily in the treatment of herpes labialis (penciclovir) and herpetic keratitis (idoxuridine, trifluridine). Acyclovir can be used orally, intravenously, or topically, whereas valaciclovir and famciclovir are administered orally, in the treatment of both HSV and VZV infections. Brivudin (available in some European countries) is used orally for the treatment of herpes zoster, but is also effective against HSV-1 infections.
4 Acyclovir (ACV)  
Zovirax

5 Valaciclovir (VACV)  
Zelitrex, Valtrex

6 Penciclovir (PCV)  
Denavir, Vectavir

7 Famciclovir (FCV)  
Famvir

8 Idoxuridine  
Herpid, Stoxil

9 Trifluridine  
Viroptic

10 (E)-5-(2-Bromovinyl)-2'-deoxyuridine  
BVDU  
Brivudin  
Zostex, Brivirac
While acyclovir (and its oral prodrug valaciclovir) have remained the gold standard for the treatment of HSV and VZV infections, few attempts have been made to bring other anti-HSV (or anti-VZV) agents into the clinic, with the exception of the H2G prodrug (11), which after quite a number of years is still in clinical development for the treatment of herpes zoster. Any attempt for clinical development as an anti-HSV (or anti-VZV) agent are a number of carbocyclic guanosine analogs, such as A-5021 (12), cyclohexenylguanine, and the methylene cyclopropane synguanol (13). All these compounds owe their selective antiviral activity to a specific phosphorylation by the HSV- or VZV-encoded thymidine kinase (TK); upon phosphorylation to their triphosphate form, they act as chain terminators in the DNA polymerization reaction. In the (rare) circumstances that HSV or VZV becomes resistant to the acyclic (or carbocyclic) guanosine analogs due to TK deficiency (TK−), the pyrophosphate analog foscarnet (14) could be useful for treating TK− HSV or TK− VZV infections (in immunocompromised patients).

Recently, a second generation of methylene cyclopropane analogs, the 2,2-bihydroxymethyl derivatives, has been synthesized. These compounds may have potential, not only for the treatment of HSV-1, HSV-2, and VZV, but also beta-herpes (CMV, HHV-6, HHV-7) and gamma-herpes (EBV, HHV-8) infections. In particular, ZSM-I-62 (Cyclopropavir) (15) has been reported to be very effective in reducing mortality of mice infected with murine CMV. New anti-HSV agents targeting the viral helicase–primase complex, the thiazolylphenyl derivatives BILS 179BS and BAY 57-1293 (16), were recently reported to have in vivo efficacy in animal models of HSV-1 and HSV-2 infections. These compounds seem to function by diminishing the affinity of the helicase–primase complex for the HSV DNA. The antiviral potency of BAY 57-1293 was claimed to be superior to all compounds that are currently used to treat HSV infections. If so, this lead should be further pursued from a clinical viewpoint.
A new class of anti-VZV compounds are the bicyclic furo (2,3-d)pyrimidine nucleoside analogs (BCNAs), represented by Cf 1742 and Cf 1743 (17). These compounds are exquisitely active against VZV. They inhibit the replication of VZV, but not that of other viruses (including HSV), at subnanomolar concentrations, with a selectivity index in excess of 100,000. Given the extremely high potency and selectivity of the BCNAs they warrant to be further developed toward clinical use, e.g., against herpes zoster.

7.10.6.2 Beta-Herpesviruses (CMV, HHV-6, HHV-7)

Five compounds have been licensed to treat CMV infections: ganciclovir (18), its oral prodrug valganciclovir (19), foscarnet (14), cidofovir (1), and fomivirsen (20). With the exception of fomivirsen (an antisense oligonucleotide) which targets the CMV immediate–early mRNA, all other licensed anti-CMV drugs target the viral DNA polymerase. Ganciclovir must first be phosphorylated by the CMV-encoded protein kinase (the UL97 gene product) which is also the principal site for mutations engendering resistance toward this compound. Toxic side effects (i.e., bone-marrow suppression for ganciclovir, nephrotoxicity for foscarnet and cidofovir) have prompted the search for new inhibitors of CMV.

Several benzimidazole ribonucleosides, including maribavir (previously also known as 1263W94) (21), have been accredited with specific activity against human CMV. Maribavir seems to target the UL97 protein kinase, and, as the
UL97 gene product has been shown to account for the release of CMV nucleocapsids from the nucleus,\textsuperscript{20} maribavir may be assumed to target a stage in the viral life cycle that follows viral DNA maturation and packaging. Preclinical pharmacokinetic and toxicological studies have shown that maribavir has a favorable safety profile and excellent oral bioavailability.\textsuperscript{21} Phase I/II dose-escalation trials in HIV-infected men with asymptomatic CMV shedding further indicated that maribavir is rapidly absorbed following oral dosing and reduces CMV titers in semen.\textsuperscript{22}

While maribavir is primarily active against CMV, 2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV 423) (22) has potent and selective in vitro activity against all three human beta-herpesviruses, CMV, HHV-6, and HHV-7.\textsuperscript{23} As compared to ganciclovir and foscarnet, CMV 423 has higher antiviral potency and lower cytotoxicity. It is targeted at an early stage of the viral replication cycle (following viral entry but preceding viral DNA replication), which is regulated by a cellular process that may involve protein tyrosine kinase activity. The in vitro antiviral action profile of CMV 423 is such that it deserves to further explored for its in vivo potential in the treatment of CMV and HHV-6 infections.

There is, at present, no standardized antiviral treatment for HHV-6 infections. From a comparative study, A-5021, foscarnet, S2242, and cidofovir emerged as the most potent compounds with the highest antiviral selectivity against HHV-6.\textsuperscript{24} The latter three also proved to be the most potent against HHV-7.\textsuperscript{24} However, indications for the clinical use of anti-HHV-6 and anti-HHV-7 agents remain ill-defined.

\section{7.10.6.3 Gamma-Herpesviruses (EBV, HHV-8)}

Although a number of the aforementioned approved antitherpetic drugs, such as acyclovir, ganciclovir, brivudin, and cidofovir, have proven to be effective against the in vitro replication of EBV and HHV-8,\textsuperscript{24} none of these (or any other) antiviral drugs has been formally approved for the treatment of diseases associated with EBV (e.g., mononucleosis infectiosa, B-cell lymphoma, lymphoproliferative syndrome, Burkitt’s lymphoma, nasopharyngeal carcinoma) or HHV-8 (e.g., Kaposi’s sarcoma, primary effusion lymphoma, multicentric Castleman’s disease). It would seem appealing to further examine established antitherpetic drugs, such as cidofovir, and other acyclic nucleoside phosphonates such as HPMPA, or prodrugs thereof, for their potential in the therapy of EBV- and HHV-8-associated malignancies. Also, new nucleoside analogs, such as \textit{north}-methanocarbathymidine (N-MCT) (23),\textsuperscript{25} which have been previously shown to block the replication of HSV-1 and HSV-2, should be further explored for their potential in the prevention and treatment of HHV-8-associated malignancies: in particular, N-MCT, which is specifically triphosphorylated in HHV-8-infected cells undergoing lytic replication, efficiently blocks HHV-8 DNA replication in these cells.\textsuperscript{25}

\section{7.10.7 Poxviruses (Variola, Vaccinia, Monkeypox, Molluscum Contagiosum, Orf, etc.)}

Several nucleoside analogs (e.g., S2242, 8-methyladenosine, idoxuridine) and nucleotide analogs (e.g., cidofovir, HPMPA-DAPy) have proven to be effective in various animal models of poxvirus infections.\textsuperscript{26} In particular, cidofovir has shown high efficacy, even after administration of a single systemic (intraperitoneal) or intranasal (aerosolized) dose, in protecting mice from a lethal respiratory infection with either vaccinia or cowpox. Cidofovir has demonstrated high effectiveness in the treatment of disseminated progressive vaccinia in athymic-nude mice.\textsuperscript{27} In humans, cidofovir has been used successfully, by both the topical and intravenous route, in the treatment of orf and recalcitrant molluscum contagiosum in immunocompromised patients.\textsuperscript{28} Given the in vitro activity of cidofovir against variola (smallpox), and the in vivo efficacy of cidofovir against various poxvirus infections in animal models and humans, it can be reasonably
assumed that cidofovir should be effective in the therapy and/or prophylaxis of smallpox in case of an inadvertent outbreak or biological attack with the variola virus.

Being a phosphonate analog, cidofovir only has limited oral bioavailability. In case of an outbreak of smallpox, it would be useful to have an orally active drug at hand. To this end, hexadecyloxypropyl-cidofovir (HDP-CDV, 24) and octadecyloxyethyl-cidofovir (ODE-CDV, 25) were designed as potential oral prodrugs of cidofovir. These alkylxalkyl esters of cidofovir were found to significantly enhance inhibition of the replication of orthopoxviruses (e.g., vaccinia, cowpox) in vitro. HDP-CDV and ODE-CDV given orally were as effective as cidofovir given parenterally for the treatment of vaccinia and cowpox infections. HDP-CDV has also proven effective in the treatment of a lethal vaccinia virus respiratory infection in mice. Furthermore, HDP-CDV and ODE-CDV, when given orally, proved highly efficacious in a lethal (aerosol) mousepox (ectromelia) virus model, further attesting as to the potential usefulness of the alkylxalkyl esters of cidofovir in the oral therapy and prophylaxis of poxvirus infections.

In fact, as attested by the most recent findings with cidofovir in mice infected with ectromelia (mousepox) virus encoding interleukin-4, and monkeys infected with monkeypox, cidofovir (CDV) (and HDP-CDV and/or ODE-CDV) still provides the best current hope for effective control of virulent poxvirus infections.

In addition to the nucleotide analog cidofovir, which primarily acts as a viral DNA chain terminator (for vaccinia virus DNA polymerase after it has been incorporated at the penultimate position), antiviral strategies for poxvirus infections may also be based on inhibitors of cellular processes, i.e., signal transduction pathways. In this respect, the 4-anilinoquinazoline CI-1033 (26), an ErbB tyrosine kinase inhibitor, was found to block variola virus replication in vitro and vaccinia virus infection in vivo.

Likewise, Gleevec (STI-571, Imatinib), an Abl-family kinase inhibitor used to treat chronic myelogenous leukemia in humans, was shown to suppress poxviral dissemination in vivo by several orders of magnitude and to promote survival in infected mice, suggesting possible use for this drug in treating smallpox or complications associated with vaccination against smallpox. Because the drug targets host rather than viral molecules, it is less likely to engender resistance compared to more specific antiviral agents. Collectively, inhibitors of host-signaling pathways exploited by poxviral pathogens may represent potential antiviral therapies.

Recently, a new antipoxvirus compound (ST-246, 27) has been described, which is orally bioavailable, acts according to a novel mechanism of action, targeting a specific viral product (i.e., vaccinia virus F13L) required for extracellular
virus particle formation, and protecting mice from a lethal orthopoxvirus challenge.\textsuperscript{39} These properties make ST-246 an attractive candidate for development as a smallpox antiviral drug that could be stockpiled for use in the treatment and prevention of smallpox virus infection in the event of a bioterrorist threat.

7.10.8 Hepadnaviruses (HBV)

An estimated 400 million people worldwide are chronically infected with the hepadnavirus HBV; approximately 1 million die each year from complications of infection, including cirrhosis, hepatocellular carcinoma, and end-stage liver disease. Formally approved for the treatment of chronic hepatitis B are lamivudine (28), adefovir dipivoxil (29), (pegylated) interferon-\(\alpha\), and entecavir (30). Whereas lamivudine, adefovir, and entecavir (and other nucleoside analogs that are still in (pre)clinical development) act as genuine antiviral agents at the HBV-associated reverse transcriptase, interferon, in the chronic hepatitis B setting, primarily acts as an immunomodulator. Pegylated interferon-\(\alpha\)b is effective in the treatment of hepatitis B e-antigen (HBeAg)-positive chronic hepatitis B, but no additional benefit is achieved if it is combined with lamivudine.\textsuperscript{40}

Whereas interferon therapy, also because of its unavoidable side effects (influenza-like symptoms) is not recommended for treatment lasting longer than 1 year, the nucleos(t)ide analogs can, in principle, be administered for quite a number of years.

For lamivudine (3TC), however, this prolonged treatment is compounded by the emergence of both virological and clinical resistance at an accumulating rate of approximately 20\% of patients per year. Resistance to adefovir dipivoxil may also emerge, but much less frequently (not more than 6\% after 3 years).\textsuperscript{41} Adefovir dipivoxil is the oral prodrug of adefovir (PMEA, 9-(2-phosphonylmethoxyethyl)adenine), which, after intracellular conversion to the diphosphate form, acts as a competitive inhibitor or alternative substrate for the HBV reverse transcriptase, and, when incorporated into the DNA, acts as a chain terminator, thereby preventing DNA chain elongation.\textsuperscript{42}
In patients with chronic HBV infection who were either positive or negative for HBeAg, 48 weeks of treatment with a dose of adefovir dipivoxil as low as 10 mg day$^{-1}$ resulted in significant improvement of all parameters of the disease (histological liver abnormalities, serum HBV DNA titers, and serum alanine aminotransferase levels). In patients with HBeAg-negative chronic hepatitis B, the benefits achieved from 48 weeks of adefovir dipivoxil were lost when treatment was discontinued, but maintained if treatment was continued through week 144.

Entecavir, one of the most recent antiviral drugs launched for clinical use, has in vitro and in vivo potency that seems to be greater than that of lamivudine; in patients with chronic hepatitis B infection it has proven efficacious at a dose as low as 0.5 mg day$^{-1}$. The active (triphosphate) metabolite of entecavir would accumulate intracellularly at concentrations that are inhibitory to 3TC-resistant HBV DNA polymerase. How this translates to the clinical efficacy of entecavir the treatment of 3TC-resistant HBV infections remains to be followed up.

In addition to entecavir, a number of L-nucleosides, e.g., β-L-thymidine (L-dT, Telbivudine, 31), the 3′-valine ester of β-L-2′-deoxyctydine (Val-L-dC, Valtorcitabine, 32) and 1-(2-fluoro-5-methyl-β-L-arabinosyl)uracil (L-FMAU, Clevudine, 33) are in clinical development (Clevudine involves the role of deoxythymidylate (dTMP) kinase for the treatment of chronic hepatitis B. Other compounds in preclinical development include 2′,3′-dideoxy-3′-fluoroguanosine (FLG, 34), racivir, and L-Fd4C. These compounds are also in preclinical development for HIV (see below).

Moreover, tenofovir disoproxil fumarate (TDF, 35) and emtricitabine ((-)-FTC, the 5-fluoro-substituted counterpart of lamivudine, 36)), which have both been licensed, individually and in combination, for the treatment
of HIV infections (AIDS), may also be considered and further pursued, most likely in combination, for use in the
treatment of chronic hepatitis B. In fact, TDF has been considered an important new therapeutic tool for the induction
of complete remission in patients with lamivudine-resistant HBV infection.49

An interesting recommendation has been proposed for the care of patients with chronic HBV and HIV coinfection.50
They should be put on the combination of TDF with (-) FTC, which would cover both the HBV and HIV infection.
Only if no antiretroviral therapy is used in these patients, adefovir dipivoxil and/or pegylated interferon could be
installed depending on whether they are HBeAg-negative or -positive, respectively.50

7.10.9 **Picornaviruses (Entero- and Rhinoviruses)**

Among the enteroviruses, polio and hepatitis A can be efficiently controlled by vaccination: for polio both a live
tenuated and an inactivated (‘killed’) virus vaccine available, whereas for hepatitis A an inactivated virus vaccine is
available. The other enteroviruses (Coxsackie A and B and echoviruses) and the rhinoviruses need to be approached by
chemotherapeutic agents. No single antiviral drug has ever been licensed for clinical use against entero- or rhinovirus
infections. The most extensively studied for its potential against enteroviruses has been pleconaril (37). This
compound binds to a hydrophobic pocket beneath the ‘canyon floor’ of the VP1 capsid protein of picornaviruses,51
thereby ‘freezing’ the viral capsid and preventing its dissociation (uncoating) from the viral RNA genome. The clinical
efficacy of pleconaril has been evaluated in experimentally induced enterovirus (Coxsackie A21) respiratory infections
in adult volunteers52 and, on a compassionate basis, against potentially life-threatening enterovirus infections.53
Pleconaril has also been shown to reduce the duration and severity of picornavirus-associated viral respiratory illnesses
in adolescents and adults.54,55
For the prevention and/or treatment of rhinovirus infections (common colds) inhibitors of the human rhinovirus (HRV) 3C protease have been extensively investigated. Ruprintrivir (38) is an irreversible 3C protease inhibitor,\(^5\) which, upon intranasal administration in human volunteers, appeared to be safe and well tolerated.\(^5\) In experimentally induced rhinovirus colds in healthy volunteers, ruprintrivir prophylaxis reduced the proportion of subjects with positive viral cultures but did not decrease the frequency of colds.\(^5\) Another, irreversible inhibitor of HRV 3C protease, here referred to as a pyrrolidinyl pentenoic acid ethyl ester (compound 3\(^9\) or compound 1\(^6\)) (39), offers the advantage of being orally bioavailable: in healthy volunteers, single oral doses of this compound appeared to be safe and well tolerated, although the compound is currently not progressing toward clinical development.\(^6\)

In great need of antiviral treatment are the often severe complications of Coxsackie B virus infections, such as myocarditis which may lead to idiopathic dilated cardiomyopathy. In mice, Coxsackie B3 virus-induced myocarditis is inhibited by the immunosuppressive agent mycophenolic acid (MPA) mofetil (40).\(^6\) This beneficial outcome must apparently result from the immunosuppressive effect of MPA (through inhibition of IMP dehydrogenase and, hence, GTP supply), since MPA did not reduce the infectious virus titers in the myocarditis. A more pronounced inhibitory effect on Coxsackie B3 virus-induced myocarditis, accompanied by a marked reduction in the virus titers in the heart, was obtained with the interferon inducer poly(I),poly(C) and poly(I),poly(C\(_{12}\)U) (also known as Ampligen), and to a lesser extent with (pegylated) interferon-\(\alpha\)2b.\(^6\) Combination of an inhibitor of viral replication (such as Ampligen) with an immunosuppressant (such as MPA mofetil) could be an ideal treatment strategy for viral myocarditis, whether due to Coxsackie B or other viruses. How to implement such as treatment regimen in the clinical setting should be further addressed.
Flaviviruses (Yellow Fever, Dengue, West Nile, etc.)

No antivirals are currently available for the treatment of flavivirus infections (although there is a live virus vaccine routinely used for the prophylaxis of yellow fever), and the prospects for an effective therapy of flavivirus infections do not seem encouraging.\textsuperscript{63} Antiviral compounds such as ribavirin (41) have only weak activity against flaviviruses. Greater hope may be vested in interferon and interferon inducers. Based on infection of hamsters with the murine Modoc virus, an experimental flavivirus encephalitis model has been developed, which is reminiscent of Japanese encephalitis virus infection in humans.\textsuperscript{64} In a related model with Modoc virus in SCID mice, both interferon-\(\alpha\)2b (whether pegylated or not) and interferon inducers (poly(I).poly(C) and Ampligen) were shown to significantly delay virus-induced morbidity (paralysis) and mortality (due to progressive encephalitis).\textsuperscript{65} Ribavirin did not provide any beneficial effect in this model, whether given alone or in combination with interferon.

Hepaciviruses (HCV)

Current, approved therapy for chronic hepatitis C consists of pegylated interferon-\(\alpha\)-2 (180\,\mu g, parenterally, once weekly) combined with ribavirin (1000 or 1200 orally, daily).\textsuperscript{66,67} This treatment regimen is associated with a sustained viral response in at least 50\% of the patients infected with HCV genotype 1, and of 80\% in patients infected with another genotype (2, 3, or 4) of HCV. Duration of treatment is 48 weeks (or longer) for patients infected with HCV genotype 1, but may be reduced to 24 weeks for patients infected with another genotype. Interferon appears to be targeted at the phosphoprotein encoded by the nonstructural NS5A gene of the HCV genome,\textsuperscript{68} whereas ribavirin, akin to MPA, primarily acts as an inhibitor of IMP dehydrogenase, thus reducing the biosynthesis of GTP.

The combination of pegylated interferon \(\alpha\)-2a with ribavirin has also been advocated for the therapy of HCV infection in patients with HIV coinfection.\textsuperscript{69} However, it should not be forgotten that, should these patients be treated (for their HIV infection) with azidothymidine (zidovudine, ZDV, 42), the latter may be antagonized by ribavirin.\textsuperscript{70} Therefore it was reassuring to note that ribavirin (at 800\,mg\,day\(^{-1}\)) administered in combination with pegylated interferon \(\alpha\)-2a did not significantly affect the intracellular phosphorylation or plasma pharmacokinetics of ZDV (or other pyrimidine dideoxynucleosides such as 3TC or d4T) in HIV/HCV coinfected patients.\textsuperscript{71}
to develop more selective anti-HCV agents, targeted at specific viral proteins such as the NS3 protease and RNA helicase, and the NS5B RNA replicase (RNA-dependent RNA polymerase). Also the HCV p7 protein, which forms an ion channel and can be blocked by long-alkyl-chain iminosugar derivatives, has been considered as a potential target for antiviral therapy.

Proof of principle that compounds targeted at the NS3 protease could reduce plasma concentrations of HCV RNA has already been delivered with BILN 2061 (44) administered orally for no longer than 2 days in patients infected with HCV genotype 1. BILN 2061 (Ciluprevir) was able to reduce HCV RNA levels by 2-3 log_{10} in patients infected with HCV genotype 1, after 2 days of treatment, but in patients infected with HCV genotypes 2 or 3, it proved less effective, apparently due to a lower affinity of BILN 2061 for the HCV protease of these genotypes.

Another NS3 protease inhibitor, which differs in its in vitro resistance profile from BILN 2061, is VX-950 (45): i.e., the major BILN 2061-resistant mutations at Asp168 are fully susceptible to VX-950, and similarly, the dominant resistant mutation against VX-950 at Ala156 remains sensitive to BILN 2061. Thus, VX-950 and BILN 2061 elicit resistance to HCV protease (NS3A) by different mechanisms. VX-950 (750 mg every 8 h) was found to achieve, at the end of a 14-day treatment, a main reduction of HCV RNA of 4.4 log_{10}. (In some patients dosed with VX-950, the virus became undetectable at day 14 of dosing.) The overall preclinical profile of VX-950 supports its candidacy as a novel oral therapy against hepatitis C. Other HCV NS3 protease inhibitors may be announced in the future as behaving similarly. Various other 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid-based macrocyclic inhibitors of HCV NS3 protease as well as SCH 503034 (46), a mechanism-based inhibitor of HCV NS3 protease, are in preclinical development. In fact, the latter was found to act synergistically with α-interferon in suppressing HCV replicon synthesis.

In addition to the NS3 protease, the NS5B RNA replicase has also been perceived as an attractive target for the development of HCV inhibitors. Highly potent and selective antiviral agents targeted at the viral RNA replicase
have been described to inhibit the replication of bovine viral diarrhea virus (BVDV), a pestivirus which could be considered as a surrogate virus for HCV. We have recently described a novel series of compounds (prototype: 5-[(4-bromophenyl)methyl]-2-phenyl-5H-imidazo[4,5-c]pyridine (BPIP: 47)) that act as ‘nonnucleoside’ RNA replicase inhibitors (NNRRIs) and effect a highly potent and selective inhibition of the replication of BVDV. From the BPIP class of compounds, new congeners have been derived that act equally efficiently against HCV replication. In future treatment strategies for HCV infections, these NNRRIs may likely be combined with ‘nucleoside’ RNA replicase inhibitors (NRRIs), in analogy with the strategy followed for the treatment of HIV infections, where NRTIs are combined with NNRTIs (see below).

The sole NRRI which has already proceeded to phase I/II clinical trials for the therapy of hepatitis C is the 3'-O-valine ester of 2'-C-methylcytidine (NM-283, valopicitabine, 48), which can be administered by the oral route and shows enhanced antiviral efficacy if combined with pegylated interferon. In addition to 2'-C-methylcytidine, several other ribonucleoside analogs (NRRIs) have been reported to inhibit HCV replication: 2'-O-methylcytidine (49), 2'-C-methyladenosine (50), 7-deaza-2'-C-methyladenosine (51), and 2'-C-methylguanosine (52). All these compounds act as nonobligate chain-terminating nucleoside analogs. It would seem interesting to prepare and evaluate the corresponding 3'-deoxyribonucleoside analogs for their anti-HCV activity.

A new class of HCV NS5B RNA replicase inhibitors is represented by the alpha, gamma-diketo acids (DKAs), one of the more active DKAs being DKA compound 30 (53). These compounds are reminiscent of the DKA type of HIV integrase inhibitors (see below) and are assumed to inhibit the HCV polymerase activity via chelation of the active site Mg$^{2+}$ ions. In a certain sense they may be considered as ‘pyrophosphate mimics,’ acting as product-like inhibitors of the polymerase reaction.
In recent years, a constellation of NNRRIs has been described as acting in a very similar (‘allosteric’) fashion with HCV NS5B RNA replicase as NNRTIs do with respect to the HIV reverse transcriptase: benzimidazole-based derivatives (54), indole-N-acetamide derivatives (55), benzo-1,2,4thiadiazine derivatives (56), benzimidazole derivative (53), indole-N-acetamide derivative (55), benzothiadiazine derivative (56), phenylalanine derivatives (57), thiophene 2-carboxylic acid derivatives (58), dihydropyranone derivatives (59), the tetrahydropyranodolyl acetic acid derivative HCV-371 (60) and the N-1-aza-4-hydroxyquinolone benzothiadiazine A-782759 (61). The allosteric binding site for some of these compounds have already been identified by crystallographic studies. It corresponds to a narrow cleft on the protein’s surface in the ‘thumb’ domain, about 3.0–3.5 nm from the enzyme’s catalytic center. Curiously, most of the NNRRIs that are active against the HCV NS5B polymerase contain, besides a large hydrophobic region, a carboxylic acid group (or a similar motif) that allows hydrogen bonding with main chain amide nitrogen atoms (i.e., Ser476 and Tyr477, as demonstrated for the phenylalanine derivative).
Mutations conferring resistance to both the HCV NS5B RNA replicase (i.e., H95Q, N411S, M414L, M414T, or Y448H) and NS3 protease (i.e., A156 V or D168 V) have been identified. These mutations conferred high levels of resistance to A-782759 and BILN 2061, respectively. However, the A-782759-resistant mutants remained susceptible to the NRRIs and other classes of NNRRIs, as well as interferon. In addition, the dually (A-782759- and BILN 2061-) resistant mutants displayed significantly reduced replicative ability as compared to the wild-type. These findings support a rationale for drug combinations in the therapy of HCV infections.

As recently reviewed, several other approaches, including ribozymes, antisense oligonucleotides, and RNA interference (RNAi) based on small interfering (si)RNAs could be envisaged to target the HCV genome. In particular, siRNAs aimed at posttranscriptional gene silencing may be considered an attractive approach.

7.10.12 Coronaviruses (SARS)

As for HCV, there are several proteins encoded by the SARS coronavirus which could be considered as targets for chemotherapeutic intervention: i.e., the spike (S) protein, the 3C-like main protease, the NTPase/helicase, the RNA-dependent RNA polymerase (RNA replicase), and, possibly, other viral (or cellular) protein-mediated processes. The severe acute respiratory syndrome (SARS) coronavirus S protein mediates infection of permissive cells through interaction with its receptor, the angiotensin-converting enzyme 2 (ACE 2), and monoclonal antibody to the S1 domain was found to neutralize the virus by blocking its association with the receptor ACE 2.

Also the fusion of the SARS coronavirus with the cell could be considered an attractive target. To the extent that this fusion process bears resemblance to the fusogenic mechanism of HIV, i.e., with regard to heptad repeat interactions and six-helix bundle formation, it might be feasible to develop SARS coronavirus inhibitors, analogous to the HIV fusion inhibitor enfuvirtide.

Following receptor binding and induced conformational changes in the spike glycoprotein, a third step would be involved in the viral entry process, namely cathepsin L proteolysis within endosomes. The cathepsin-L-specific inhibitor, MDL 28170 (also known as calpain inhibitor III (62), or Z-Val-Phe(CHO)), at the same time inhibited cathepsin L activity and S protein-mediated infection (IC50 of 2.5 nM and 0.1 μM, respectively). In addition to calpain inhibitor III, some other calpain inhibitors have been described as inhibitors of SARS coronavirus replication, the most selective (selectivity index > 100) being calpain inhibitor VI (4-fluorophenylsulfonyl-Val-Leu(CHO),63).
The crystal structure of the SARS coronavirus protease has been revealed.\(^\text{113,114}\) This offers a solid basis for the rational drug design of SARS protease inhibitors. For other potential targets such as the NTPase/helicase and the RNA replicase (RNA-dependent RNA polymerase) such structural basis still has to be delineated.

Of a number of peptidomimetic compounds (aziridinyl peptides, keto-glutamine analogs, chymotrypsin-like protease inhibitors, and peptide anilides) that have been reported as inhibitors of the SARS coronavirus main protease, the niclosamide anilide (64), with a \(K_i = 0.03\ \mu\text{M} \quad \text{(IC}_{50} = 0.06\ \mu\text{M})\), proved to the most potent (competitive) inhibitor.\(^\text{115}\) There are only a few cases where the 3C-like protease inhibitors were shown to inhibit both the SARS coronavirus protease activity and virus replication in cell culture. For example, the phe–phe dipeptide inhibitor (65) was found to inhibit the 3C-like protease at an IC\(_{50}\) of 1 \(\mu\text{M}\) and inhibited virus replication in Vero cells at an EC\(_{50}\) of 0.18 \(\mu\text{M}\), while not being toxic to the host cells at a concentration of 200 \(\mu\text{M}\) (selectivity index >1000).\(^\text{116}\) An octapeptide specifically designed for the SARS coronavirus main protease, namely AVLQSGFR, was reported to inhibit SARS coronavirus replication in Vero cells at an EC\(_{50}\) of 0.027 \(\mu\text{g mL}^{-1}\), while not being cytotoxic at 100 \(\mu\text{g mL}^{-1}\), thus establishing a selectivity index of >3700.\(^\text{117}\) Whether this highly selective antiviral effect was actually mediated by an inhibition of the SARS coronavirus main protease was not ascertained in this study.\(^\text{117}\)

The SARS coronavirus NTPase/helicase has been considered a potential target for the development of anti-SARS agents.\(^\text{118}\) Bananin (66) and three of its derivatives (iodobananin, vanillinbananin, and eubananin) were shown to inhibit both the ATPase and helicase activity of the SARS coronavirus NTPase/helicase, with IC\(_{50}\) values (for the ATPase activity) in the range of 0.5–3 \(\mu\text{M}\).\(^\text{119}\) Bananin was also found to inhibit SARS-CoV replication in fetal rhesus kidney (FRhK-4) cells at an EC\(_{50}\) of less than 10 \(\mu\text{M}\) and a CC\(_{50}\) of over 300 \(\mu\text{M}\), thus exhibiting a selectivity index of over 30.\(^\text{119}\) Whether the antiviral effect obtained in cell culture was causally linked to inhibition of the NTPase/helicase was not ascertained.

The SARS coronavirus RNA-dependent RNA polymerase (RdRp), because of its pivotal role in viral replication, represents another potential target for anti-SARS therapy. This enzyme does not contain a hydrophobic pocket for nonnucleoside inhibitors similar to those that have proven effective against the HCV polymerase or HIV-1 reverse transcriptase.\(^\text{120}\) In fact, nonnucleoside HIV-1 reverse transcriptase inhibitors were shown to have no evident inhibitory effect on SARS coronavirus RdRp activity.\(^\text{121}\) At present, few, if any, nucleoside analogs have been recognized as specific inhibitors of the SARS coronavirus RdRp. There is N\(^4\)-hydroxycytidine, which has been accredited with both anti-HCV
and anti-SARS coronavirus effects. Against SARS coronavirus it proved active at an EC$_{50}$ of 10$\mu$M (selectivity index $\geq 10$).$^{112}$ Whether this antiviral effect was mediated by an inhibition of the viral RdRp was not ascertained, however.

A wide variety of 'old' and 'new' compounds have been reported to inhibit the in vitro replication of the SARS coronavirus at relatively high concentration ($\geq 1$ $\mu$M).$^{122}$ There is no shortage of small molecules that inhibit the replication of the SARS virus within the 1–10 $\mu$M (or higher) concentration range,$^{123}$ but whether any of these molecules would be able to prevent or suppress SARS in vivo, remains to be determined. Typical examples of such miscellaneous compounds, often with an ill-defined mode of action but selectivity indexes up to 100, that have been reported to inhibit SARS coronavirus replication are valinomycin (67),$^{123}$ glycyrrhizin (68),$^{124}$ chloroquine (69),$^{125}$ niclosamide (70),$^{126}$ and nelfinavir (71).$^{127}$
SiRNAs have been developed that target the replicase \(^{126}\) and spike (S) \(^{129}\) genes of the SARS coronavirus genome, thereby silencing their expression in cell culture. Potent siRNA inhibitors of SARS coronavirus in vitro (i.e., the siRNA duplexes siSC2 (forward sequence: 5'-GCUCUAUUACACUCACdTdT-3') and siSC5 (forward sequence: 5'-GGAUGAGGAAGGCAAUUAdtAdt-3'), targeting the SARS coronavirus genome at S protein-coding and nonstructural protein-12-coding regions, respectively) were further evaluated for their efficacy in a rhesus macaque SARS model\(^ {130}\), and found to provide relief from SARS coronavirus infection-induced fever, diminish SARS-CoV levels, and reduce acute diffuse alveolar damage. Whether SARS can be conquered by the siRNA approach remains to be proven, however.

Shortly after SARS coronavirus was identified as the causative agent of SARS, interferons were shown to inhibit the replication of SARS coronavirus in cell culture in vitro, interferon-\(\beta\) being more potent than either interferon-\(\alpha\) or -\(\gamma\)\(^ {131}\). These observations were subsequently confirmed and extended in several other studies\(^ {132,134}\). Interferon-\(\beta\), in conjunction with interferon-\(\gamma\), was found to synergistically inhibit the replication of SARS coronavirus in Vero cells\(^ {134}\). Being a prophylactic rather than therapeutic agent, interferon(s) may have their highest utility in the prophylaxis or early postexposure management of SARS. Pegylated interferon-\(\alpha\) has been shown to reduce viral replication and excretion, viral antigen expression by type 1 pneumocytes, and the attendant pulmonary damage in cynomolgous macaques that were infected experimentally with SARS coronavirus\(^ {135}\). Pegylated interferon-\(\alpha\) is commercially available for the treatment of hepatitis C (where it is generally used in combination with ribavirin) and hepatitis B. Pegylated interferon-\(\alpha\) as well as the other commercially available interferons (interferon-\(\beta\), alfacon-1, etc.) could be considered for prevention and/or early postexposure treatment of SARS should it reemerge.

### 7.10.13 Orthomyxoviruses (Influenza A, B)

For many years, amantadine (72) and rimantadine (73) have been used for the prophylaxis and therapy of influenza A virus infections, but they never gained wide acceptance, primarily because of the risk of rapid emergence of drug-resistant virus mutants. These compounds interact specifically with the matrix M2 protein, which through its function as an hydrogen ion (H\(^+\)) channel, helps in the decapsidation (uncoating) of the influenza A virus particles. Influenza A (H1N1) viruses harboring amantadine resistance mutations are as virulent as wild-type virus strains\(^ {136}\) and can be readily transmitted during antiviral pressure in the clinical setting.
In recent years, the neuraminidase inhibitors zanamivir (74) and oseltamivir (75) have become available for the therapy and/or prophylaxis of influenza A and B virus infections. Influenza has adopted a unique replication strategy by using one of its surface glycoproteins, hemagglutinin (H), to bind to the target cell receptor (which contains a terminal sialic acid, N-acetylneuraminic acid (NANA)), and another surface glycoprotein neuraminidase (N), to cleave off the terminal sialic acid, thus allowing the virus particles to leave the cells after the viral replicative cycle has been completed. Neuraminidase inhibitors block the release of progeny virus particles from the virus-infected cells, thus preventing virus spread to other host cells.

When used therapeutically, neuraminidase inhibitors lead to a reduction in illness by 1–2 days, a reduction in virus transmission to household or healthcare contacts, a reduction in the frequency and severity of complications (such as sinusitis and bronchitis) and a diminished use of antibiotics. When used prophylactically, neuraminidase inhibitors significantly reduced the number of new influenza cases. Although resistance of human influenza viruses to neuraminidase inhibitors can develop, there is no evidence of naturally occurring resistance to either zanamivir or oseltamivir. Zanamivir and oseltamivir should be effective against both influenza A and B, and, among influenza A, the prevailing variants H1N1, H3N2, and also the ‘avian flu’ H5N1. Zanamivir, which must be taken through (oral) inhalation, and, in particular, oseltamivir, which can be more conveniently administered as oral capsules, should be stockpiled to confront a potential influenza pandemic in the future. With the increasing threat of the avian flu, the need for a sufficient supply of neuraminidase inhibitors, such as oseltamivir and zanamivir, has become extremely urgent. In comparison with the neuraminidase inhibitors, the existing influenza vaccines are likely to be of limited value against newly emerging influenza virus strains.

Following zanamivir and oseltamivir, similar structure-based neuraminidase inhibitors have been developed, such as the cyclopentane derivative RWJ-270201 (peramivir, 76) and the pyrrolidines A-192558 (77) and A-315675 (78). These novel neuraminidase inhibitors may themselves be considered as potential drug candidates, and, while being amenable to further optimization, lead to the development of yet newer compounds with improved activity, bioavailability and/or resistance profiles.
In fact, both peramivir (RWJ-270201) and A-315675 proved effective against a panel of five zanamivir-resistant and six oseltamivir-resistant A and B influenza virus strains. Oseltamivir resistance in clinical isolates of human influenza A has been associated with mutations at positions 119, 198, 274, 292, or 294 of the neuraminidase. Recently, resistance of avian influenza A H3N1 against oseltamivir was shown to be caused by the H274Y mutation. Prominent among the other neuraminidase inhibitor-resistant influenza A (H3N2) virus mutations (not yet demonstrated for H5N1) are E119V and R292K; whereas the R292K mutation was associated with compromised virus growth and transmissibility, the growth and transmissibility of the E119V variant were comparable to those of wild-type virus.

Are there other antiviral agents, besides amantadine, rimantadine, and the neuraminidase inhibitors, that may be considered for their potential, in the prevention and/or therapy, of influenza A virus infections, including avian influenza? Ribavirin has since long been recognized as a broad-spectrum antiviral agent, with particular activity against both ortho- and paramyxoviruses. Recently, viramidine, the carboxamidine analog of ribavirin, was shown to have efficacy similar to ribavirin against influenza virus infections, and considering its lesser toxicity, viramidine may warrant further evaluation as a possible therapy for influenza, including H1N1. Yet, other recently described compounds with specific activity against influenza A, B, and C viruses are T-705 (6-fluoro-3-hydroxy-2-pyrazine carboxamide, T-705) and the 2,6-diketopiperazine flutimide (80), which would target the viral polymerase and cap-dependent endonuclease, respectively.

### 7.10.14 Paramyxoviruses (Parainfluenza, Measles, Mumps, RSV, hMPV, Nipah, etc.)

Of the paramyxoviruses, parainfluenza (types 1–5) has received little attention from either a preventative or curative viewpoint; mumps and measles, like the rubellivirus rubella, are now sufficiently contained by vaccination, which makes respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and Nipah the paramyxoviruses with the greatest need for antiviral therapy. For RSV the only approved antiviral therapy is aerosol administration of ribavirin. In practice, however, ribavirin is rarely used owing to the technical burden of delivery by aerosol under the given circumstances (RSV bronchopneumonitis in young infants). Given the high incidence of RSV infections (which are...
often diagnosed as influenza), there is a high (and as yet unmet) medical need for an appropriate therapy (and prophylaxis) of RSV infections; the same holds for hMPV infections, which usually occur during the same (winter) season as RSV, mainly in young children, elderly people, and immunocompromised individuals. Ribavirin certainly holds promise for the treatment of hMPV infections, as has recently been demonstrated in the mouse model for hMPV. Also, as has been mentioned above for HCV, siRNA may also be applicable, if properly designed and administered (intranasally) in the treatment of respiratory virus infections.

Recently, a number of small molecules, e.g., VP-14637 (81) and JNJ-2408068 (82) (formerly known as R-170591), although structurally dissimilar, have been shown to fit into a small hydrophobic cavity in the inner core of the RSV fusion (F) protein, thereby interacting with the heptad repeats HR1 and HR2 domains, and to inhibit RSV fusion. Although the therapeutic potential of these compounds in the treatment of RSV infections is presently unclear, there is no doubt that further exploration of the mechanism of interaction between these inhibitors and the F protein should facilitate the design of new RSV fusion inhibitors.

BMS-433771 (83) was found to be a potent inhibitor of RSV replication in vitro; it exhibited excellent potency against multiple laboratory and clinical isolates of both A and B RSV with an average EC_{50} of 20 nM. BMS-433771 inhibits fusion the (viral and cellular) lipid membranes during both the early and virus entry stage and late-stage syncytium formation. BMS-433771 was shown to be orally active against RSV in BALB/c mice and cotton rats, even if administered as a single oral dose 1 h prior to intranasal RSV inoculation. It could be considered the prototype of low-molecular-weight inhibitors that target the formation of the six helical coiled-coil bundles as a prelude to virus–cell fusion, not only of RSV but also HIV.

Although human parainfluenza viruses are important respiratory tract pathogens, especially in children, they have received little attention from either prophylactic (vaccine) or therapeutic viewpoint. Yet, they contain a unique target, the major surface glycoprotein hemagglutinin-neuraminidase (HN) that serves, at the same time, for cell attachment and virus spread. The HN inhibitors BCX-2798 (84) and BCX-2855 (85) were found to inhibit both functions, and to block infection with parainfluenza viruses both in vitro and in vivo. These compounds may limit parainfluenza virus infections in humans. Other compounds that may be further pursued for their activity against parainfluenza viruses, RSV, as well as influenza viruses, include flavonoids, uncinosides, and polyoxotungstates.
7.10.15  **Arenas-, Bunya-, Rhabdo-, and Filoviruses**

7.10.15.1  **Arenaviruses**

Of the 23 arenaviruses known, five are associated with viral hemorrhagic fever: Lassa, Junin, Machupo, Guanarito, and Sabia. Ribavirin has proven to be effective in postexposure prophylaxis and therapy of experimental arenavirus infections in animal models, and anecdotal reports suggest that it might also be effective in the treatment of arenavirus infections (i.e., Machupo and Sabia) in humans. The most convincing evidence for the (clinical) efficacy was obtained in the case of Lassa fever, where it was found to reduce the case-fatality rate, irrespective of the time point in the illness when treatment was started.

7.10.15.2  **Bunyaviruses**

Of the bunyaviruses, one of the most feared (because it is highly infectious, easily transmitted between humans, and associated with a case-fatality rate of approximately 30%) is Crimean–Congo hemorrhagic fever virus. Bunyaviruses are sensitive to ribavirin, and this has also been demonstrated in experimental animal models. Also, interferon and interferon inducers have proved effective in the treatment of experimental bunyavirus infections, and, likewise, interferon-α should be considered for the treatment of arenavirus infections, as warranted by its efficacy in the therapy of Pichindi virus infection in hamsters.

7.10.15.3  **Rhabdoviruses**

Of the rhabdoviruses, rabies, which is almost invariably fatal if no control measures are taken, can be contained by repeated injections of specific immunoglobulin and/or the inactivated (‘killed’) rabies vaccine as soon as possible after the infection. For the filovirus infections Ebola and Marburg no vaccine is (yet) available. Specific immunoglobulin or interferon-α may only be of limited value in the treatment of filovirus infections, as indicated by experimental findings in rhesus macaques infected with Ebola (Zaire) virus.

7.10.15.4  **Filoviruses**

No antiviral drugs that are currently in clinical use, including ribavirin, provide meaningful protection against filoviruses in vivo. A possible therapeutic strategy may be based on the use of S-adenosylhomocysteine (SAH) hydrolase inhibitors. SAH hydrolase inhibitors, such as 3-deazaneplanocin A (86), interfere with S-adenosylmethionine (SAM)-dependent methylation reactions, particularly those involved in the ‘capping’ of viral mRNA. Some viruses, such as the rhabdovirus vesicular stomatitis virus (VSV), rely heavily on mRNA capping and are particularly sensitive to inhibition by SAH hydrolase inhibitors, including 3-deazaneplanocin A. As, biochemically, filo- and rhabdoviruses are quite similar in their replication machinery, both requiring 5'-capping of their mRNAs, SAH hydrolase inhibitors such as 3-deazaneplanocin A may logically be expected to be effective in the treatment of Ebola virus infections. In fact, when administered as a single dose of 1 mg kg⁻¹, 3-deazaneplanocin A was found to protect mice against a lethal infection with Ebola virus (Zaire strain). This protective effect was accompanied, and probably mediated, by the production of high concentrations of interferon in the Ebola virus-infected mice. It can be hypothesized that, by blocking the 5'-capping of the nascent (+)RNA viral strands (and, hence, their maturation toward mRNAs), 3-deazaneplanocin A stimulated the formation of double-stranded (±)RNA complexes, which have long been known to be excellent inducers of interferon.

Like SAH hydrolase, inosine monophosphate (IMP) dehydrogenase is another cellular enzyme that may be envisaged as a target for antiviral agents. IMP dehydrogenase is a crucial enzyme involved in the biosynthesis of GTP, and, although ribavirin may act against distinct viruses by distinct mechanisms (e.g., IMP dehydrogenase inhibition, immunomodulatory effect, RNA capping interference, polymerase inhibition, lethal mutagenesis), the predominant mechanism by which ribavirin exerts its antiviral activity in vitro against flaviviruses and paramyxoviruses is mediated by inhibition of IMP dehydrogenase.

Recently, a new class of compounds, phosphorodiamidate morpholino oligomers (PMO), conjugated to arginine-rich cell-penetrating peptides (P-PMO) and designed to base pair with the translation start region of Ebola virus VP35 positive-sense RNA, were reported to inhibit Ebola virus replication and to protect mice against a lethal Ebola virus infection.
7.10.16  Reoviruses

Of the reoviruses, rotavirus, which is associated with viral gastrointestinal infections, is by far the most clinically important pathogen. Several attempts have been, and are still being, made to develop an effective vaccine for rotavirus infections. Current treatment for rotavirus diarrhea is mainly based on the administration of fluids to prevent dehydration. There are no attempts to develop an antiviral drug for this disease, although it is worthy to note that the replication of reo- (and rota)viruses is exquisitely sensitive to SAH hydrolase inhibitors such as 3-deazaneplanocin A.179

7.10.17  Retroviruses (HIV)

There are at present some 20 compounds available for the treatment of HIV infections.186 These compounds fall into five categories: (1) nucleoside reverse transcriptase inhibitors (NRTIs): zidovudine, didanosine (87), zalcitabine (88), stavudine (89), lamivudine, abacavir (90) and emtricitabine; (2) nucleotide reverse transcriptase inhibitors (NRTIs): tenofovir disoproxil fumarate; (3) nonnucleoside reverse transcriptase inhibitors (NNRTIs): nevirapine (91), delavirdine (92), and efavirenz (93); (4) protease inhibitors (PIS): saquinavir (94), ritonavir (95), indinavir (96), nelfinavir, amprenavir (97), lopinavir (98) (combined at a 4-to-1 ratio with ritonavir), atazanavir (99), fosamprenavir (100), and tipranavir (101); and (5) fusion inhibitors (FIs): enfuvirtide (102). Several of these compounds are also available as fixed dose combinations: zidovudine with lamivudine, lamivudine with abacavir, and emtricitabine with tenofovir disoproxil fumarate. A triple-drug fixed dose combination, containing efavirenz, emtricitabine, and tenofovir disoproxil fumarate is forthcoming.
91 Nevirapine
Viramune

92 Delavirdine
Rescriptor

93 Efavirenz
Sustiva, Stocrin

94 Saquinavir
hard gel capsules, Invirase
soft gelatin capsules, Fortovase

95 Ritonavir
Norvir
96 Indinavir
    Crixivan

97 Amprenavir
    Agenerase, Prozei

98 Lopinavir
    combined with ritonavir at 4/1 ratio
    Kaletra

99 Atazanavir
    Reyataz
In addition to the 21 licensed anti-HIV compounds, various others are (or have been) in clinical (phase II or III) development: the HIV-1 attachment inhibitors BMS-378806 (103) and BMS-488043 (104), \(^{187}\) the CXCR4 antagonist AMD-3100 (105) (as stem cell mobilizer for stem cell transplantation in patients with non-Hodgkin lymphoma or multiple myeloma), \(^{188}\) the CCR5 antagonists \(^{189}\) SCH-C (106), vicriviroc (SCH-D, SCH 417690) (107), \(^{190}\) aplaviroc (873140) (108), \(^{191}\) and maraviroc (UK-427857) (109), \(^{192}\) the NRTIs Racivir (110), (-)-dOTC (AVX-754 (SPD-754) (111), which has been accredited with activity against most other NRTI-resistant HIV-1 strains \(^{193}\), Reverset (112), elvucitabine (113), alovudine (114), and amdoxovir (115), the NNRTIs capravirine (116) and etravirine (117), the protease inhibitor TMC-114 (118), \(^{186,194}\) and the gag (p24) maturation inhibitor PA-457 (119). \(^{195}\) Also, a prodrug of the benzophenone GW678248 (120) has recently progressed to phase II clinical trials. \(^{196}\)
110 Racemic (±)FTC (FdOTC)
Racivir

111 AVX-754 ((−)-dOTC)

112 DPC-817 (β-D-Fd4C)
Reverset

113 ACH-126443 (β-L-Fd4C)
Elvucitabine

114 MIV-310 (FddThd, FLT)
Alovudine

115 Diaminopurine dioxolane (DAPD)
Amdoxovir

116 Capravirine (S-1153, AG1549)

117 Etravirine (TMC-125, R-165335)
Yet other compounds are in preclinical development and/or may soon proceed to clinical phase I/II clinical trials: the CD4 (HIV receptor) downmodulator cyclotriazadisulfonamide (CADA) (121) \(^{197}\); the HIV gp120 envelope-binding protein cyanovirin-N (122) as a topical microbicide \(^{198}\); KRH-2731, a CXCR4 antagonist, structurally related to KRH-1636 (123) \(^{199}\); the CXCR4 antagonist AMD-070 (a derivative of the bicyclam AMD3100, which is currently being pursued in phase II/III clinical trials, in combination with granulocyte colony-stimulating factor (G-CSF), for the mobilization of autologous hematopoietic progenitor cells) \(^{200}\); TAK-220 (124), a CCR5 antagonist, structurally related to TAK-779 (125) \(^{201}\) which has proved to be a highly potent (orally bioavailable) inhibitor of CCR5-using (R5) HIV-1 strains \(^{202,203}\) and acts synergistically with other antiretrovirals \(^{204}\); TAK-652 (126), another orally bioavailable inhibitor of CCR5-mediated HIV infection \(^{205}\); MIV-210 (127), a produg of the NRTI 3'-fluoro-2',3'-dideoxyguanosine; the thymine dioxolane DOT (128), another NRTI \(^{206}\); 4'-Ed4T (2',3'-didehydro-3'-deoxy-4'-ethynyl-2'-deoxythymidine) (129), which has favorable oral bioavailability and a unique drug resistance profile, different from the other NRTIs \(^{207}\); the NtRTIs 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines PMPO-DAPy, PMEO-DAPy, and 5-substituted derivatives thereof (130) \(^{2}\); the deoxythreosyl nucleoside phosphonates phosphonomethyldéoxythreosyladenine (PMDTA, 131) and -thymine (PMDTT, 132) \(^{206}\); the NNRTIs thiocarboxanilide UC-781 (133) and dapivirine (TMC-120) (134), both as topical microbicides, and rilpivirine (R-278474) (135), one of the most potent anti-HIV agents ever described \(^{209}\); GW678248, a novel benzophenone NNRTI \(^{210,211}\) which has activity at 1 nM against the K103N and Y181C RT HIV-1 mutants associated with clinical resistance to efavirenz and nevirapine, respectively \(^{196}\); and a number of compounds, including the 1,6-naphthyridine-7-carboxamides L-870810 (136) and L-870812 (137), which are targeted at the HIV-1 integrase \(^{212,213}\). The 3,7-dihydroxytropolones (138) represent an interesting platform for the design of inhibitors of both the reverse transcriptase (and RNase H) as well as the HIV integrase \(^{214}\). Similarly, indolyl aryl sulfone (139) may serve as a platform for the design of new NNRTIs effective against K103N HIV-1 variants \(^{215}\). Recently, diketo acids bearing a nucleobase scaffold have been described as highly potent HIV integrase inhibitors \(^{216}\); the prototype compound, 4-(1,3-dibenzyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidin-5-yl)-2-hydroxy-4-oxo-but-2-enoic acid (140), exhibited an anti-HIV selectivity index in cell culture of >4000.
(H₂N)Leu — Gly — Lys — Phe — Ser — Gln — Thr — Cys — Tyr — Asn — Ser — Ala —
— Ile — Gln — Gly — Ser — Val — Leu — Thr — Ser — Thr — Cys — Glu — Arg — Thr — Asn — Gly — Gly — Tyr — Asn — Thr — Ser —
— Ser — Ile — Asp — Leu — Asn — Ser — Val — Ile — Glu — Asn — Val — Asp — Gly — Ser — Leu — Lys — Trp — Gln — Pro — Ser —
— Asn — Phe — Ile — Glu — Thr — Cys — Arg — Asn — Thr — Gln — Leu — Ala — Gly — Ser — Glu — Leu — Ala — Ala — Glu —
— Cys — Lys — Thr — Arg — Ala — Gin — Gin — Phe — Val — Ser — Thr — Lys — Ile — Asn — Leu — Asp — Asp — His — Ile — Ala —
— Asn — Ile — Asp — Gly — Thr — Leu — Lys — Tyr — Glu(COOH)
127 MIV-210 [FLG (3’-fluoro-2’,3’-dideoxyguanosine) prodrug]

128 1-[(β-D-dioxolane)thymine DOT

129 4’-Ed4T

130 R = CH₃ : PMPO-DAPy
   R = H : PMEO-DAPy

131 PMDTA

132 PMDTT

133 Thiocarboxanilide UC-781
In addition to the aforementioned cyanovirin-N, thiocarboxanilide UC-781, and dapivirine, there are some other compounds that could be further developed as topical (e.g., vaginal) microbicides, namely the aglycons of the glycopeptide antibiotics vancomycin, teicoplanin, and eremomycin which specifically interact with the gp120 glycoprotein. Also the plant lectins, e.g., *Galanthus nivalis* agglutinin (GNA) and *Hippeastrum* hybrid agglutinin (HHA), represent potential candidate anti-HIV microbicides: they show marked stability at relatively low pH and high temperatures for prolonged time periods, they directly interact with the viral envelope and prevent entry of HIV into its target cells. Upon prolonged exposure of HIV in cell culture to HHA or GNA, the virus acquires resistance mutations in the gp120 glycoprotein which are predominantly located at the N-glycosylation (asparagine) sites.

An avenue to be further explored is the combination of different microbicides, such as the NNRTI thiocarboxanilide UC-781 with the cellulose acetate 1,2-benzenedicarboxylate (CAP) viral entry inhibitor, which exhibit synergistic and complementary effects against HIV-1 infection. There is, in addition, no shortage of sulfated and sulfonated polymers (starting off with suramin, the first polysulfonate ever shown to be active against HIV) which could be considered as topical anti-HIV microbicides.
7.10.18 Conclusion

About 40 compounds are registered as antiviral drugs, at least half of which are used to treat HIV infections. An even greater number of compounds are under clinical or preclinical development, with again, as many targeting HIV as all the other viruses taken together. This implies that HIV, since its advent, has remained the main target in antiviral drug development. Antiviral agents can, as guided by the anti-HIV agents as examples, be divided in roughly five categories: (1) nucleoside analogs, (2) nucleotide analogs (or acyclic nucleoside phosphonates), (3) nonnucleoside analogs, (4) protease inhibitors, and (5) virus–cell fusion inhibitors. Molecular targets are for (1) and (2) the viral DNA polymerase (whether DNA-dependent as in the case of herpesviruses, or RNA-dependent as in the case of HIV or HBV); for (3) RNA-dependent DNA polymerase (reverse transcriptase), associated with HIV, or RNA-dependent RNA polymerase (RNA replicase) associated with HCV; for (4) the proteases associated with HIV and HCV; and for (5) the fusion process of HIV (and, potentially, other viruses such as the SARS coronavirus and RSV). Antiviral agents may also exert their antiviral effects through an interaction with cellular targets such as IMP dehydrogenase (ribavirin) and SAH hydrolase (3-deazaneplanocin A). The latter enzymes are essential for viral RNA synthesis (through the supply of GTP) and viral mRNA maturation (through 5'-capping), respectively. Finally, interferons (now generally provided in their pegylated form) may be advocated in the therapy of those viral infections (actually, HBV and HCV; prospectively, Coxsackie B, SARS, ...) that, as yet, cannot be sufficiently curbed by other therapeutic measures.

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