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Recent highlights in the development of new antiviral drugs

Erik De Clercq

Twenty antiviral drugs, that is about half of those that are currently approved, are formally licensed for clinical use in the treatment of human immunodeficiency virus infections (acquired immune deficiency syndrome). The others are used in the treatment of herpesvirus (e.g. herpes simplex virus, varicella zoster virus and cytomegalo virus), hepatitis B virus, hepatitis C virus or influenza virus infections. Recent endeavours have focussed on the development of improved antiviral therapies for virus infections that have already proved amenable to antiviral drug treatment, as well as for virus infections for which, at present, no antiviral drugs have been formally approved (i.e. human papilloma viruses, adenoviruses, human herpesvirus type 6, poxviruses, severe acute respiratory syndrome coronavirus and hemorrhagic fever viruses).

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Introduction

Of the ~40 antiviral drugs that have been formally licensed for clinical use [1], 20 are used in the treatment of human immunodeficiency virus (HIV) infections (acquired immune deficiency syndrome; AIDS). The anti-HIV compounds fall into five categories: first, the nucleoside reverse transcriptase inhibitors (NRTIs), zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine; second, the nucleotide reverse transcriptase inhibitor, tenofovir disoproxil fumarate; third, the non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine, delavirdine and efavirenz; fourth, the protease inhibitors (PIs), saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir (combined at a 4 to 1 ratio with ritonavir), atazanavir and fosamprenavir; and fifth, the fusion inhibitor, enfuvirtide. Lamivudine and adefovir dipivoxil have been approved for the treatment of hepatitis B virus infections. Of the anti-herpesvirus agents, acyclovir, valaciclovir, famciclovir, brivudin,

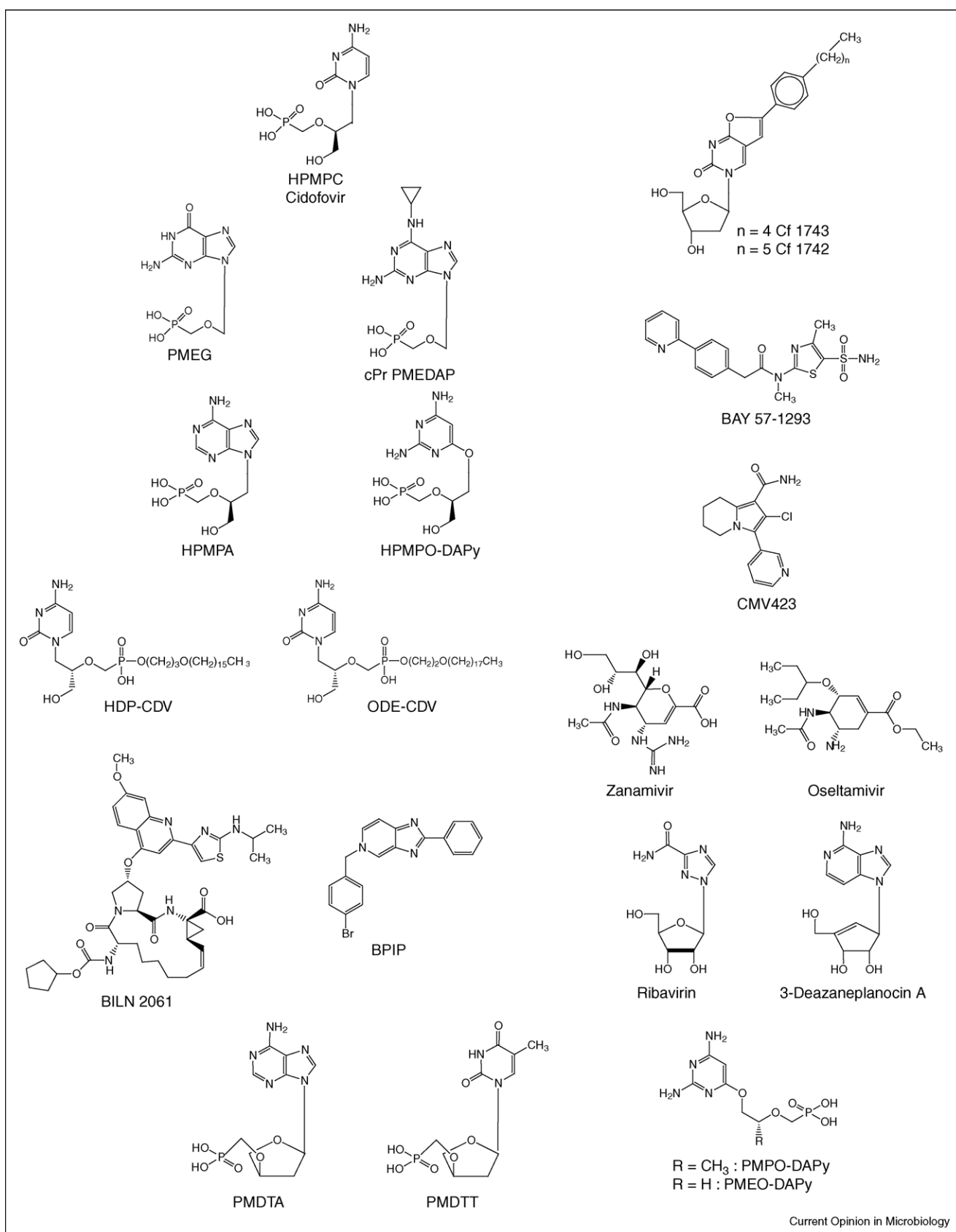
peniclovir, idoxuridine and trifluridine are used (the latter three only topically) in the treatment of herpes simplex virus (HSV) and/or varicella-zoster virus (VZV) infections; ganciclovir, valganciclovir, foscarnet, cidofovir and fomivirsen (the latter only by intravitreal injection) have proven useful in the treatment of cytomegalovirus (CMV) infections in immunocompromised patients (i.e. AIDS patients that have CMV retinitis). Following the progression of amantadine and rimantadine (matrix 2 protein blockers) onto the market, the neuraminidase inhibitors zanamivir and oseltamivir have become available for the therapy (and prophylaxis) of influenza virus infections. Ribavirin has been used (topically as an aerosol) in the treatment of respiratory syncytial virus infections, and the combination of ribavirin with (pegylated) interferon- α has received increased acceptance for the treatment of hepatitis C virus (HCV) infections.

There are, however, several other important virus infections for which no antiviral drugs have been developed; even for those that are amenable to antiviral drug therapy there is still considerable room for improvement in terms of higher potency and/or increased selectivity or safety. In this review, I will highlight some of the most recent approaches towards the treatment of human papilloma virus (HPV) infections, adenovirus infections, HSV and VZV infections, human herpesvirus type 6 (HHV-6) infections, poxvirus infections, hepatitis C virus infections, corona virus infections (i.e. severe acute respiratory syndrome [SARS]), influenza virus infections, hemorrhagic fever virus infections and HIV infections (AIDS).

Human papilloma virus infections

Although cidofovir, which is also known as (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC; Figure 1), is formally licensed only for the intravenous treatment of CMV retinitis in AIDS patients, 'off label' it has been used successfully in the systemic and topical treatment of several other DNA virus infections, such as polyoma-, papilloma-, adeno-, herpes- and pox-virus [2]. Particularly striking are the effects that have been obtained with cidofovir (topical gel application or direct intralesional injection) in the treatment of HPV-associated papillomatous lesions, such as hypopharyngeal and esophageal papilloma, laryngeal papilloma, recurrent respiratory papillomatosis (in children), anogenital HPV infections (i.e. condylomata acuminata), Bowenoid papulosis (perianal intraepithelial neoplasia), cervical and vulvar intraepithelial neoplasia grade III, recalcitrant warts (including plantar warts), and other mucocutaneous HPV lesions that did not respond to conventional therapies [3^{*}]. In most of

Figure 1



Structures of the compounds indicated in text.

these cases, a virtually complete and durable resolution of the lesions was achieved following the application of cidofovir (as a 1% gel or cream).

Although the (compelling) evidence for the effectiveness of cidofovir against HPV infections stems from *in vivo* observations in an increasing number of patients, *in vitro* experiments with HPV-infected keratinocytes have ascertained that cidofovir specifically 'kills' HPV-positive cells by the induction of apoptosis. This effect might be related to the ability of cidofovir 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 [3^{*}]. In addition to cidofovir, there are several other acyclic nucleoside phosphonates, for example 9-(2-phosphonylmethoxyethyl)guanine (PMEG; Figure 1) and 9-(2-phosphonylmethoxyethyl)-N⁶-cyclopropyl-2,6-diaminopurine (cPr PMEDAP; Figure 1), which, like cidofovir, are able to specifically induce apoptosis in HPV-infected keratinocytes. PMEG and cPr PMEDAP are currently being investigated for their potential in the treatment of HPV-associated papillomatous lesions.

Adenovirus infections

Adenovirus infections in immunocompetent individuals are generally self-limiting, but in immunocompromised patients and, in particular, allogeneic hematopoietic stem cell transplant recipients, adenovirus infections can be severe and life-threatening. At present, cidofovir (HPMPC) appears to be the only licensed antiviral drug that can be successfully used to treat adenovirus infections [3^{*}].

In addition to cidofovir, a few other acyclic nucleoside phosphonates such as (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA; Figure 1) and (*S*)-2,4-diamino-6-[3-hydroxy-2-phosphonomethoxypropoxy]pyrimidine (HPMPO-DAPy; Figure 1) have proved to be potent and selective inhibitors of the *in vitro* replication of adenoviruses [4]. Also, the N7-substituted acyclic nucleoside 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine S-2242 and the 2',3'-dideoxynucleoside analogues zalcitabine (2',3'-dideoxycytidine; ddC) and alovudine (3'-fluoro-2',3'-dideoxythymidine; also known as FddT and FLT) were found to inhibit *in vitro* adenovirus replication, whereas no anti-adenovirus activity was observed for several other antiviral compounds, such as ribavirin, foscarnet, acyclovir, penciclovir and brivudin [4]. It might be worthwhile to further examine HPMPA, HPMPO-DAPy, S-2242, ddC and FLT in both experimentally and clinically oriented studies.

Also, the ether lipid ester (i.e. hexadecyloxypropyl [HDP] and octadecyloxyethyl [ODE]) prodrugs of cidofovir (HPMPC) and HPMPA should be evaluated in the future for their potential to treat adenovirus infections in humans; these compounds were found to inhibit adenovirus repli-

cation *in vitro* at significantly lower concentrations and with significantly higher selectivity indices than those noted for the parent compounds (HPMPC and HPMPA) [5].

Herpes simplex virus and varicella-zoster virus infections

Standard antiviral drugs currently used in the treatment of HSV and/or VZV infections include acyclovir, valaciclovir (the oral prodrug of acyclovir), famciclovir (the oral prodrug of penciclovir) and brivudin (BVDU). The latter is only available in some European countries, and, although exquisitely active against VZV, its use is restricted to patients that have herpes zoster or HSV-1 infections. BVDU cannot be administered concomitantly with 5-fluorouracil (which is a cytostatic agent used in the treatment of certain cancers) because through the release of its degradation product, BVU, BVDU can potentiate the toxicity of 5-fluorouracil (through inhibition of dihydropyrimidine dehydrogenase, a key enzyme in the degradation of 5-fluorouracil). The newly discovered bicyclic furo(2,3-d)pyrimidine nucleoside analogues (BCNAs; i.e. Cf 1368, Cf 1369, Cf 1742 [Figure 1] and Cf 1743 [Figure 1]) do not have this drawback. Although the BCNAs have proven to be as active, and Cf 1742 and Cf 1743 are 10-fold more active against VZV than BVDU [6^{*},7], they are unlikely to enhance the toxicity of 5-fluorouracil for two reasons: first, they are not degraded to the free pyrimidine base, and second, the latter was shown not to interfere with the dihydropyrimidine dehydrogenase that initiates the catabolism of 5-fluorouracil [6]. BCNAs are extremely selective in their anti-VZV activity, in that they inhibit only VZV replication and not the replication of any other viruses such as HSV. With a selectivity index (ratio of 50% cytotoxic concentration to 50% inhibitory concentration against viral replication) in excess of 100 000, the BCNAs offer an unprecedented potential for the treatment of VZV infections.

New anti-HSV agents that target the viral helicase–primase complex, such as the thiazolylphenyl derivatives BILS 179BS and, especially, BAY 57-1293 (Figure 1), were recently reported to have *in vivo* efficacy in animal models of HSV-1 and HSV-2 infections [8,9]. These compounds appear to function by enhancing the affinity of the helicase–primase complex for the HSV DNA. This complex comprises three viral proteins — the HSV UL5, UL-8 and UL52 gene products — which together unwind the double-stranded viral DNA and generate the primer/templates for DNA synthesis by the viral DNA polymerase. It has been claimed that the antiviral potency of BAY-1293 is superior to all other compounds currently used for the treatment of HSV infections [10]. This by itself validates the further pursuit of helicase–primase inhibitors for the treatment of HSV infections.

A further extension of this approach involves the molecules that interact with the formation of the HSV DNA

polymerase complex (i.e. BP5, which inhibits the interaction between UL-30 and UL42) [11]. Protein–protein interactions constitute a new target for the design of antiviral agents to be further explored for their potential as antiviral drugs.

Human herpesvirus type 6 infections

HHV-6, like CMV, has been increasingly recognized as an important pathogen in immunocompromised patients (in which it might cause life-threatening complications); however, unlike for CMV infections, no compounds have been formally approved for the treatment of HHV-6 infections [12^{*}]. The drugs clinically used against HHV-6 are the same as those used in CMV therapy (or prophylaxis) and consist of ganciclovir, valganciclovir, and, to a lesser extent, acyclovir, valaciclovir, cidofovir and foscarnet. All these compounds are targeted at the viral DNA polymerase and can be considered (following phosphorylation) as substrate analogues (except for foscarnet, which acts as a pyrophosphate analogue).

Another class of compounds, the 4-oxo-dihydroxyquinolines, act as non-nucleoside inhibitors of herpesvirus DNA polymerases. They exhibit broad-spectrum antiviral activity against most human herpesviruses (including HCMV), with the exception of HHV-6 and HHV-7. Their lack of activity against HHV-6 is owing to a single amino acid change in the conserved domain III of the HHV-6 DNA polymerase [13].

We have recently discovered a new antiviral agent, CMV423 (2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide; Figure 1), which demonstrated potent and selective *in vitro* activity against all three human β -herpesviruses — CMV, HHV-6 and HHV-7 [14]. Compared to ganciclovir and foscarnet, CMV423 showed a higher potency and lower cytotoxicity. However, its antiviral action appeared to be cell-dependent. CMV423 must be targeted at an event that follows viral entry but that precedes viral DNA replication. CMV423 was also found to inhibit total cellular protein tyrosine kinase activity. It was concluded that CMV423 exerts its activity against HHV-6 through inhibition of a cellular process that is crucial in the early stage of viral replication and that might involve protein tyrosine kinase activity [14].

Poxvirus infections

The family of poxviridae encompasses orthopoxviruses (e.g. variola, vaccinia, cowpox, monkeypox and camelpox), parapoxviruses (e.g. orf) and mollusciviruses (e.g. molluscum contagiosum virus). Several nucleoside analogues (i.e. S2242, 8-methyladenosine and idoxuridine) and nucleotide analogues (including HPMPC [cidofovir] and HPMPD-DAPy) are effective in various animal models of poxvirus infections [15]. In particular, cidofovir has shown high efficacy in protection of mice from a lethal respiratory infection of either vaccinia or cowpox, even

after administration of a single systemic (intraperitoneal) or intranasal (aerosolized) dose. Cidofovir has demonstrated high effectiveness in the treatment of disseminated progressive vaccinia in athymic-nude mice [16]. 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 (reviewed by De Clercq [17]). These data indicate that cidofovir will be effective in the therapy and prophylaxis of smallpox (variola) and related poxviruses in humans, as well as in the treatment of the complications of vaccinia that can arise in immunocompromised patients inadvertently inoculated with the smallpox vaccine (vaccinia) [17].

However, as cidofovir is a phosphonate analogue it has limited oral bioavailability. In an outbreak of smallpox, it would be useful to have an orally active drug that could be self-administered [18^{*}]. Therefore, hexadecyloxypropyl-cidofovir (HDP-CDV) and octadecyloxyethyl-cidofovir (ODE-CDV) were designed and developed as potential (oral) drugs for the prophylaxis and treatment of variola virus infection (Figure 1) [18^{*}]. These alkyloxyalkyl esters of cidofovir were found to significantly enhance inhibition of orthopoxvirus replication (i.e. vaccinia and cowpox) *in vitro* [19]. When given orally, HDP-CDV and ODE-CDV were as effective as cidofovir is when administered parenterally for the treatment of vaccinia and cowpox infections [20]; HDP-CDV has also proven effective in the treatment of lethal vaccinia virus respiratory infections in mice [21]. Furthermore, when administered orally, HDP-CDV and ODE-CDV proved highly efficacious in a lethal mousepox (aerosol ectromelia virus) model, further attesting the potential usefulness of other lipid prodrugs of cidofovir in the oral therapy and prophylaxis of poxvirus infections.

Hepacivirus infections (hepatitis C)

The present therapy for chronic hepatitis C consists of pegylated interferon (IFN)- α 2a (180 μ g, once weekly) combined with ribavirin (1000 or 1200 mg, daily) [22,23]. This treatment regimen produces a sustained virologic response in at least 50% of the patients infected with HCV genotype 1 and 2 (and 80% of the patients infected with another genotype of HCV). Patients infected with HCV genotype 1 should be treated for at least 48 weeks [24], but this length of time might be reduced to 24 weeks for patients infected with HCV genotypes 2 or 3. In this combination, ribavirin is assumed to act as an immunomodulator, whereas the IFN would act as an antiviral agent targeted at a phosphoprotein encoded by the non-structural NS5A gene of the HCV genome [25].

In addition to the NS5A gene product, there are a few other non-structural proteins such as the NS3-encoded NTPase–helicase and serine protease and the NS5B-encoded RNA-dependent RNA polymerase (RdRp,

which is also known as RNA replicase) that could be envisaged as targets for inhibitors of HCV replication. Proof-of-principle that compounds targeted at the NS3 protease could reduce plasma concentrations of HCV RNA has been provided for the protease inhibitor BILN 2061 (Figure 1) [26*].

Equally attractive as a target for the development of HCV inhibitors is the HCV RNA replicase. Highly selective antiviral agents targeted at the viral RNA replicase have been described to inhibit the replication of bovine viral diarrhoea virus (BVDV), a pestivirus that could be considered as a surrogate virus for HCV [27,28]. We have recently described a novel series of compounds (e.g. prototype 5-[(4-bromophenyl)methyl]-2-phenyl-5*H*-imidazo[4,5-*c*]pyridine [also known as BPIP; Figure 1]) that act as 'non-nucleoside' RdRp inhibitors; these inhibit the replication of BVDV with high efficiency [29]. From this class of compounds, based on a different structure-activity relationship, new congeners have been derived that act equally efficiently against HCV replication. In the future treatment of HCV infections, these non-nucleoside RdRp inhibitors are likely to be combined with the 'nucleoside' RdRp inhibitors. An example of the latter, albeit a relatively weak inhibitor of BVDV and HCV, is N^4 -hydroxycytidine [30].

Coronavirus infections

There are several HCV proteins encoded by the SARS coronavirus (SARS-CoV) that could be considered as targets for chemotherapeutic intervention: the spike (S) protein, the SARS-CoV main protease (also known as the 3C-like protease), the NTPase/helicase, the RNA-dependent RNA polymerase (RdRp) and, possibly, other viral (or cellular) protein-mediated processes [31]. The SARS-CoV S protein mediates infection of permissive cells by controlling the interaction of its S1 domain with cells that express the SARS-CoV receptor angiotensin-converting enzyme 2 (ACE2) [32]. A 193-amino acid fragment of the S protein (that corresponds to amino acid residues 318–510) was found to block S protein-mediated infection [33], and a human monoclonal antibody to the S1 domain was found to neutralize SARS-CoV by blocking its association with the SARS-CoV receptor ACE2 [34]. In addition to ACE2, the C-type lectin CD209L (also called L-SIGN) was identified recently as a receptor for SARS-CoV (as well as for other enveloped viruses, including Ebola); thus, CD209L, like ACE2, might be a target for blocking SARS-CoV infection. Also, to the extent that the fusogenic mechanism of HIV is similar to that of SARS-CoV (e.g. with regard to heptad repeat interactions and six-helix bundle formation), it should be feasible to develop SARS-CoV fusion inhibitors analogous to enfuvirtide, which is used for treatment of HIV [35].

Knowledge of the crystal structure of the SARS-CoV main protease offers a solid basis for rational drug design of

SARS-CoV protease inhibitors [36]. For other potential targets, for example the NTPase/helicase and RNA replicase, such a structural basis still has to be elaborated. Meanwhile, a variety of 'old' and 'new' compounds have been reported to inhibit the *in vitro* replication of SARS-CoV when present in relatively high concentrations ($\geq 1 \mu\text{M}$) [37]. There is no shortage of small molecules that are active in the 1–10 μM concentration range [38], but whether any of these molecules will prevent or suppress the infection *in vivo* remains to be established.

Should SARS re-emerge, now two years after its first apparition (and subsequent disappearance), perhaps the pegylated form of IFN- α would be a logical choice for the prophylaxis and early post-exposure management of SARS: IFN- α inhibits SARS-CoV replication *in vitro* [39], and pegylated IFN- α was recently shown to reduce viral replication and excretion, to decrease viral antigen expression by type-1 pneumocytes, and to reduce the attendant pulmonary damage in cynomolgus macaques infected experimentally with SARS-CoV [40*].

Influenza virus infections

Given the persistent epidemics of influenza A strains H_3N_2 and H_1N_1 and the threat of a pandemic with the avian influenza A strain H_5N_1 , combined with the fact that no vaccination is available for the latter, much emphasis has been recently put on the chemotherapy (and prophylaxis) of influenza virus infections. There are, in principle, three classes of compounds that could be considered for this purpose: ribavirin, amantadine and rimantadine, and the neuraminidase inhibitors zanamivir and oseltamivir (Figure 1).

Although ribavirin is active *in vitro* against the replication of influenza virus, it has not been actively pursued for the management of influenza virus infections *in vivo*. Instead, it has been developed for inhalation as a small-particle aerosol formulation in patients (primarily young infants) that are at (high) risk of bronchopneumonitis caused by respiratory syncytial virus infection.

The matrix (M2) hydrogen ion channel blockers amantadine and rimantadine have been used for many years for the prophylaxis and therapy of influenza A virus infections. However, in the past they did not gain wide acceptance, mainly for the following reasons: they rapidly lead to the emergence of drug-resistant virus mutants; they are not active against influenza B; and it has been demonstrated, particularly for amantadine, that they exert side effects on the central nervous system.

At present, only the neuraminidase inhibitors zanamivir and oseltamivir are practically available for the therapy and/or prophylaxis of influenza A and B virus infections. Influenza virus has adopted a unique replication strategy: it uses one of its surface glycoproteins, hemagglutinin

(H), to bind to the target cell receptor, which contains a terminal sialic acid (*N*-acetylneuraminic acid), and another surface glycoprotein, neuraminidase, to cleave off the terminal sialic acid of the host cell receptor, allowing the virus particles to leave the cells after the viral replicative cycle has been completed [37,41]. Neuraminidase inhibitors block the release of newly formed virus particles, thus preventing their further spread to other host cells.

The benefits offered by the neuraminidase inhibitors are substantial. When used therapeutically, they lead to a reduction in illness in 1–2 days, a reduction in virus transmission to household or healthcare contacts, and a reduction in the frequency and severity of complications (such as sinusitis and bronchitis); they therefore decrease the requirement for antibiotics. When used prophylactically, they significantly reduce the number of new cases that have influenza. Although resistance to neuraminidase inhibitors can develop [42], there is no evidence of naturally occurring resistance to either zanamivir or oseltamivir [43]. Zanamivir and oseltamivir are both able to block influenza A and B viruses, and would, in theory, also be effective against the 1918 pandemic influenza A (H₁N₁) virus [44^{*}]. Zanamivir, which must be taken by (oral) inhalation, and in particular oseltamivir, which can be more conveniently administered as oral capsules, should be stockpiled to affront a potential influenza A pandemic in the future.

Hemorrhagic fever virus infections

The most important hemorrhagic fever viruses fall within the families of the flaviviridae (i.e. yellow fever and dengue), arenaviridae (i.e. Lassa, Junin, Machupo, Guanarito and Sabia), bunyaviridae (i.e. Rift Valley, Crimean-Congo and Hantaan) and filoviridae (i.e. Ebola, Marburg).

Prospects for the therapy of flavivirus infections are not overwhelming [37]. Ribavirin has only weak activity against flaviviruses. At present, IFN- α , whether pegylated or not, and IFN inducers, such as poly(I)-poly(C) and amplitgen, offer an appreciable potential for the prophylaxis and/or therapy of flavivirus infections as they decrease virus-induced morbidity (paralysis) and mortality (caused by progressive encephalitis) in an experimental flavivirus encephalitis model in severe combined immunodeficient mice [45]. Another approach, based on the conjugation of phosphordiamidate morpholino oligomers with arginine-rich peptides to increase their cellular uptake, has been described to inhibit the replication of several RNA viruses *in vitro*, including dengue virus types 1–4 [46], but it remains to be demonstrated whether or not this antisense approach also offers *in vivo* efficacy.

For arenaviruses and bunyaviruses, ribavirin (Figure 1) can be accredited with some efficacy in experimental

animal models as well as in humans (in the latter case, against Lassa fever [37]). Various mechanisms of action have been proposed to explain the antiviral activity of ribavirin. The most fascinating of these is the theory of ‘error catastrophe’ [47,48]. According to this hypothesis, ribavirin would act as an RNA virus mutagen, forcing the RNA virus into gaining a lethal accumulation of errors. This error catastrophe has been shown only with poliovirus; it has not been demonstrated with other RNA viruses such as HIV or HCV. For flaviviruses (such as yellow fever) and paramyxoviruses (such as parainfluenza), the predominant mechanism by which ribavirin exerts its antiviral activity *in vitro* appears to be based on inhibition of inosine 5'-monophosphate dehydrogenase [41,49].

For the treatment of Ebola (or Marburg) virus infections, *S*-adenosylhomocysteine hydrolase inhibitors (such as carbocyclic 3-deazaadenosine and 3-deazaneplanocin A; Figure 1) might be worth pursuing: even when administered as a single dose on the first or second day after infection, 3-deazaneplanocin A was found to protect almost all the mice against a lethal Ebola virus infection, and this protective effect was accompanied, and probably mediated, by an enhanced production of IFN in the Ebola virus-infected animals [50,51]. A totally different approach towards the treatment of Ebola virus infection is based on the use of a recombinant nematode anticoagulant protein C2, a potent inhibitor of tissue factor-initiated blood coagulation. This strategy targets the disease process rather than viral replication [52].

Human immunodeficiency virus infections

HIV has generated more efforts towards antiviral drug development than any other virus. In recent years, we have witnessed the development of novel approaches as well as newly emerging (candidate) drugs for anti-HIV therapy [53^{*},54]. The compounds that are currently under (pre)clinical investigation target the same specific viral proteins as the licensed compounds, target specific novel viral proteins, or target cellular proteins [54]. Also, new acyclic nucleoside phosphonates (i.e. the 6-[2-(phosphonomethoxy)alkoxy]-2,4-diamino pyrimidines PMPO-DAPy and PMEO-DAPy [55] and deoxythreosyl nucleoside phosphonates (i.e. phosphonomethyldeoxythreosyladenine [PMDTA] and -thymine [PMDTT]; Figure 1) [56^{*}] have been described as potent and selective anti-HIV agents.

Some compounds, such as cyanovirin-N and thiocarboxanilide UC-781 are being actively pursued as topical (i.e. vaginal) microbicides. Also, the plant lectins (i.e. *Galanthus nivalis* agglutinin and *Hippeastrum* hybrid agglutinin) 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 they prevent entry of HIV into its target cell [57]. Upon prolonged exposure (in cell culture, *in vitro*) of HIV to *Hippeastrum* hybrid agglutinin or to *Galanthus nivalis* agglutinin, the virus acquires resistance mutations in the gp120 glycoprotein that are predominantly located at the *N*-glycosylation (asparagine) sites [58]. In addition to their role as topical microbicides, plant lectins might, due to their propensity to deplete the viral envelope of its glycan shield [59], force the virus to convert to a gp120 phenotype that can no longer escape the immune surveillance of the host.

As a final highlight in the search for new anti-HIV agents, I would like to cite the multidisciplinary approach that, over a period of 17 years, led to the identification of what can now be claimed to be (one of) the most potent anti-HIV agents ever discovered: the NNRTI R-278474, which is also known as rilpivirine [60**]. This chemical evolution starting from TIBO R-86183 in 1987 has yielded compound R-278474, which shows activity against both wild-type and NNRTI mutant (K103N + Y181C) HIV-1 strains at sub-nanomolar concentrations. The discovery of R-278474 emanated from a coordinated multidisciplinary effort that involved medicinal chemists, virologists, crystallographers, molecular modellers, toxicologists, analytical chemists and pharmacists, among others [60**]. R-278474 highlights the important requirements of novel anti-HIV drugs: high antiviral potency against wild-type and mutant viruses, high oral bioavailability (allowing once-daily administration), minimal side effects, and ease of synthesis and formulation.

Conclusions

In recent years, considerable progress has been made towards the treatment of a wide variety of virus infections, irrespective of whether or not they are currently amenable to antiviral therapy. Highlighted in this review are: the potential of cidofovir and other acyclic nucleoside phosphonates for the treatment of HPV-associated papillomatous lesions, future prospects for the treatment of adenovirus, HSV, VZV and HHV-6 infections, and the potential of oral cidofovir prodrugs for the prophylaxis and therapy of poxvirus infections. I have also discussed therapeutic approaches, both present and future, for HCV infections, antiviral strategies for the management of SARS, and the importance of having neuraminidase inhibitors at hand to affront future influenza virus epidemics. Possible strategies for the prevention and treatment of hemorrhagic fever virus infections are identified, and the many new (candidate) antiviral drugs that have been developed for the treatment of HIV infections (AIDS) are also reviewed.

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