

STRATEGIES IN THE DESIGN OF ANTIVIRAL DRUGS

Erik De Clercq

A decade ago, just five drugs were licensed for the treatment of viral infections. Since then, greater understanding of viral life cycles, prompted in particular by the need to combat human immunodeficiency virus, has resulted in the discovery and validation of several targets for therapeutic intervention. Consequently, the current antiviral repertoire now includes more than 30 drugs. But we still lack effective therapies for several viral infections, and established treatments are not always effective or well tolerated, highlighting the need for further refinement of antiviral drug design and development. Here, I describe the rationale behind current and future drug-based strategies for combating viral infections.

VIRION
A mature infectious virus particle.

Effective vaccines have led, or might lead, to the eradication of important viral pathogens, such as smallpox, polio, measles, mumps and rubella. But other viral diseases, particularly human immunodeficiency virus (HIV) and hepatitis C virus (HCV), have so far proved to be intractable to the vaccine approach. The need for effective antiviral drugs is further emphasized by the lack of vaccines for most respiratory-tract virus infections (adenovirus, rhinovirus, parainfluenza virus and respiratory syncytial virus (RSV)), the widely occurring human papilloma viruses (HPV) and herpesviruses (herpes simplex virus types 1 and 2 (HSV-1, -2), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses types 6, 7 and 8 (HHV-6, -7, -8)), and the vast array of haemorrhagic fever viruses. And although vaccines have been developed for hepatitis B virus (HBV) and influenza virus types A and B, their use has not eliminated the need for effective chemotherapeutic agents.

Many new antiviral drugs have been licensed in recent years (TABLE 1), most of which are used for the treatment of HIV infections; of the current repertoire of more than 30 drugs, 16 are anti-HIV, 5 are anti-CMV, 5 are anti-HSV and anti-VZV, 1 is anti-RSV, 3 are anti-hepatitis and 4 are anti-influenza¹. But there is considerable room for improvement, as these compounds are not always efficacious or well tolerated. The emergence of viral resistance to drugs and drug-related

side effects are among the main reasons for further refinement of antiviral drug design and development.

Antiviral drug design could, in principle, be targeted at either viral proteins or cellular proteins. The first approach is likely to yield more specific, less toxic compounds, with a narrow spectrum of antiviral activity and a higher likelihood of virus drug-resistance development, whereas the second approach might afford antiviral compounds with a broader activity spectrum and less chance of resistance development, but higher likelihood of toxicity. Both routes are worth exploring, the preferred route being dictated by both the nature of the virus and the targets that the virus or its host cell have to offer.

As exemplified for HIV (FIG. 1), the viral life cycle encompasses several crucial steps, starting with the attachment of the virus to the cell and finishing with the release of the progeny VIRIONS from the cell. The replicative cycle of retroviruses, such as HIV, becomes closely associated with the host cell; after reverse transcription (RNA→DNA), the resulting proviral DNA becomes integrated into the cellular genome and then follows the ‘classical’ transcription and translation processes. By contrast, ‘normal’ cytotolytic viruses, such as herpesviruses, replicate their genome and express their genes autonomously, independent of the host cell metabolism. Here, I focus primarily on approaches targeted at specific processes in viral infection (FIG. 1), including virus adsorption, virus–cell fusion, viral

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.
e-mail: erik.declercq@regal.kuleuven.ac.be
DOI: 10.1038/nrd703

Table 1 | The antiviral repertoire

| Approach | Target virus(es) | Compounds approved | Selected compounds in development for the indicated target virus |
|---|--|--|--|
| Virus adsorption inhibitors | HIV, HSV, CMV, RSV and other enveloped viruses | | Polysulphates, polysulphonates, polycarboxylates, polyoxometalates, chicoric acid, zintevir, cosalane derivatives, negatively charged albumins |
| Virus–cell fusion inhibitors | HIV, RSV and other paramyxoviruses | | HIV: AMD3100, TAK779 and T20 derivatives |
| Viral DNA polymerase inhibitors | Herpesviruses (HSV-1, -2, VZV, CMV, EBV, HHV-6, -7, -8) | Acyclovir, valaciclovir, ganciclovir, valganciclovir, penciclovir, famciclovir, brivudin*, foscarnet | Bicyclic furopyrimidine nucleoside analogues, A5021, cyclohexenylguanine |
| Reverse transcriptase inhibitors | HIV | NRTIs: zidovudine, didanosine, zalcitabine, stavudine, lamivudine‡, abacavir NNRTIs: nevirapine, delavirdine, efavirenz | Emtricitabine, amdoxovir Emtricitabine, UC781, DPC083, TMC125 (R165335) |
| Acyclic nucleoside phosphonates | DNA viruses (polyoma-, papilloma-, herpes-, adeno- and poxviruses), HIV, HBV | CMV: cidofovir HIV: tenofovir | HBV: adefovir |
| Inhibitors of processes associated with viral RNA synthesis | HIV, HCV | | |
| Viral protease inhibitors | HIV, herpesviruses, rhinoviruses, HCV | HIV: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir | HIV: atazanavir, mozenavir, tipranavir Human rhinovirus: AG7088 |
| Viral neuraminidase inhibitors | Influenza A and B virus | Zanamivir, oseltamivir§ | RWJ270201 |
| IMP dehydrogenase inhibitors | HCV, RSV | Ribavirin | Mycophenolic acid, EICAR, VX497 |
| S-adenosylhomocysteine hydrolase inhibitors | (-)RNA haemorrhagic fever viruses (for example, Ebola) | | |

* Brivudin is approved in some countries; for example, Germany.

† Lamivudine is also approved for the treatment of HBV.

§ In addition to zanamivir and oseltamivir, amantadine and rimantadine have been approved as anti-influenza drugs, but these compounds are targeted at the viral uncoating process, not the viral neuraminidase.

|| Ribavirin is used in combination with interferon- α for HCV.

CMV, cytomegalovirus; EBV, Epstein–Barr virus; EICAR, 5-ethynyl-1- β -D-ribofuranosylimidazole-4-carboxamide; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IMP, inosine 5'-monophosphate; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

DNA or RNA synthesis (although host cellular components are also associated with these processes), and viral enzymes, such as protease and neuraminidase. Two host cellular enzymes — inosine 5'-monophosphate (**IMP**) **dehydrogenase** and **S-adenosylhomocysteine (SAH) hydrolase** — could also be targets for certain classes of antiviral agents.

Virus adsorption inhibitors

Numerous polyanionic compounds (for example, polysulphates such as polyvinylalcohol sulphate, polysulphonates such as polyvinylsulphonate (FIG. 2), polycarboxylates, polynucleotides such as zintevir, polyoxometalates and negatively charged albumins) have been shown to inhibit HIV replication by preventing virus attachment (adsorption) to the surface of the host cell. All of these negatively charged polymers might be expected to interact with the positively charged amino acids in the **V3 LOOP** of the HIV glycoprotein, gp120, which is rich in arginine (R) and lysine (K) residues. In doing so, the polyanions shield the V3 loop and therefore hamper the binding of the HIV virions to heparan sulphate², the primary binding site at the cell surface, before more specific binding occurs with the **CD4** receptor on CD4⁺ cells.

Heparan sulphate is widely expressed on animal

cells, and is involved in virus–cell binding for a broad array of ENVELOPED viruses, including HSV³ and dengue virus⁴. So, polysulphates, polysulphonates and other polyanionic substances that interfere with the binding of these enveloped viruses to target cells could be effective in both the treatment and prevention of such infections. Polyanionic substances could be important as vaginal microbicides as, in an appropriate formulation, they might prevent sexual transmission of HIV infection. Moreover, these polyanions are not only active against HIV, but also against HSV and other sexually transmitted disease (STD) pathogens, such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*⁵.

Although polyanions might have several sites of interaction, virus–cell attachment would be the preferred target from a therapeutic viewpoint, as it is the first opportunity to curtail the viral life cycle, and the polyanions do not need to enter the cells, which would be problematic. The interaction of polyanionic substances at this level can also be considered specific, as repeated passage of the HIV virus in the presence of polyanions can lead to resistance mediated by mutations in the envelope glycoprotein gp120, particularly in the V3 loop (K269E, Q278H, N293D), as originally shown for dextran sulphate⁶, and subsequently for zintevir⁷ and negatively charged albumins⁸.

V3 LOOP
The gp120 protein has eleven defined loop segments, five of which are termed variable (designated V1–V5).

ENVELOPE
A lipoprotein-bilayer outer membrane of many viruses. Envelope proteins often aid in identifying and attaching the virus to a cell-surface receptor, whereby viral entry can occur.

Virus-cell fusion inhibitors

Enveloped viruses, as a rule, enter their host cells by fusion between the viral envelope and cellular plasma membrane (FIG. 1). This fusion process is basically similar for several enveloped virus families (that is, retro-, paramyxo- and herpesviruses), but for HIV it is preceded by the interaction of gp120 with its co-receptor on the host cell — the chemokine (C-X-C) motif receptor 4 (**CXCR4**) for T-tropic or X4 HIV strains, or the chemokine (C-C) motif receptor 5 (**CCR5**) for M-tropic or R5 HIV strains. CXCR4 and CCR5 normally act as the receptors for the C-X-C chemokine, **SDF1** (stromal-cell-derived factor 1), and the C-C chemokines **RANTES** (regulated upon activation, normal T-cell expressed and secreted) and **MIP1** (macrophage inflammatory protein 1), respectively. The coincidental use of both CXCR4 and CCR5 by HIV as co-receptors to enter cells has prompted the search for CXCR4 and CCR5 antagonists, which, through blockade of the corresponding co-receptor, might be able to block HIV entry into the cells.

This has now been shown with several compounds, the most prominent among the CXCR4 antagonists being the bicyclam AMD3100 (REFS 9,10 and FIG. 2), and the best documented among the CCR5 antagonists being TAK779 (REFS 11,12 and FIG. 2). The site of interaction of TAK779 with the transmembrane helices of CCR5 has been mapped¹² (FIG. 3), and, likewise, crucial amino-acid residues involved in the binding of AMD3100 to CXCR4 have been identified¹³. Recently, a new CCR5 antagonist, SCH-C (SCH351125), was announced as an orally bioavailable inhibitor of M-tropic R5 strains that is capable of suppressing R5 HIV-1 infection both *in vitro* and *in vivo* (SCID-hu Thy/Liv mice)¹⁴. The clinical potential of the CXCR4 and CCR5 antagonists in the management of HIV infections remains to be proved. To ensure maximal coverage of both X4 and R5 strains, dual CXCR4/CCR5 antagonists should be developed, or single CCR5 and CXCR4 antagonists should be combined.

The interaction of gp120 with its co-receptor (CCR5 or CXCR4) triggers a series of conformational changes in the gp120–gp41 complex that ultimately lead to the formation of a ‘trimer-of-hairpins’ structure in gp41 — a bundle of six α -helices: three α -helices formed by the carboxy-terminal regions packed in an antiparallel manner with three α -helices formed by the amino-terminal regions. The fusion-peptide region, located at the extreme amino terminus, will insert into the cellular membrane, whereas the carboxy-terminal region remains anchored in the viral envelope. In this sense, the trimer-of-hairpins motif brings the two membranes together, so agents that interfere with the formation of the gp41 trimer-of-hairpins structure might be expected to inhibit the fusion process¹⁵.

Several constructs have been designed to interfere with the gp41-mediated fusion process: the so-called ‘5-helix’, which binds the carboxy-terminal region of gp41 (REF. 15); D-peptide inhibitors, which dock into the pocket formed by the α -helices of gp41 (REF. 16); and T20 (pentafuside, previously called DP178, a synthetic 36-amino-acid

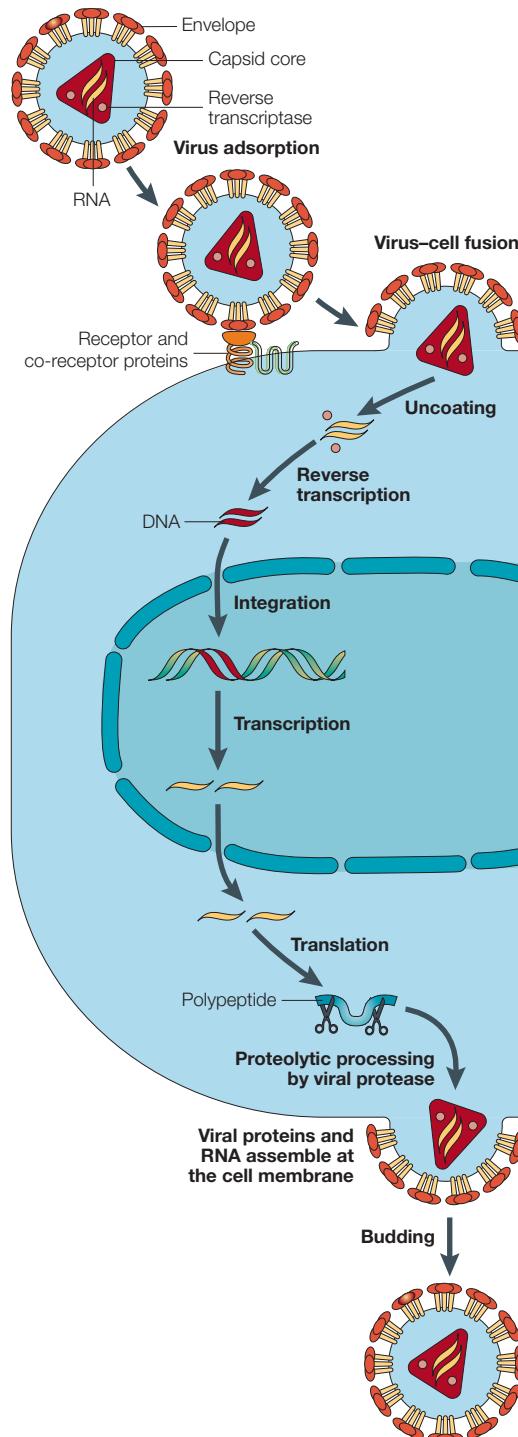


Figure 1 | The viral life cycle, as exemplified by HIV. Viral life cycles have several specific steps, many of which are targets for antiviral drugs. After virus adsorption, enveloped viruses enter the cell by virus-cell fusion. For human immunodeficiency virus (HIV), which is a retrovirus with an RNA (yellow) genome, replication of the genome occurs after reverse transcription and integration into the host cell chromosome. For DNA viruses, such as herpesviruses, the genome is replicated by a viral DNA polymerase. After transcription to RNA, followed by translation and proteolytic processing of the precursor polypeptide, viral proteins assemble at the cell membrane, from which they bud to release new virions.

Animated online

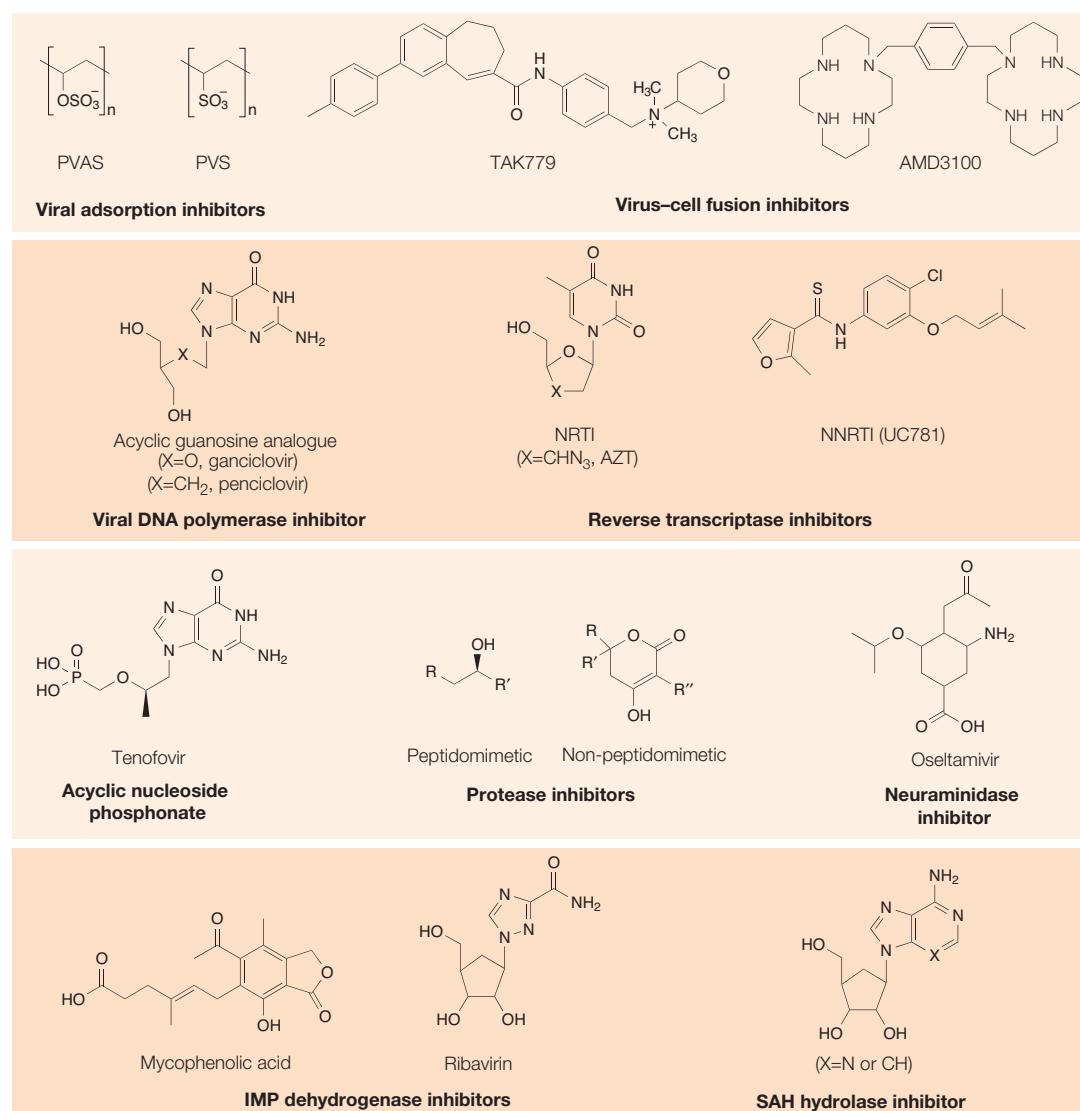


Figure 2 | Basic (skeletal) pharmacophores or prototypic compounds of the classes of antiviral agents described in this review. AZT, azidothymidine; IMP, inosine monophosphate; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PVAS, polyvinylalcohol sulphate; PVS, polyvinylsulphonate; SAH, S-adenosylhomocysteine.

peptide that corresponds to residues 127–162 of the ectodomain of gp41). T20 has proved effective in reducing plasma HIV levels in humans, providing the proof of concept that viral entry can be successfully blocked *in vivo*¹⁷.

Insight into the HIV fusion process should help in designing fusion inhibitors for other viruses as well, as trimer-of-hairpins motifs could also be predicted for other virus families¹⁵, including paramyxoviridae, such as parainfluenza virus, measles and respiratory syncytial virus. In fact, for each of these paramyxoviruses, peptides similar to T20 have been shown to block viral fusion¹⁸. Also, a cobalt-chelating complex (CTC96) that inhibits infection by HSV-1 through blocking fusion¹⁹ might have an extended antiviral activity spectrum, given the premise that enveloped viruses belonging to different families share an analogous process of membrane fusion.

PRODRUG

A pharmacologically inactive compound that is converted to the active form of the drug by endogenous enzymes or metabolism. It is generally designed to overcome problems associated with stability, toxicity, lack of specificity or limited (oral) bioavailability.

Inhibitors of viral DNA or RNA synthesis

Viral DNA polymerase inhibitors. In contrast to retroviruses (FIG. 1), herpesviruses do not have a reverse transcription step in their replicative cycle, which means that their DNA genome can be replicated by the viral DNA polymerase after the latter has been expressed in the virus-infected cell. At present, all the antiviral agents that are available for the treatment of herpesvirus infections are nucleoside analogues: either acyclic guanosine analogues (that is, **acyclovir**, **penciclovir**, **ganciclovir**, and their oral PRODRUG forms **valaciclovir**, **famciclovir** and **valganciclovir**, respectively), or thymidine analogues (**brivudin**) (FIG. 2 and TABLE 1). All of these compounds target the viral DNA polymerase, but before they can interact with viral DNA synthesis, they need to be phosphorylated intracellularly to the triphosphate form. The first (and, for brivudin, also the second) phosphorylation step is ensured by the HSV- or VZV-encoded

thymidine kinase, or the CMV-encoded protein kinase, and is therefore confined to virus-infected cells, which explains the specific antiviral action of the established antiherpetic compounds. Subsequent phosphorylations are achieved by host cellular kinases. In their triphosphate form, the nucleoside analogues interact with the viral DNA polymerase, either as competitive inhibitors or as alternative substrates with respect to the natural substrate (dGTP for the guanosine analogues, dTTP for the thymidine analogues). If the acyclic guanosine analogues act as alternative substrates, their incorporation prevents further chain elongation (FIG. 4a).

So, is there room for improvement? The moderate oral bioavailability of the acyclic guanosine analogues has been improved by formulating them as prodrugs. The success obtained with acyclovir, valacyclovir and famciclovir in the treatment of HSV and VZV infections has impeded further progress in this area. However, brivudin, which is considerably more potent than acyclovir and penciclovir as an anti-VZV agent, represents an important alternative for the treatment of VZV infections. Furthermore, although brivudin is active in the nanomolar concentration range against VZV replication, its potency can be superseded by bicyclic furo-pyrimidine nucleoside analogues bearing a long alkyl or alkylaryl side chain attached to the furane ring^{20,21}. The mechanism of action of these exquisitely potent and selective anti-VZV agents remains to be elucidated, although there is no doubt that their specificity for VZV is governed by the virus-encoded thymidine kinase.

In the guanosine analogue class, several new CONGENERS have been described; namely A5021 (REFS 22,23) and the D- and L-enantiomers of cyclohexenylguanine²⁴. These compounds seem to have an activity spectrum and mode of action similar to that of acyclovir, but further studies are warranted to verify whether these new guanosine analogues might have an extended spectrum of activity (that is, against HHV-6, -7 and -8, which are not particularly sensitive to acyclovir), increased *in vivo* efficacy, or improved pharmacokinetics.

Reverse transcriptase inhibitors. As is evident from FIG. 1, reverse transcriptase is essential in the replicative cycle of retroviruses, such as HIV, as it synthesizes the proviral DNA, which will then be integrated into the host cell genome and passed on to all of the progeny cells. The substrate (dNTP) binding site of HIV reverse transcriptase (RT) has proved to be an attractive target for nucleosidic HIV inhibitors: six nucleoside analogues — **zidovudine** (azidothymidine, AZT), **didanosine** (dideoxyinosine), **zalcitabine** (dideoxycytidine), **stavudine** (didehydrodideoxythymidine, d4T), **lamivudine** (3'-thiadideoxycytidine) and **abacavir** — have been licensed as anti-HIV drugs (FIG. 2 and TABLE 1), and several others, such as emtricitabine and amdoxovir, are in advanced development. All of these dideoxynucleoside analogues act according to a similar ‘recipe’, as exemplified for AZT (FIG. 4b). They must be phosphorylated consecutively inside the host cell by three cellular kinases — a nucleoside kinase, a nucleoside 5'-monophosphate kinase and a nucleoside 5'-diphosphate kinase — to form the

corresponding 5'-triphosphate derivative, which can interact, as a chain terminator, with the reverse transcription (RNA → DNA) reaction. One of the mechanisms by which resistance to AZT might arise is through removal of the chain-terminating residue, a kind of repair reaction involving pyrophosphorolysis, which can be regarded as the opposite of the reverse transcriptase reaction. Not all chain terminators are readily removed; for example, the acyclic nucleoside phosphonate derivative tenofovir (PMPA; FIG. 2) is not, and, in this sense, PMPA should be less prone to resistance development than the regular nucleoside analogues.

The first phosphorylation step that converts the 2',3'-dideoxynucleoside analogues to their 5'-monophosphate can be considered as the bottleneck in the overall metabolic pathway leading to the formation of the active 5'-triphosphate metabolites. If certain dideoxynucleoside analogues (for example, 2',3'-dideoxyuridine) are not active against HIV under conditions in which others are, this stems from their poor, or lack of, phosphorylation at the nucleoside-kinase level. Therefore, attempts have been made at constructing prodrugs of 2',3'-dideoxynucleoside 5'-monophosphate that deliver the 5'-monophosphate derivatives on cellular uptake, which can then be converted into the corresponding 5'-di- and 5'-triphosphate derivatives. This approach bypasses the initial nucleoside-kinase dependency and has been validated by the design of the phosphoramidate^{25,26} and cyclosaligenyl^{27,28} prodrugs of d4T 5'-monophosphate. Both prodrugs were found to deliver d4T 5'-monophosphate efficiently within the cells, which, after conversion to its 5'-triphosphate, afforded anti-HIV

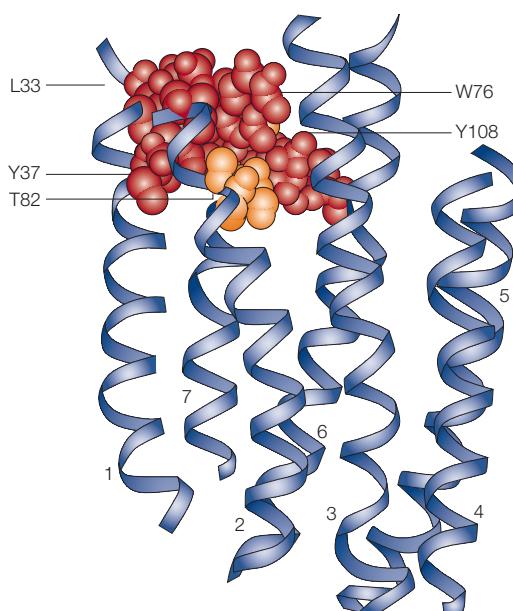


Figure 3 | Interaction of CCR5 with TAK779. A structural model of CCR5 complexed with TAK779 (FIG. 2), viewed from within the plane of the membrane¹². The indicated cluster of amino acids in the TAK779 binding site includes several aromatic residues (Y37, W86, Y108) that might form favourable interactions with the aromatic rings of TAK779. (Reprinted with permission from REF. 12 © 2000, National Academy of Sciences, USA.)

CONGENER
Any member of the same chemical family.

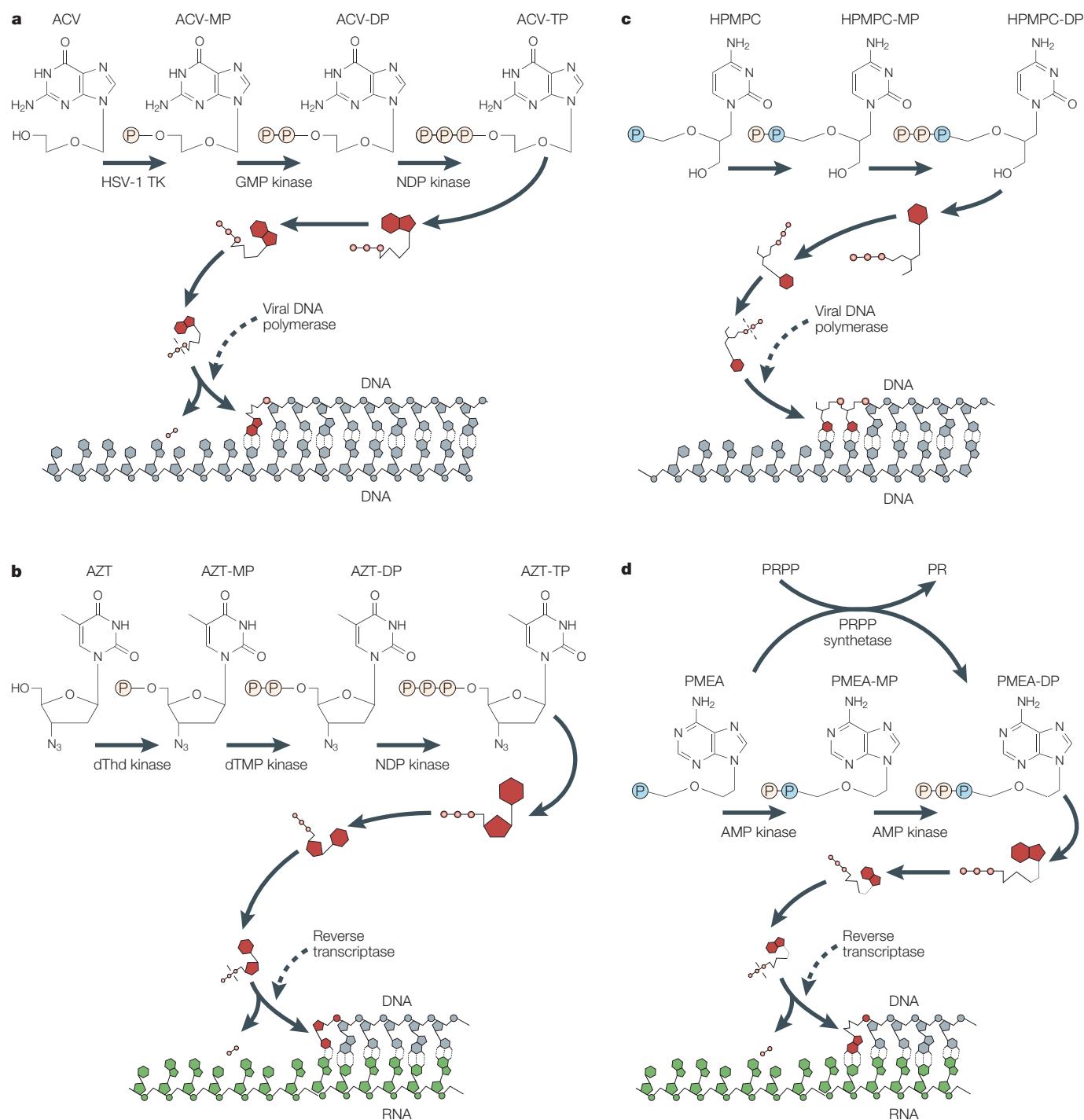


Figure 4 | Examples of antiviral nucleoside analogues acting by a chain termination mechanism. **a** | Acyclovir (ACV) targets viral DNA polymerases, such as the herpesvirus (HSV) DNA polymerase. Before it can interact with viral DNA synthesis, it needs to be phosphorylated intracellularly, in three steps, into the triphosphate form. The first phosphorylation step is ensured by the HSV-encoded thymidine kinase (TK), and is therefore confined to virus-infected cells. **b** | Azidothymidine (AZT) targets the human immunodeficiency virus (HIV) reverse transcriptase, and also needs to be phosphorylated, in three steps, to the triphosphate form before it can interfere with reverse transcription. **c** | Cidofovir (S-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; HPMPC), an acyclic nucleotide analogue, which can be viewed as an acyclic nucleoside analogue extended by a phosphonate moiety, targets viral DNA polymerases, and is active against DNA viruses, whether or not they encode a specific viral thymidine kinase. In contrast to acyclovir and azidothymidine, cidofovir requires only two phosphorylations to be converted to the active (triphosphate) form. **d** | Adefovir (9-(2-phosphonylmethoxyethyl)adenine; PMEA) — also an acyclic nucleoside phosphonate — is active against retroviruses and hepadnaviruses; similar to cidofovir, adefovir needs only two phosphorylations and so can bypass the nucleoside-kinase reaction that limits the activity of dideoxynucleoside analogues such as AZT. DP, diphosphate; dThd, (2'-deoxy)-thymidine; MP, monophosphate; NDP, nucleoside 5'-diphosphate; PR, 5-phosphoribose; PRPP, 5-phosphoribosyl-1-pyrophosphate; TP, triphosphate.

activity under conditions in which the d4T nucleoside, owing to inefficient phosphorylation, did not. It remains to be established whether this nucleoside-kinase bypass strategy will also yield increased antiviral efficacy *in vivo*.

All of the aforementioned 2',3'-dideoxynucleoside analogues, in their 5'-triphosphate form, act as competitive substrates/inhibitors with respect to the natural substrates (dNTPs) at the catalytic site of HIV RT, and, as HBV uses a similar RT in its life cycle, these compounds might also be able to inhibit HBV replication. This premise has been borne out in particular for lamivudine, which is licensed for the treatment of chronic HBV infections.

Such an extended activity spectrum cannot be anticipated for a second class of RT inhibitors — the non-nucleoside reverse transcriptase inhibitors (NNRTIs) — which interact with an ALLOSTERIC, non-substrate binding ('pocket') site on HIV-1 RT²⁹. This 'pocket' does not exist in ligand-free RT, and does not occur in RTs other than HIV-1 RT, or, if it does, only the HIV-1 RT pocket offers the necessary interactions with NNRTIs. These interactions are: stacking interactions with the aromatic amino acids Y181, Y188, W229 and Y318; electrostatic interactions with K101, K103 and E138; van der Waals interactions with L100, V106, Y181, G190, W229, L234 and Y318; and hydrogen bonding with the main-chain peptide bonds³⁰. A model for the interaction of a representative NNRTI, the thiocarboxanilide UC781 (FIG. 2), with HIV-1 RT³¹ is shown in FIG. 5.

NNRTIs are notorious for rapidly eliciting virus resistance resulting from mutations at amino-acid residues that surround the NNRTI-binding site. In the clinic, the most prominent mutations engendering resistance to NNRTIs are the K103N and Y181C mutations. At present, only three NNRTIs (**nevirapine**, **delavirdine** and **efavirenz**) have been formally licensed, although several others are in the developmental stage. However, it is obvious that in the future design of new NNRTIs, not only potency and safety, but also resilience to drug-resistance mutations should be taken into account³². It is noteworthy that some amino acids that surround the NNRTI-binding site, such as W229 and Y318, do not seem apt to mutate, or, if they do, they lead to a 'suicidal' loss of RT activity³³. Such immutable amino acids could be prime targets for the rational design of new NNRTIs.

Acyclic nucleoside phosphonates. The acyclic nucleoside phosphonates can be viewed as acyclic nucleoside analogues that are extended by a phosphonate moiety. The phosphonate group is equivalent to a phosphate group, but, unlike phosphate, phosphonate can no longer be cleaved by the esterases that would normally convert nucleoside monophosphates back to their nucleoside form. Consequently, acyclic nucleoside phosphonates might show a broadened antiviral activity spectrum compared with those of acyclic nucleoside analogues such as acyclovir, and dideoxynucleoside analogues such as zidovudine. On the one hand, they should be active against those DNA viruses that do not encode a specific viral thymidine kinase (TK) or protein kinase (PK), or that have become resistant to the

ALLOSTERIC SITES
Two or more topologically distinct binding sites within a protein can interact functionally with each other. So, two sites in different positions can bind ligands (substrates, inhibitors and so on), and binding of a ligand at one site alters the properties of the other(s).

CPK COLOURING
The CPK colour scheme for elements is based on the colours of the popular plastic space-filling models developed by Corey, Pauling and Kultun, and is conventionally used by chemists. In this scheme, carbon is represented in light grey, oxygen in red, nitrogen in blue, sulphur in yellow, hydrogen in white and chlorine in green.

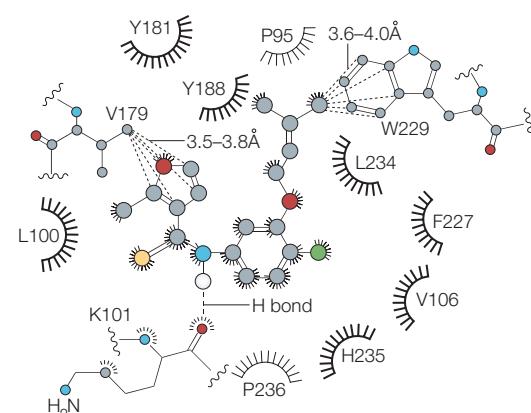


Figure 5 | Interaction of HIV-1 RT with UC781.

Features stabilizing the complex between the human immunodeficiency virus 1 (HIV-1) reverse transcriptase (RT) and the non-nucleoside reverse transcriptase inhibitor UC781 (FIG. 2). The hydrogen bond with K101, and the two methyl-group-aromatic-ring interactions are shown explicitly. Other main hydrophobic contacts are shown with bold lines; minor ones are shown with faint lines³¹. Standard CPK COLOURING is used.

nucleoside analogues through TK or PK deficiency. On the other hand, they should also be able to bypass the nucleoside-kinase reaction that limits the activity of the dideoxynucleoside analogues against retroviruses such as HIV, and hepadnaviruses such as HBV.

These objectives have been fulfilled on both scores, albeit by different types of acyclic nucleoside phosphonate: **cidofovir** (HPMPC) has broad-spectrum activity against DNA viruses; and **adefovir** (PMEA) and **tenofovir** (PMPA) have activity against retro- and hepadnaviruses.

Although their eventual activity spectrums are different, both types of compound share a common strategy in their modes of action: they both need two (instead of three) phosphorylation steps to be converted into their active (diphosphorylated) metabolites, which then act as chain terminators in the DNA polymerase reaction (HPMPC; FIG. 4c) or reverse transcriptase reaction (PMEA, PMPA; FIG. 4d). For HPMPC to shut down viral DNA synthesis, the incorporation of two consecutive HPMPC units is required³⁴, whereas for PMEA, one such incorporation suffices³⁵. In both cases, the acyclic nucleotides remain stably incorporated, presumably because the phosphonate group prevents repair enzymes from excising these nucleotides.

The 'era' of the acyclic nucleoside phosphonates started in 1986, with the description of the broad-spectrum anti-DNA virus activity of the adenine derivative HPMPA³⁶. Its cytosine counterpart, cidofovir (HPMPC), which seemed less harmful to the host in preliminary toxicity experiments, was then developed as an antiviral drug³⁷, and was approved for clinical use in the treatment of CMV retinitis in AIDS patients. Cidofovir also holds great potential for the treatment of several other DNA virus infections. These include: TK-deficient HSV and VZV infections, which are resistant to acyclovir (or brivudin); herpesvirus infections, such as EBV, HHV-6, HHV-7 and HHV-8; HPV infections,

including pharyngeal, oesophageal and laryngeal papillomatosis, plantar and genital warts, and cervical intraepithelial neoplasia; polyomavirus infections (progressive multifocal leukoencephalopathy); adenovirus infections (epidemic keratoconjunctivitis); and poxvirus infections³⁸, such as smallpox, monkeypox, cowpox, orf virus and molluscum contagiosum. Adefovir and tenofovir, the two other leading acyclic nucleoside phosphonates, have both progressed, in their oral prodrug forms, adefovir dipivoxil and tenofovir disoproxil, to Phase III clinical trials for the treatment of HBV and HIV, respectively. Tenofovir disoproxil fumarate has recently been approved in the United States for the treatment of HIV infections.

In contrast to all other antiviral drugs, acyclic nucleoside phosphonates have a particularly long intracellular half-life (1 to several days), allowing infrequent dosing (once daily for adefovir and tenofovir, or once weekly or every other week for cidofovir). Furthermore, they do not lead easily to resistance, even after prolonged treatment for more than one or two years. No drug metabolic interactions are known for the acyclic nucleoside phosphonates, which means that they can readily be added to any drug regimen, as has been shown in particular for tenofovir in the treatment of HIV infections.

Inhibitors of associated processes. Gene expression (that is, transcription to viral RNA) of the genome of retroviruses, such as HIV, is not possible without integration of the proviral DNA into the host chromosome (FIG. 1). So, the enzyme involved — integrase — has been considered an attractive target for chemotherapeutic intervention. Numerous integrase inhibitors have been described^{39,40}, although none has sufficient specificity to be further pursued as an integrase-targeted drug. The problem with integrase inhibitors is that, although they might be effective in enzyme-based assays, their anti-HIV activity in cell culture can be masked by cytotoxicity. And even if selective anti-HIV activity in cell culture is noted, caution should be exercised in unconditionally attributing this activity to inhibition of the integration process, as the compounds concerned might owe their anti-HIV activity to an action targeted elsewhere. This has proved to be the case for the anionic compounds zintevir⁷ and L-chicoric acid⁴¹, two integrase inhibitors that owe their anti-HIV activity primarily to an interaction with the viral envelope protein gp120, and so fall into the category of the polyanionic inhibitors of virus adsorption discussed above. At present, the only compounds that qualify as genuine integrase inhibitors are the diketo acids L731,988 and L708,906 (REFS 42,43). In cell culture, these compounds were shown to inhibit both the replication of HIV-1 and the strand transfer function of the integrase (the other catalytic function of the enzyme being endonucleolytic cleavage of the terminal dinucleotide GT from the 3' end of the substrate DNA). Furthermore, these two events could be causally linked, as mutations in the HIV-1 integrase conferred resistance to the inhibitory effects of the compounds on both strand transfer and HIV-1 infectivity⁴².

At the transcription level, HIV gene expression could be inhibited by compounds that interact with cellular factors that bind to the long terminal repeat (LTR) promoter, and which are needed for basal-level transcription, such as NF- κ B inhibitors⁴⁴. However, greater specificity might be achieved using compounds that specifically inhibit the transactivation of the HIV LTR promoter by the viral *trans*-acting transactivator (tat) protein. The tat protein interacts specifically with the tat responsive element, which is located at the beginning of the viral messenger RNA that is transcribed from the LTR promoter, thereby enhancing the transcription process. Several compounds have been described as inhibitors of the transcription process; for example, fluoroquinolines⁴⁵, and bistriazoloacridone derivatives, such as temacrazine⁴⁶. The latter was found to block HIV-1 RNA transcription that starts from the HIV LTR promoter, without interfering with the transcription of any cellular genes. The peptide analogue CGP64222, which is structurally reminiscent of amino acids 48–56 (RKKRRQRRR) of the tat protein, was also designed to act as a tat antagonist⁴⁷. However, although CGP64222 is able to interact with the tat-driven transcription process, its anti-HIV activity in cell culture is mediated primarily by an interaction with CXCR4, the co-receptor for X4 HIV strains⁴⁸.

Viral RNA transcription might also be affected by targeting cyclin-dependent kinases (CDKs), which are required for the replication of many viruses, including HIV. Indeed, flavopiridol, a typical inhibitor of CDKs (in particular, CDK9, which is involved in the tat-driven transcription process), has proved to be effective in blocking HIV infectivity⁴⁹.

One of the virus infections in the greatest need of antiviral therapy is HCV. In this case, two specific enzymatic functions associated with viral RNA synthesis could be predicted to be targets for the design of new antiviral agents: first, the non-structural protein 3 (NS3)-associated helicase; and second, the non-structural protein 5B (NS5B) RNA-dependent RNA polymerase. Crystal structures of both NS5B and the NS3 helicase are available⁵⁰, and both enzymatic activities have been characterized in sufficient detail^{51,52} to facilitate the development of effective HCV therapeutics. For the helicase, there is no precedent, but for the RNA polymerase there is, and the experience gathered from studies of the HIV RT inhibitors might be of paramount importance when targeting the HCV RNA polymerase, especially if, as seems possible, this enzyme shows similar kinetics to the HIV RT⁵².

Viral protease inhibitors

Viral proteases are crucial in the life cycle of many viruses, including retroviruses such as HIV, herpesviruses, picornaviruses such as rhinovirus, and flaviviruses such as HCV. Viral proteases have therefore been favoured as targets for antiviral agents⁵³. Proteases cleave newly expressed precursor polyproteins into smaller, mature viral proteins, termed ‘functional’ (if endowed with enzymatic activity) or ‘structural’ (if part of the virion structure). For example, in HIV replication, HIV protease cleaves the glycosaminoglycan (gag) and gag-polymerase

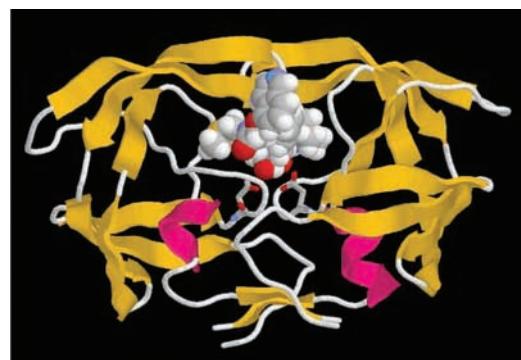


Figure 6 | Interaction of HIV protease with KNI272.
Ribbon diagram of human immunodeficiency virus (HIV) protease complexed with the peptidomimetic protease inhibitor KNI272; derived from the crystal structure⁵⁴. The inhibitor is shown as a space-filling model, and the two active-site aspartic acids are shown as sticks; both have standard CPK colouring.

(pol) precursor proteins to structural proteins (p17, p24, p9 and p7) and functional proteins (protease, reverse transcriptase/RNase H and integrase). HIV protease inhibitors have been tailored to the peptidic linkages (for example; F-P, F-L and F-T) in the gag and gag-pol precursor proteins that are cleaved by the protease, and have been extensively modelled in the active site of the enzyme, which is formed at the interface of two homodimeric subunits and contains two catalytic aspartic residues (each belonging to the DTG motif; FIG. 6)⁵⁴. All of the protease inhibitors licensed at present for the treatment of HIV infection, namely **saquinavir**, **ritonavir**, **indinavir**, **nelfinavir**, **amprenavir** and **lopinavir** (TABLE 1), share the same structural determinant (FIG. 2) — a hydroxyethylene core (instead of the normal peptidic linkage) that makes them non-scissile peptidomimetic substrate analogues for the HIV protease. The HIV protease inhibitors have proved to be valuable therapeutics in combination with NRTIs and NNRTIs (a drug combination schedule known as 'highly active antiretroviral therapy' (HAART)) in the treatment of HIV infections. However, they are met by confounding factors, such as difficulties in drug adherence, drug–drug interactions, overlapping resistance patterns and long-term side effects, including lipodystrophy, cardiovascular disturbances and metabolic disturbances, such as diabetes. This has prompted the search for new, non-peptidic inhibitors of HIV protease, with cyclic urea, 4-hydroxycoumarin, L-mannanic acid or 4-hydroxy-5,6-dihydro-2-pyrone as the central scaffold instead of the peptidomimetic hydroxyethylene core^{55,56} (FIG. 2); for example, tipranavir. Such compounds should show little, if any, cross-resistance with the peptidomimetic inhibitors. At present, however, their *in vivo* efficacy, pharmacokinetic profile and short- and long-term safety remain to be established.

Whether the protease-inhibitor approach would be as successful for tackling herpes-, picorna- and flaviviruses as for HIV remains to be seen. Whereas the HIV protease is an aspartate protease, herpesvirus proteases are serine proteases that have SHH as the catalytic triad⁵⁷. Several

non-peptidic inhibitors of the herpesviral protease CMV protease, which is also referred to as 'assemblin', because of its role in the CMV assembly process, have been described; for example, thieno[2,3-*D*]oxazinones⁵⁸, aryl hydroxylamine derivatives⁵⁹, monobactams⁶⁰, pyrrolidine-5,5-*trans*-lactams⁶¹, 1,4-dihydroxynaphthalene and naphthoquinones⁶². Although a useful exercise in targeting the herpesviral protease, all of these efforts should be viewed as a prelude to further investigations on the *in vitro* and *in vivo* inhibitory effects of these compounds on virus replication.

Further advanced is the structure-assisted design of mechanism-based irreversible inhibitors of the human rhinovirus 3C protease — a cysteine protease involved in the proteolytic cleavage of the viral precursor polyprotein to both capsid and functional proteins required for RNA replication. This work has yielded a wealth of compounds with potent activity against several rhinovirus SEROTYPES^{63–67}. One compound of the series, AG7088, which was shown to inhibit rhinovirus replication even when added late in the virus life cycle⁶⁸, has proceeded into clinical trials.

The HCV protease is a serine protease that is encoded by the non-structural NS3 domain, and is responsible for the proteolytic cleavage of the non-structural NS3, NS4A, NS4B, NS5A and NS5B proteins from the viral polyprotein (the NS4A protein then binds to the NS3 protein and enhances its proteolytic activity). The HCV NS3–4A protease is remarkably similar to the pestiviral NS3–4A protease, which is found in bovine viral diarrhoea virus (BVDV)⁶⁹, and has been intensively pursued as a target for the design of inhibitors. Again, as for the herpesvirus serine protease, several inhibitors, both peptide based^{70,71} and non-peptidic⁷², of the HCV NS3–4A protease have been identified, but as there is no cell-culture assay available for HCV, their activity against HCV could not be assessed. Given the similarities of the HCV and BVDV NS3–4A proteases, it seems advisable to evaluate putative HCV protease inhibitors for their activity against BVDV replication, for which cell-culture assay systems have been established.

Viral neuraminidase inhibitors

Influenza virus (both A and B) has adopted a unique replication strategy by using one of its surface glycoproteins, haemagglutinin, to bind to the target-cell receptor, which contains a terminal sialic acid. The other surface glycoprotein, neuraminidase, cleaves off the terminal sialic acid of the host cell receptor, allowing virus particles to leave the cell after the viral replicative cycle has been completed. The viral neuraminidase is therefore needed for the elution of newly formed virus particles from the cells. In addition, the viral neuraminidase might also promote viral movement through the respiratory tract mucus, thereby enhancing viral infectivity.

So, influenza viral neuraminidase has been envisaged as a suitable target for the design of specific inhibitors. Computer-assisted drug design, based on the crystal structure of the influenza viral neuraminidase, led to the identification of **zanamivir** (GG167) as a specific and

SEROTYPE
Variety of a species (usually bacteria or virus) characterized by its antigenic properties.

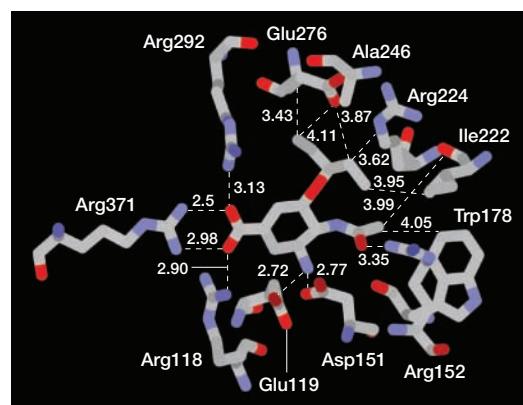


Figure 7 | Interaction of influenza neuraminidase with oseltamivir. Shows binding interactions of the neuraminidase inhibitor oseltamivir (FIG. 2) with influenza neuraminidase; derived from the crystal structure. (Adapted with permission from REF. 76 © (1997) American Chemical Society.)

potent inhibitor of the enzyme, and of the *in vitro* and *in vivo* replication of both influenza A and B virus⁷³. Zanamivir was tailored to interact with conserved amino-acid residues within the active site of influenza A and B viral neuraminidase, and its inhibitory effect on the enzyme has proved to be predictive of the susceptibility of clinical isolates to the drug⁷⁴. Meanwhile, zanamivir was shown to be efficacious and safe in the treatment (by inhalation) of influenza virus infections⁷⁵, and the drug has been licensed for clinical use.

Zanamivir has to be given topically (by inhalation), owing to its poor oral bioavailability. In attempts to identify potentially orally bioavailable inhibitors, a series of carbocyclic transition-state-based analogues were developed, in which the polar glycerol and guanidino groups of zanamivir were replaced by a lipophilic (3-pentyloxy) side chain and amino group, respectively, to give GS4071 (REF. 76). X-ray crystallographic studies showed that these groups of **GS4071** could be accommodated within the active site of neuraminidase (FIG. 7). As aimed for, GS4071, when administered as the ethyl-ester prodrug (GS4104; **oseltamivir**; FIG. 2), is orally bioavailable and was found to be effective in protecting mice and ferrets against influenza infection⁷⁷. Subsequently, oseltamivir was found to be effective and safe in the oral treatment and prevention of influenza virus infections^{78,79}, and has been licensed for clinical use.

Zanamivir and oseltamivir have paved the way for the development of similar structure-based neuraminidase inhibitors^{80–82}, such as the cyclopentane derivative RWJ270201, which have a comparable, or even better, efficacy profile in the mouse model of influenza. The clinical potential of RWJ270201 in the prevention and/or treatment of human influenza virus infections still needs to be established.

IMP dehydrogenase inhibitors

IMP dehydrogenase is a key enzyme in the *de novo* biosynthesis of purine mononucleotides: it is responsible for the NAD-dependent oxidation of IMP to xanthosine

5'-monophosphate (XMP), which is then further converted to GMP, GDP and GTP, and also from GDP, through dGDP, to dGTP. Inhibitors of IMP dehydrogenase might affect both RNA and DNA synthesis by reducing the intracellular pools of GTP and dGTP, respectively. Although IMP dehydrogenase is a cellular target, inhibitors targeted at this enzyme might inhibit viral RNA and/or DNA synthesis preferentially, as there is an increased need for such syntheses in virus-infected cells.

IMP dehydrogenase can be targeted by two types of inhibitor: competitive or uncompetitive with respect to the normal substrate, IMP. To the first category belongs **ribavirin**, which has been approved for clinical use as an aerosol for the treatment of RSV infections, and in combination with **interferon-α** for the treatment of HCV infections. To the second category belongs mycophenolic acid (FIG. 2), an immunosuppressing agent that has been approved, as its morpholinoethyl ester prodrug, for the prevention of acute allograft rejection following kidney transplantation. The X-ray crystal structure of **IMP dehydrogenase**, complexed with mycophenolic acid at the active site, has been reported at high resolution (2.6 Å)⁸³. New congeners of both ribavirin (EICAR)⁸⁴ and mycophenolic acid (VX497)⁸⁵ have an activity spectrum as broad as ribavirin, but considerably greater potency. This activity spectrum encompasses both DNA and RNA viruses, including, among the latter, picorna-, togavirus-, flavivirus-, bunyavirus-, arena-, reo-, rhabdo-, and, in particular, ortho- and paramyxoviruses.

Mycophenolic acid has marked activity against yellow fever virus and also markedly potentiates the inhibitory effects of the acyclic guanosine analogues acyclovir, penciclovir and ganciclovir against HSV, VZV and CMV infections, which could be of great clinical utility in organ-transplant recipients with these infections⁸⁶. Furthermore, mycophenolic acid potentiates the activity of guanine-derived dideoxynucleoside analogues, such as abacavir, against HIV⁸⁷, which could be further exploited as a new combination strategy in the treatment of HIV infections.

Although IMP dehydrogenase inhibitors should, in their own right, be explored further for their potential in the treatment of various (+)RNA and (−)RNA viral infections, including haemorrhagic fever virus infections, current interest is mainly focused on their use in combination with (PEGYLATED) interferon-α in the treatment of HCV infections. This stems from the successful responses that have been seen following treatment of chronic hepatitis C with ribavirin in combination with interferon-α, in patients who did not respond to interferon alone⁸⁸.

Recently, ribavirin was shown to act as an RNA-virus mutagen, forcing RNA viruses into a lethal accumulation of errors, dubbed 'error catastrophe'^{89,90}. The antiviral activity of ribavirin might, therefore, result from the lethal mutagenic effect following incorporation of ribavirin into the viral genome, and, obviously, this lethal mutagenesis might be enhanced by the inhibitory effect of ribavirin (in its 5'-monophosphate form) on IMP dehydrogenase and the consequent decrease in cellular

PEGYLATION

Addition of poly(ethylene glycol) (PEG) groups to proteins can increase their resistance to proteolytic degradation, improve their water solubility and reduce their antigenicity.

GTP pools (as mentioned above). The ability of ribavirin to force RNA viruses into error catastrophe has so far been shown only with poliovirus^{89,90}, and it remains to be verified whether the theory also holds for other RNA viruses, such as HCV, and other substrate analogues, such as EICAR.

S-adenosylhomocysteine hydrolase inhibitors

S-adenosylhomocysteine (SAH) hydrolase is a key enzyme in methylation reactions that depend on S-adenosylmethionine (SAM) as the methyl donor, including those methylation reactions that are required for the maturation of viral mRNA. In particular, (–)RNA viruses are crucially dependent on these methylations for the stability and functioning of their messenger RNA. SAH is both a product and an inhibitor of the methyltransferase reactions; however, SAH is rapidly hydrolysed by SAH hydrolase into homocysteine and adenosine, and this prevents the accumulation of SAH, which would otherwise lead to an inhibition of the SAM-dependent methylation reactions. Inhibitors of the SAH hydrolase could therefore lead to an accumulation of SAH, and consequent inhibition of the methylation reactions. Again, as noted for IMP dehydrogenase, SAH hydrolase is a cellular target, but as virus replication increases the need for such methylations, SAH hydrolase inhibitors might confer selective antiviral activity that could vary from one virus to another depending on their individual methylation needs.

Various adenosine analogues, such as carbocyclic adenosine, carbocyclic 3-deazaadenosine, neplanocin A, 3-deazaneplanocin A and their 5'-nor derivatives, have been described as potent inhibitors of SAH hydrolase⁹¹. All of these compounds have a characteristic antiviral activity spectrum, encompassing, in particular, poxviruses, (±)RNA viruses (reoviruses) and (–)RNA viruses (bunya-, arena-, rhabdo-, filo-, ortho- and paramyxoviruses). This includes several haemorrhagic

fever viruses, such as Ebola haemorrhagic fever virus. In fact, a mouse model for Ebola haemorrhagic fever has been developed⁹², and the SAH hydrolase inhibitors carbocyclic 3-deazaadenosine⁹³ and 3-deazaneplanocin A⁹⁴ were found to protect the animals against an otherwise lethal Ebola virus infection. So, SAH hydrolase inhibitors offer real potential for the treatment of haemorrhagic fever virus infections.

Conclusion

The strategies reviewed here for interfering with the key events in the viral replicative cycle have the potential to target virtually all important human viral pathogens. Several of these strategies, such as those aimed at viral DNA synthesis, viral polyprotein cleavage, and viral release from the cells (by means of the viral neuraminidase), have already provided a number of effective and useful therapeutics for the treatment of herpesvirus (HSV-1, HSV-2, VZV and CMV), retrovirus (HIV), hepadnavirus (HBV) and influenza virus infections. Further improvements along these lines could yield more efficacious and more selective antiviral compounds. This should by no means detract from other approaches, not addressed here, that might also be predicted to target viral compounds or virus-associated events. For example, agents that specifically bind to the picornaviral capsids (pleconaril), the HIV nucleocapsid, p7 (2,2'-dithiobisbenzamides), or the influenza virus A matrix (adamantanamine derivatives); glycosylation inhibitors (deoxynojirimycin derivatives); antisense oligonucleotides or ribozymes targeted at selected viral mRNAs; gene therapy approaches; immunotherapy; and so on. Whatever approach or strategy is followed to combat viral infections, the highest profit is likely to be obtained if two or more of these strategies are combined, especially in the treatment of chronic viral infections, such as HIV, HBV and HCV.

1. De Clercq, E. Antiviral drugs: current state of the art. *J. Clin. Virol.* **22**, 73–89 (2001).
2. Gallaher, W. R., Ball, J. M., Garry, R. F., Martin-Amedee, A. M. & Montelaro, R. C. A general model for the surface glycoproteins of HIV and other retroviruses. *AIDS Res. Hum. Retroviruses* **11**, 191–202 (1995).
3. Shukla, D. *et al.* A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* **99**, 13–22 (1999).
4. Chen, Y. *et al.* Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Med.* **3**, 866–871 (1997).
5. Herold, B. C. *et al.* Poly(sodium 4-styrene sulfonate): an effective candidate topical antimicrobial for the prevention of sexually transmitted diseases. *J. Infect. Dis.* **181**, 770–773 (2000).
6. Esté, J. A. *et al.* Development of resistance of human immunodeficiency virus type 1 to dextran sulfate associated with the emergence of specific mutations in the envelope gp120 glycoprotein. *Mol. Pharmacol.* **52**, 98–104 (1997).
7. Esté, J. A. *et al.* Human immunodeficiency virus glycoprotein gp120 as the primary target for the antiviral action of AR177 (zintevir). *Mol. Pharmacol.* **53**, 340–345 (1998).
8. Cabrera, C. *et al.* Resistance of the human immunodeficiency virus to the inhibitory action of negatively charged albumins on virus binding to CD4. *AIDS Res. Hum. Retroviruses* **15**, 1535–1543 (1999).
9. Schols, D. *et al.* Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J. Exp. Med.* **186**, 1383–1388 (1997).
10. De Clercq, E. Inhibition of HIV infection by bicyclams, highly potent and specific CXCR4 antagonists. *Mol. Pharmacol.* **57**, 833–839 (2000).
11. Baba, M. *et al.* A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. USA* **96**, 5698–5703 (1999).
12. Dragic, T. *et al.* A binding pocket for a small molecule inhibitor of HIV-1 entry within the transmembrane helices of CCR5. *Proc. Natl. Acad. Sci. USA* **97**, 5639–5644 (2000).
13. Hatse, S. *et al.* Mutation of Asp171 and Asp262 of the chemokine receptor CXCR4 impairs its coreceptor function for human immunodeficiency virus-1 entry and abrogates the antagonistic activity of AMD3100. *Mol. Pharmacol.* **60**, 164–173 (2001).
14. Strizki, J. M. *et al.* SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA* **98**, 12718–12723 (2001).
- The most recent report on a potential anti-HIV drug candidate that blocks HIV infection through an antagonistic effect on the CCR5 co-receptor, which is used by macrophage-tropic (R5) HIV strains to enter cells.**
15. Root, M. J., Kay, M. S. & Kim, P. S. Protein design of an HIV-1 entry inhibitor. *Science* **291**, 884–888 (2001).
16. Eckert, D. M., Malashkevich, V. N., Hong, L. H., Carr, P. A. & Kim, P. S. Inhibiting HIV-1 entry: discovery of D-peptide inhibitors that target the gp41 coiled-coil pocket. *Cell* **99**, 103–115 (1999).
17. Kilby, J. M. *et al.* Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nature Med.* **4**, 1302–1307 (1998).
18. Lambert, D. M. *et al.* Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proc. Natl. Acad. Sci. USA* **93**, 2186–2191 (1996).
19. Schwartz, J. A., Liou, E. K. & Silverstein, S. J. Herpes simplex virus type 1 entry is inhibited by the cobalt chelate complex CTC-96. *J. Virol.* **75**, 4117–4128 (2001).
20. McGuigan, C. *et al.* Potent and selective inhibition of varicella-zoster virus (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* **42**, 4479–4484 (1999).
21. McGuigan, C. *et al.* Highly potent and selective inhibition of varicella-zoster virus by bicyclic furopyrimidine nucleosides bearing an aryl side chain. *J. Med. Chem.* **43**, 4993–4997 (2000).
- References 20 and 21 provide an example of how classical medicinal chemistry can lead to the discovery of an entirely new class of nucleoside analogues with exquisite antiviral activity (that is, inhibition of varicella-zoster virus replication at subnanomolar concentrations).**

22. Iwayama, S. *et al.* Antiherpesvirus activities of (1'S,2'R)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl] (A-5021) in cell culture. *Antimicrob. Agents Chemother.* **42**, 1666–1670 (1998).
23. Ono, N. *et al.* Mode of action of (1'S,2'R)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]guanine (A-5021) against herpes simplex virus type 1 and type 2 and varicella-zoster virus. *Antimicrob. Agents Chemother.* **42**, 2095–2102 (1998).
24. Wang, J. *et al.* The cyclohexene ring system as a furanose mimic: synthesis and antiviral activity of both enantiomers of cyclohexylguanine. *J. Med. Chem.* **43**, 736–745 (2000).
25. Siddiqui, A. Q. *et al.* Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for *in vitro* activity and QSAR. *J. Med. Chem.* **42**, 4122–4128 (1999).
26. Sabouard, D. *et al.* Characterization of the activation pathway of phosphoramidate triester prodrugs of stavudine and zidovudine. *Mol. Pharmacol.* **56**, 693–704 (1999).
27. Meier, C., Lorey, M., De Clercq, E. & Balzarini, J. CycloSal-2',3'-dideoxy-2',3'-didehydrothymidine monophosphate (cycloSal-d4TMP): synthesis and antiviral evaluation of a new d4TMP delivery system. *J. Med. Chem.* **41**, 1417–1427 (1998).
28. Balzarini, J. *et al.* Cyclosaligenyl-2',3'-didehydro-2',3'-dideoxythymidine monophosphate: efficient intracellular delivery of d4TMP. *Mol. Pharmacol.* **58**, 928–935 (2000).
29. De Clercq, E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antivir. Res.* **38**, 153–179 (1998).
30. Jonckheere, H., Anné, J. & De Clercq, E. The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med. Res. Rev.* **20**, 129–154 (2000).
31. Esnouf, R. M., Stuart, D. I., De Clercq, E., Schwartz, E. & Balzarini, J. Models which explain the inhibition of reverse transcriptase by HIV-1-specific (thio)carboxanilide derivatives. *Biochem. Biophys. Res. Commun.* **234**, 458–464 (1997).
32. Ren, J. *et al.* Binding of the second generation non-nucleoside inhibitor S-1153 to HIV-1 reverse transcriptase involves extensive main chain hydrogen bonding. *J. Biol. Chem.* **275**, 14316–14320 (2000).
- Reference 32 emphasizes that future design of new non-nucleoside HIV-1 reverse transcriptase inhibitors could be rationally guided by resilience to drug-resistance mutations.**
33. Pelemans, H., Esnouf, R., De Clercq, E. & Balzarini, J. Mutational analysis of Trp-229 of human immunodeficiency virus type 1 reverse transcriptase (RT) identifies this amino acid residue as a prime target for the rational design of new non-nucleoside RT inhibitors. *Mol. Pharmacol.* **57**, 954–960 (2000).
34. Xiong, X., Smith, J. L. & Chen, M. S. Effect of incorporation of cidofovir into DNA by human cytomegalovirus DNA polymerase on DNA elongation. *Antimicrob. Agents Chemother.* **41**, 594–599 (1997).
35. Balzarini, J., Hao, Z., Herdejewin, P., Johns, D. G. & De Clercq, E. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. *Proc. Natl. Acad. Sci. USA* **88**, 1499–1503 (1991).
36. De Clercq, E. *et al.* A novel selective broad-spectrum anti-DNA virus agent. *Nature* **323**, 464–467 (1986).
37. De Clercq, E. Therapeutic potential of HPMPC as an antiviral drug. *Rev. Med. Virol.* **3**, 85–96 (1993).
38. De Clercq, E. Vaccinia virus inhibitors as a paradigm for the chemotherapy of poxvirus infections. *Clin. Microbiol. Rev.* **14**, 382–397 (2001).
- Describes several compounds that are effective against vaccinia virus, and that could, therefore, be considered for the therapy of poxvirus infections, including smallpox, should the need arise. The prominent candidate is cidofovir, which has already proved to be effective in the treatment of poxvirus infections, such as molluscum contagiosum, in humans.**
39. Pommier, Y., Pilon, A. A., Bajaj, K., Mazumder, A. & Neamati, N. HIV-1 integrase as a target for antiviral drugs. *Antivir. Chem. Chemother.* **8**, 463–483 (1997).
40. Pommier, Y., Marchand, C. & Neamati, N. Retroviral integrase inhibitors year 2000: update and perspectives. *Antivir. Res.* **47**, 139–148 (2000).
41. Plymmer, W. *et al.* Viral entry as the primary target for the anti-HIV activity of choricid acid and its tetra-acetyl esters. *Mol. Pharmacol.* **58**, 641–648 (2000).
- References 41 and 48 show that caution should be exercised in identifying the primary molecular target in the mode of action of antiviral compounds, in**
- particular, anti-HIV agents, which are capable of interacting at several steps in the viral replication cycle.**
42. Hazuda, D. J. *et al.* Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **287**, 646–650 (2000).
43. Wai, J. S. *et al.* 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J. Med. Chem.* **43**, 4923–4926 (2000).
44. Daemelans, D., Vandamme, A.-M. & De Clercq, E. Human immunodeficiency virus gene regulation as a target for antiviral chemotherapy. *Antiviral Chem. Chemother.* **10**, 1–14 (1999).
45. Baba, M. *et al.* Inhibition of human immunodeficiency virus type 1 replication and cytokine production by fluoroquinolone derivatives. *Mol. Pharmacol.* **53**, 1097–1103 (1998).
46. Turpin, J. A. *et al.* Inhibition of acute-, latent-, and chronic-phase human immunodeficiency virus type 1 (HIV-1) replication by a bistriazoloacridine analog that selectively inhibits HIV-1 transcription. *Antimicrob. Agents Chemother.* **42**, 487–494 (1998).
47. Hamy, F. *et al.* An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication. *Proc. Natl. Acad. Sci. USA* **94**, 3548–3553 (1997).
48. Daemelans, D. *et al.* A second target for the peptoid Tat/transactivation response element inhibitor CGP64222: inhibition of human immunodeficiency virus replication by blocking CXCR-chemokine receptor 4-mediated virus entry. *Mol. Pharmacol.* **57**, 116–124 (2000).
49. Chao, S. H. *et al.* Flavipiravir inhibits P-TEFb and blocks HIV-1 replication. *J. Biol. Chem.* **275**, 28345–28348 (2000).
50. Dymock, B. W., Jones, P. S. & Wilson, F. X. Novel approaches to the treatment of hepatitis C virus infection. *Antivir. Chem. Chemother.* **11**, 79–96 (2000).
51. Paolini, C., De Francesco, R. & Gallinari, P. Enzymatic properties of hepatitis C virus NS3-associated helicase. *J. Gen. Virol.* **81**, 1335–1345 (2000).
52. Carroll, S. S. *et al.* Only a small fraction of purified hepatitis C RNA-dependent RNA polymerase is catalytically competent: implications for viral replication and *in vitro* assays. *Biochemistry* **39**, 8243–8249 (2000).
53. Patick, A. K. & Potts, K. E. Protease inhibitors as antiviral agents. *Clin. Microbiol. Rev.* **11**, 614–627 (1998).
54. Erickson, J. W. & Burt, S. K. Structural mechanisms of HIV drug resistance. *Annu. Rev. Pharmacol. Toxicol.* **36**, 545–571 (1996).
55. Turner, S. R. *et al.* Tipranavir (PNU-140690): a potent, orally bioavailable nonpeptidic HIV protease inhibitor of the 5,6-dihydro-4-hydroxy-2-pyrone sulfonamide class. *J. Med. Chem.* **41**, 3467–3476 (1998).
56. Hagen, S. E. *et al.* 4-Hydroxy-5,6-dihydropyrones as inhibitors of HIV protease: the effect of heterocyclic substituents at C-6 on antiviral potency and pharmacokinetic parameters. *J. Med. Chem.* **44**, 2319–2332 (2001).
57. Waxman, L. & Darke, P. L. The herpesvirus proteases as targets for antiviral chemotherapy. *Antivir. Chem. Chemother.* **11**, 1–22 (2000).
58. Jarvest, R. L. *et al.* Inhibition of herpes proteases and antiviral activity of 2-substituted thieno-[2,3-d]oxazinones. *Bioorg. Med. Chem. Lett.* **9**, 443–448 (1999).
59. Smith, D. G. *et al.* The inhibition of human cytomegalovirus (hCMV) protease by hydroxylamine derivatives. *Bioorg. Med. Chem. Lett.* **9**, 3137–3142 (1999).
60. Ogilvie, W. W. *et al.* Synthesis and antiviral activity of monobactams inhibiting the human cytomegalovirus protease. *Bioorg. Med. Chem.* **7**, 1521–1531 (1999).
61. Borthwick, A. D. *et al.* Design and synthesis of pyrrolidine-5,5-trans-lactams (5-oxo-hexahydro-pyrrrolo[3,2-b]pyrroles) as novel mechanism-based inhibitors of human cytomegalovirus protease. 1. The α -methyl-trans-lactam template. *J. Med. Chem.* **43**, 4452–4464 (2000).
62. Matsumoto, M., Misawa, S., Chiba, N., Takaku, H. & Hayashi, H. Selective nonpeptidic inhibitors of herpes simplex virus type 1 and human cytomegalovirus proteases. *Biol. Pharm. Bull.* **24**, 236–241 (2001).
63. Dragovich, P. S. *et al.* Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 1. Michaelis acceptor structure–activity studies. *J. Med. Chem.* **41**, 2806–2818 (1998).
64. Dragovich, P. S. *et al.* Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 2. Peptide structure–activity studies. *J. Med. Chem.* **41**, 2819–2834 (1998).
65. Dragovich, P. S. *et al.* Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 3. Structure–activity studies of ketomethylene-containing peptidomimetics. *J. Med. Chem.* **42**, 1203–1212 (1999).
66. Dragovich, P. S. *et al.* Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 4. Incorporation of P1 lactam moieties as α -glutamine replacements. *J. Med. Chem.* **42**, 1213–1224 (1999).
67. Matthews, D. A. *et al.* Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc. Natl. Acad. Sci. USA* **96**, 11000–11007 (1999).
68. Patick, A. K. *et al.* *In vitro* antiviral activity of AG7088, a potent inhibitor of human rhinovirus 3C protease. *Antimicrob. Agents Chemother.* **43**, 2444–2450 (1999).
69. Tautz, N., Kaiser, A. & Thiel, H.-J. NS3 serine protease of bovine viral diarrhea virus: characterization of active site residues, NS4A cofactor domain, and protease-cofactor interactions. *Virology* **273**, 351–363 (2000).
70. Linès-Brunet, M. *et al.* Highly potent and selective peptide-based inhibitors of the hepatitis C virus serine protease: towards smaller inhibitors. *Bioorg. Med. Chem. Lett.* **10**, 2267–2270 (2000).
71. Bennett, J. M. *et al.* The identification of α -ketoamides as potent inhibitors of hepatitis C virus NS3–4A proteinase. *Bioorg. Med. Chem. Lett.* **11**, 355–357 (2001).
72. Sing, W. T., Lee, C. L., Yeo, S. L., Lim, S. P. & Sim, M. M. Arylalkylidene rhodanine with bulky and hydrophobic functional group as selective HCV NS3 protease inhibitor. *Bioorg. Med. Chem. Lett.* **11**, 91–94 (2001).
73. Von Itzstein, M. *et al.* Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **363**, 418–423 (1993).
- The first demonstration of how computer-assisted drug design, based on the crystal structure of the influenza viral neuraminidase, could lead to the development of new antiviral drugs.**
74. Barnett, J. M. *et al.* Zanamivir susceptibility monitoring and characterization of influenza virus clinical isolates obtained during phase II clinical efficacy studies. *Antimicrob. Agents Chemother.* **44**, 78–87 (2000).
75. Hayden, F. G. *et al.* Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. *N. Engl. J. Med.* **337**, 874–880 (1997).
76. Kim, C. U. *et al.* Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J. Am. Chem. Soc.* **119**, 681–690 (1997).
77. Mendel, D. B. *et al.* Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS 4071 protects mice and ferrets against influenza infection. *Antimicrob. Agents Chemother.* **42**, 640–646 (1998).
78. Hayden, F. G. *et al.* Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. *J. Am. Med. Assoc.* **282**, 1240–1246 (1999).
79. Nicholson, K. G. *et al.* Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. *Lancet* **355**, 1845–1850 (2000).
80. Smee, D. F., Huffman, J. H., Morrison, A. C., Barnard, D. L. & Sidwell, R. W. Cyclopentane neuraminidase inhibitors with potent *in vitro* anti-influenza virus activities. *Antimicrob. Agents Chemother.* **45**, 743–748 (2001).
81. Sidwell, R. W. *et al.* *In vivo* influenza virus-inhibitory effects of the cyclopentane neuraminidase inhibitor RWJ-270201. *Antimicrob. Agents Chemother.* **45**, 749–757 (2001).
82. Bantia, S. *et al.* Comparison of the anti-influenza virus activity of RWJ-270201 with those of oseltamivir and zanamivir. *Antimicrob. Agents Chemother.* **45**, 1162–1167 (2001).
83. Sintchak, M. D. *et al.* Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic acid. *Cell* **85**, 921–930 (1996).
84. De Clercq, E. *et al.* Antiviral activities of 5-ethynyl-1- β -D-ribofuranosylimidazole-4-carboxamide and related compounds. *Antimicrob. Agents Chemother.* **35**, 679–684 (1991).
85. Markland, W., McQuaid, T. J., Jain, J. & Kwong, A. D. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with ribavirin and demonstration of antiviral additivity with α -interferon. *Antimicrob. Agents Chemother.* **44**, 859–866 (2000).
86. Neyts, J., Andrei, G. & De Clercq, E. The novel immunosuppressive agent mycophenolate mofetil markedly potentiates the antiherpesvirus activities of acyclovir, ganciclovir, and penciclovir *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* **42**, 216–222 (1998).

87. Margolis, D. *et al.* Abacavir and mycophenolic acid, an inhibitor of inosine monophosphate dehydrogenase, have profound and synergistic anti-HIV activity. *J. Acquir. Immune Defic. Syndr.* **21**, 362–370 (1999).
88. Saracco, G. *et al.* A randomized 4-arm multicenter study of interferon- α -2b plus ribavirin in the treatment of patients with chronic hepatitis C not responding to interferon alone. *Hepatology* **34**, 133–138 (2001).
89. Crotty, S. *et al.* The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. *Nature Med.* **6**, 1375–1379 (2000).
90. Crotty, S., Cameron, C. E. & Andino, R. RNA virus error catastrophe: direct molecular test by using ribavirin. *Proc. Natl Acad. Sci. USA* **98**, 6895–6900 (2001). **References 89 and 90 afford a rather provocative account of how mutagens that induce ‘error catastrophe’ could be used in antiviral strategies, at least for some RNA viruses.**
91. De Clercq, E. *et al.* Broad-spectrum antiviral activities of neplanocin A, 3-deazaneplanocin A, and their 5'-nor derivatives. *Antimicrob. Agents Chemother.* **33**, 1291–1297 (1989).
92. Bray, M., Davis, K., Geisbert, T., Schmaljohn, C. & Huggins, J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *J. Infect. Dis.* **179** (Suppl. 1), S248–S258 (1999).
93. Huggins, J., Zhang, Z.-X. & Bray, M. Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus *in vitro* and in a lethal mouse model. *J. Infect. Dis.* **179**, (Suppl. 1) S240–S247 (1999).
94. Bray, M., Driscoll, J. & Huggins, J. W. Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. *Antiviral Res.* **45**, 135–147 (2000). **Most virus infections are amenable to antiviral therapy, and this also holds for such feared viral pathogens as Ebola.**

Acknowledgments

E.D.C. holds the Professor P. De Somer Chair of Microbiology at the School of Medicine, Katholieke Universiteit Leuven, Belgium, and thanks C. Callebaut for her invaluable editorial assistance.

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