1
Outlook of the Antiviral Drug Era, Now More Than 50 Years After Description of the First Antiviral Drug

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1.1
Introduction: The Prehistory

More than 50 years ago, the synthesis of IDU (iododeoxyuridine), a thymidine analogue, was described by Prusoff [1]. This compound would later become the first antiviral drug to be licensed for (topical) use in the treatment of herpes simplex virus (HSV) infections of the eye. In this sense, the advent of IDU marked the birth of the antiviral drug era. There are now about 50 licensed antiviral compounds, half of them are used for the treatment of AIDS, of which the viral origin was first recognized 27 years ago [2, 3] (2008 Nobel Prize for Medicine or Physiology was awarded to Françoise Barré-Sinoussi and Luc Montagnier for their discovery of human immunodeficiency virus and to Harald zur Hausen for demonstrating the link between human papilloma virus (HPV) and cervical cancer).

Was IDU truly the first antiviral? In retrospect, the antiviral chemotherapy era had a rather slow and unremarkable start. The first compounds quoted to have antiviral activity (against vaccinia virus) were the thiosemicarbazones [4, 5]. These compounds were also found effective against vaccinia virus infection in mice and rabbits [6–8], and one of the thiosemicarbazones, that is, *N*-methylisatin-β-thiosemicarbazone, even entered clinical studies for the prophylaxis of smallpox [9] just when the smallpox vaccination took over and made any further attempts to develop an antipoxvirus drug apparently superfluous.

Then came the benzimidazole derivatives as inhibitors of influenza virus multiplication [10, 11], but despite the reported effectiveness of the 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (DRB) [10, 11] against influenza virus multiplication, it was not pursued further as a potential anti-influenza virus agent. Another benzimidazole derivative, 2-(1-hydroxybenzyl)benzimidazole (HBB), was found active against the multiplication of poliovirus (and other enteroviruses) [12–14], but with the successful implementation of the poliovirus vaccine, just as we had witnessed for smallpox, interest in developing an antiviral drug for poliovirus infections vanished.

IDU, soon to be followed by TFT (trifluorothymidine), could be considered as the third, and successful, attempt to herald the antiviral chemotherapy era. IDU was first

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considered as a potential antitumor agent [15] before it was shown by Herrmann to be active against HSV and vaccinia virus [16]. That IDU and TFT finally became antiviral drugs for the topical treatment of HSV eye infections, in particular HSV keratitis, is due to the pioneering work of Kaufman [17, 18].

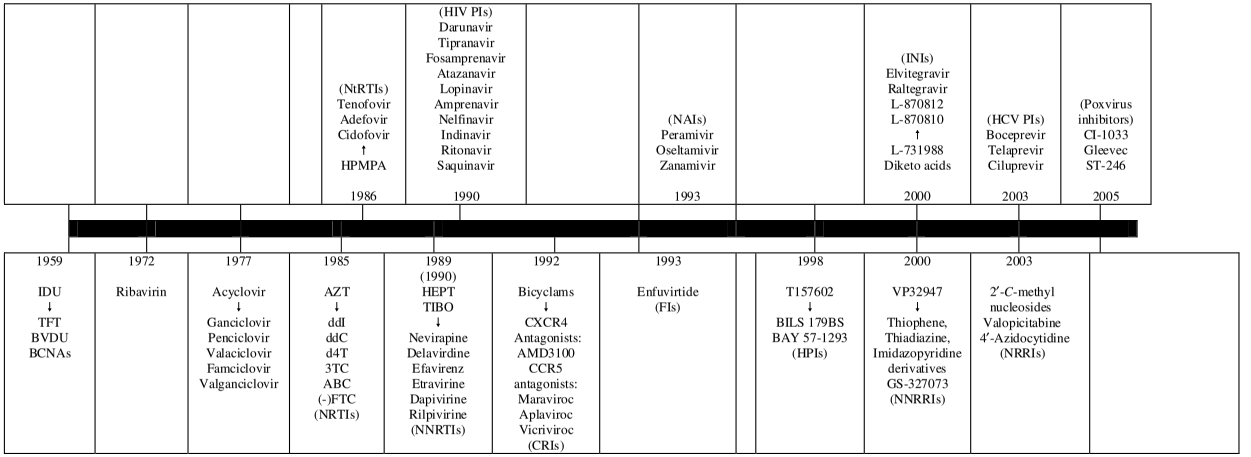
1.2 Key Events in Antiviral Drug Development

Table 1.1 presents the key events in antiviral drug discovery, 1959 being the year when IDU was first described [1]. Ribavirin was the first low molecular weight compound described as a broad-spectrum antiviral agent (in 1972) by Sidwell *et al.* [19]. The combination of ribavirin with (pegylated) interferon-α has now become a standard treatment [20] for patients with chronic hepatitis C. That virus infections could be specifically tackled, without harm to the host cell, was heralded by the advent (in 1977) of acyclovir [21, 22], which is today still considered as the gold standard for the treatment of HSV infections. Two years after the discovery of HIV, in 1985, the first antiretrovirus agent (to become a drug 2 years later), AZT (zidovudine) was described [23], and this opened the search for, and development of, a wealth of new 2′,3′-dideoxynucleoside analogues, now collectively referred to as nucleoside reverse transcriptase inhibitors (NRTIs).

In 1986, we described the first of a new class of broad-spectrum anti-DNA virus agents [24], namely, acyclic nucleoside phosphonates, several of which are active against the HIV and HBV reverse transcriptase and, therefore, referred to as nucleotide reverse transcriptase inhibitors (NtRTIs). Then followed in December 1989 and 1990 the description of a new concept for inhibiting the HIV-1 reverse transcriptase by nonnucleoside analogues (i.e., HEPT [25, 26] and TIBO [27]), giving rise to a still growing class of antiviral drugs, the nonnucleoside reverse transcriptase inhibitors (NNRTIs). With saquinavir, the year 1990 marked the birth of the rational design of HIV protease inhibitors (HIV PIs), which, in the mean time, has yielded 10 licensed drugs.

In 1992, we described an unusual class of compounds, the bicyclams as HIV inhibitors interacting with a viral uncoating event [28]. These compounds (prototype: AMD3100) would be, later on, shown to act as CXCR4 antagonists. Together with the CCR5 antagonists (the only licensed anti-HIV drug of this class of compounds being maraviroc), CXCR4 and CCR5 antagonists can be considered coreceptor inhibitors (CRIs), targeted at the coreceptor usage of X4 and R5 HIV strains, respectively. The year 1993 marked the description of two totally different strategic options: (i) that of DP-178, which later on would become known as enfuvirtide as an HIV fusion inhibitor (FI) [29] and (ii) that of 4-guanidino-Neu5Ac2en, which later on would become known as zanamivir as a neuraminidase-based inhibitor (NAI) of influenza virus replication [30]. Then followed in 1998 the seminal observation that HSV replication could be inhibited at the DNA helicase–primase level by a 2-aminothiazole (T157602) [31] that would later give impetus to the development of helicase–primase inhibitors (HPIs) as potential anti-HSV drugs.

Table 1.1 Milestones in antiviral drug discovery: year when key compounds were first described.



NRTIs: nucleoside reverse transcriptase inhibitors	FIs: fusion inhibitors	NRRIs: nucleoside RNA replicase inhibitors
NtRTIs: nucleotide reverse transcriptase inhibitors	INIs: integrase inhibitors	NNRRIs: nonnucleoside RNA replicase inhibitors
NNRTIs: nonnucleoside reverse transcriptase inhibitors	NAIs: neuraminidase inhibitors	
HIV PIs: HIV protease inhibitors	HPIs: helicase–primase inhibitors	
CRIs: coreceptor inhibitors	HCV PIs: HCV protease inhibitors	

Although considered an attractive target for two decades or so, the HIV integrase became a realistic target only when Hazuda *et al.* [32] demonstrated in 2000 it to be inhibited by the so-called diketo acids, which have yielded one integrase inhibitor (INI) that has already been formally approved (raltegravir) and another one under development (elvitegravir). Also described in 2000 was a pestivirus inhibitor (VP32947) [33] that hallmarked the search for inhibitors targeted at the RNA-dependent RNA polymerase (RdRp) of not only pestiviruses but also hepaciviruses (nonnucleoside RNA replicase inhibitors (NNRRIs)). In 2003, Lamarre *et al.* published their pioneering observation that hepatitis C virus (HCV) replication could be inhibited by ciluprevir [34], which (although the compound itself was not further developed) generated the search for other HCV PIs. Also in 2003, Migliaccio *et al.* [35] reported that 2′-*C*-methyl-substituted ribonucleosides were inhibitory to the replication of HCV and other flaviviruses by acting as nonobligate chain terminators, thus inciting the search for nucleoside RNA replicase inhibitors (NRRIs).

While, since the days of methisazone, interest in developing antivirals for poxvirus infections (i.e., smallpox) died, the advent in 2005 of ST-246 testifies to the renewed interest in this area [36], and this is further demonstrated by the observations that poxvirus infections can be successfully suppressed through inhibitors of tyrosine kinases (Gleevec [37] and CI-1033 [38]).

1.3
Antiviral Drugs: Current State of the Art

Most of the antiviral agents that have been approved, and are used in the treatment of virus infections, are targeted at HIV, HBV, HCV, influenza virus, HSV, and other herpesviruses such as varicella zoster virus (VZV) and cytomegalovirus (CMV). More compounds for the treatment of HIV, HBV, HCV, HSV, VZV, CMV, and influenza virus and several other viral infections, for example, poxvirus (e.g., variola, vaccinia, and monkeypox), respiratory syncytial virus, hemorrhagic fever virus (e.g., Lassa, Rift Valley, Ebola, yellow fever, and dengue), and enterovirus (e.g., polio, Coxsackie, and Echo), either are in clinical or preclinical development or still have to be developed. The antiviral compounds that have been approved by the US FDA (Food and Drug Administration) are listed in Table 1.2.

1.4
Antiviral Drugs Active against Herpesviruses (i.e., HSV, VZV, and so on)

Starting from IDU and TFT, many more 5-substituted 2′-deoxyuridines were synthesized [39], the most prominent antiviral drug of this class of compounds being (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) [40]. Although selectively active against both HSV-1 and VZV, BVDU has been developed specifically for the treatment of VZV infections (i.e., herpes zoster) [41].

Table 1.2 Antiviral drugs approved by the US FDA.

Registered brand name	Generic name	Manufacturer
Anti-HIV compounds		
Nucleoside reverse transcriptase inhibitors		
Retrovir	Zidovudine (AZT)	GlaxoSmithKline
Videx®, Videx® EC	Didanosine (ddI)	Bristol–Myers Squibb
Hivid®	Zalcitabine (ddC)	Roche
Zerit®	Stavudine (d4T)	Bristol–Myers Squibb
Epivir®, Zeffix®	Lamivudine (3TC)	GlaxoSmithKline
Ziagen®	Abacavir (ABC)	GlaxoSmithKline
Emtriva®	Emtricitabine ((−)FTC)	Gilead Sciences
Combivir®	Lamivudine + zidovudine	GlaxoSmithKline
Trizivir®	Abacavir + lamivudine + zidovudine	GlaxoSmithKline
Epzicom®	Abacavir + lamivudine	GlaxoSmithKline
Nucleotide reverse transcriptase inhibitors		
Viread®	Tenofovir disoproxil fumarate	Gilead Sciences
Truvada®	Tenofovir disoproxil fumarate + emtricitabine	Gilead Sciences
Atripla®	Tenofovir disoproxil fumarate + emtricitabine + efavirenz	Gilead Sciences and Bristol–Myers Squibb
Nonnucleoside reverse transcriptase inhibitors		
Viramune®	Nevirapine	Boehringer Ingelheim
Rescriptor®	Delavirdine	Pfizer
Sustiva®, Stocrin®	Efavirenz	Bristol–Myers Squibb
Intelence®	Etravirine	Tibotec
Protease inhibitors		
Fortovase®	Saquinavir	Roche
Norvir®	Ritonavir	Abbott
Crixivan®	Indinavir	Merck
Viracept®	Nelfinavir	Pfizer
Agenerase®, Prozei®	Amprenavir	GlaxoSmithKline
Kaletra®	Lopinavir + ritonavir	Abbott
Reyataz®	Atazanavir	Bristol–Myers Squibb
Lexiva®	Fosamprenavir	GlaxoSmithKline
Aptivus®	Tipranavir	Boehringer Ingelheim
Prezista®	Darunavir	Tibotec
Viral entry inhibitors		
Coreceptor inhibitors		
Selzentry®, Celsentri®	Maraviroc	Pfizer
Fusion inhibitors		
Fuzeon®	Enfuvirtide (T-20)	Roche

(*Continued*)

Table 1.2 (*Continued*)

Registered brand name	Generic name	Manufacturer
Integrase inhibitors		
Isentress®	Raltegravir	Merck
Anti-HBV compounds		
Epivir®, Zeffix®	Lamivudine (3TC)	GlaxoSmithKline
Hepsera®	Adefovir dipivoxil	Gilead Sciences
Baraclude®	Entecavir	Bristol–Myers Squibb
Tyzeka®, Sebivo®	Telbivudine	Idenix Pharmaceuticals
Viread®	Tenofovir disoproxil fumarate	Gilead Sciences
Intron A®	Interferon-α-2b	Schering-Plough
Pegasys®	Pegylated interferon-α-2a	Roche
Antiherpesvirus compounds		
HSV and VZV inhibitors		
Zovirax®	Acyclovir (ACV)	GlaxoSmithKline
Zelitrex®, Valtrex®	Valaciclovir (VACV)	GlaxoSmithKline
Denavir®, Vectavir®	Penciclovir (PCV)	Novartis
Famvir®	Famciclovir (FCV)	Novartis
Herpid®, Stoxil®, Idoxene®, Virudox®	Idoxuridine (IDU, IUdR)	Yale University
Viroptic®	Trifluridine (TFT)	King Pharmaceuticals
Zostex®, Brivirac®, Zerpex®	Brivudin (BVDU)[a]	Berlin Chemie/Menarini
CMV inhibitors		
Cymevene®, Cytovene®	Ganciclovir (GCV)	Roche
Valcyte®	Valganciclovir (VGCV)	Roche
Foscavir®	Foscarnet	Astra Zeneca
Vistide®	Cidofovir (CDV)	Pfizer
Vitravene®	Fomivirsen	Novartis
Anti-influenza virus compounds		
Symmetrel®, Mantadix®, Amantan®	Amantadine	Endo Pharmaceuticals
Flumadine®	Rimantadine	Forest Laboratories
Relenza®	Zanamivir	GlaxoSmithKline
Tamiflu®	Oseltamivir	Roche
Virazole®, Virazid®, Viramid®	Ribavirin	Valeant Pharmaceuticals
Anti-HCV compounds		
Rebetol®	Ribavirin	Schering-Plough
Copegus®	Ribavirin	Roche
Pegasys®	Pegylated interferon-α-2a	Roche
Roferon A®	Interferon-α-2a	Roche
Intron A®	Interferon-α-2b	Schering-Plough
PEG-Intron®	Pegylated interferon-α-2b	Schering-Plough
Rebetron®	Interferon-α-2b + ribavirin	Schering-Plough

a) Not formally approved by the US FDA.

BVDU owes its antiviral selectivity to a specific phosphorylation by the HSV-1- and VZV-encoded thymidine kinase, just as acyclovir does, but compared to acyclovir, BVDU is a much more potent inhibitor of VZV replication. If BVDU is further converted to a bicyclic furano[2,3-*d*]pyrimidine nucleoside analogue (BCNA) carrying an aliphatic side chain interrupted by a phenyl moiety [42, 43], as in Cf 1743, the compound becomes exquisitely and exclusively active against VZV.

Although BVDU and acyclovir belong, respectively, to the pyrimidine and purine nucleoside analogues, they share, structurally, the same carboxamide pharmacophore (Figure 1.1), which may explain why they are both specifically recognized as substrate by the HSV- and VZV-encoded thymidine kinases. The same pharmacophore is found in other acyclic guanosine analogues such as ganciclovir and

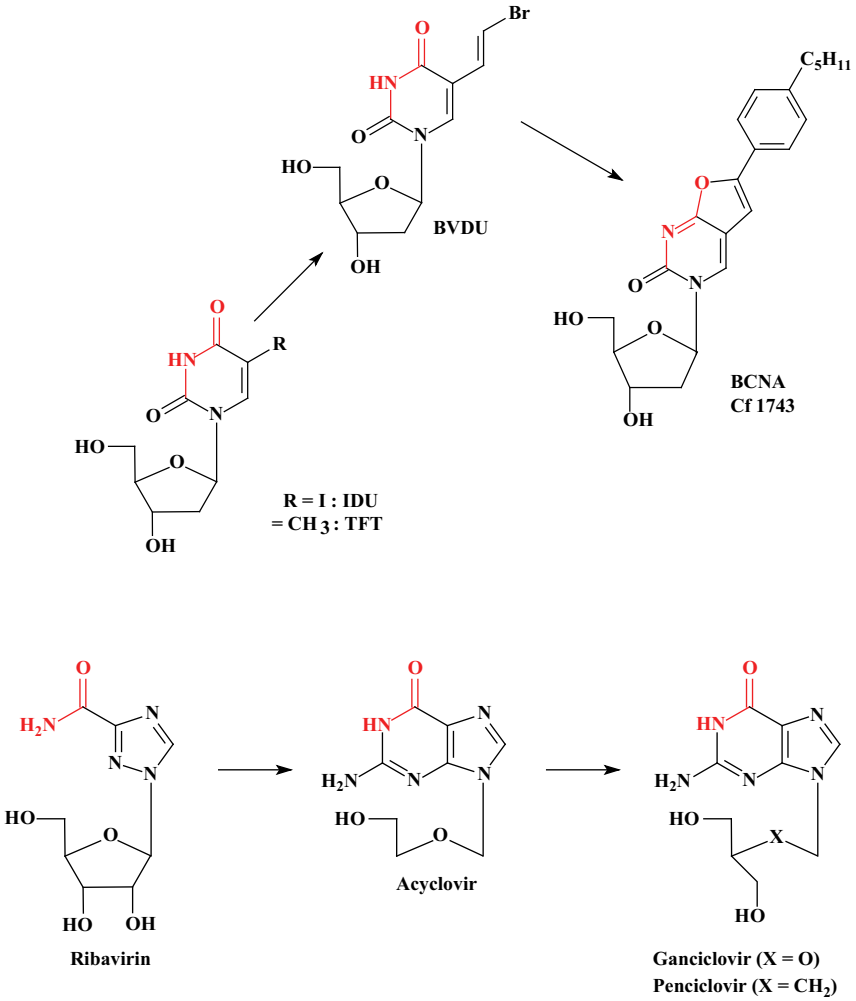


Figure 1.1 Pharmacophores in antiherpesvirus agents.

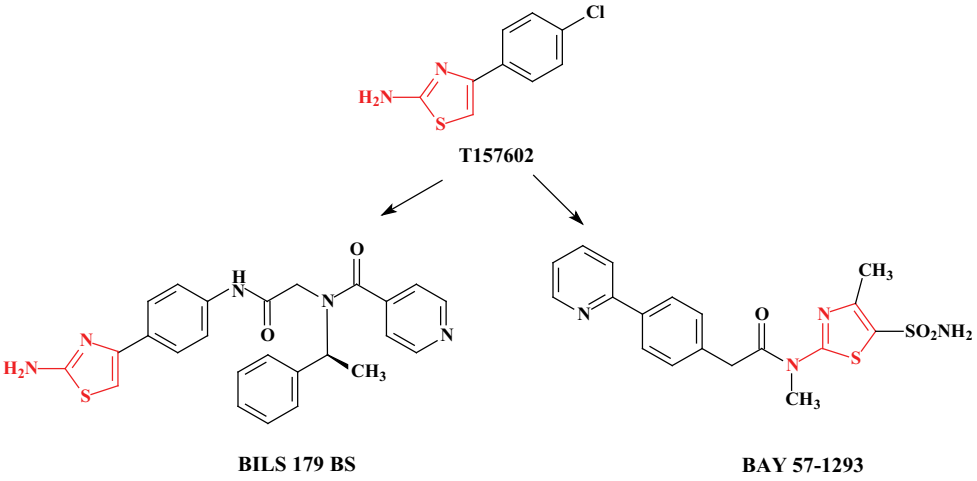


Figure 1.1 (*Continued*)

penciclovir, again explaining the specificity of these compounds against HSV and VZV. Remarkably, the same pharmacophore is also found in ribavirin, which was described as a broad-spectrum antiviral agent, 5 years before acyclovir was reported (see Table 1.1), but in the case of ribavirin, the presence of the ribofuranosyl moiety primarily directs its antiviral activity spectrum toward RNA viruses due to an inhibitory action at the level of the IMP dehydrogenase [44–46].

While BVDU and acyclovir interact in their active triphosphate form with the viral DNA polymerase, the first phosphorylation step by the viral thymidine kinase required only to initiate the activation process, the HPIs seem to be directly targeted at the HSV helicase–primase UL5–UL8–UL52 complex [47]. The first HPI reported to inhibit HSV replication via interaction with the helicase component of this complex [31] was the 2-aminothiazole T-157602. The HPIs that were subsequently described and also found to be more effective than acyclovir and famciclovir against HSV infections in murine models of HSV-1 and HSV-2 infection [48–51], namely, BILS 179BS and BAY 57-1293, are also built upon the 2-aminothiazole scaffold (Figure 1.1). HPIs represent an exciting new avenue in the development of antivirals active against herpesviruses [47], but whether they represent an alternative (or additional) strategy to acyclovir (and acyclic guanosine analogues in general) will depend on their exact spectrum of antiviral activity, whether or not encompassing VZV (an issue that presently can only be speculated upon), and the readiness by which they elicit resistance mutations [52, 53] (an issue that needs continued vigilance).

1.5
Antiviral Drugs Active against Retroviruses (HIV)

The best known class of the antiretroviral agents is that of the nucleoside reverse transcriptase inhibitors, now containing seven members – zidovudine, didanosine,

zalcitabine, stavudine, lamivudine, abacavir, and emtricitabine – that are on the market [54]. What all these compounds have in common is that they are 2′,3′-dideoxynucleoside analogues (Figure 1.2, NRTIs), which through the absence of a 3′-hydroxyl group inevitably act as chain terminators at the reverse transcriptase level. The last three of the series, namely, lamivudine (3TC, originally described as its racemic form, BCH-189) [55], abacavir (1592 U89) [56], and emtricitabine ((−)

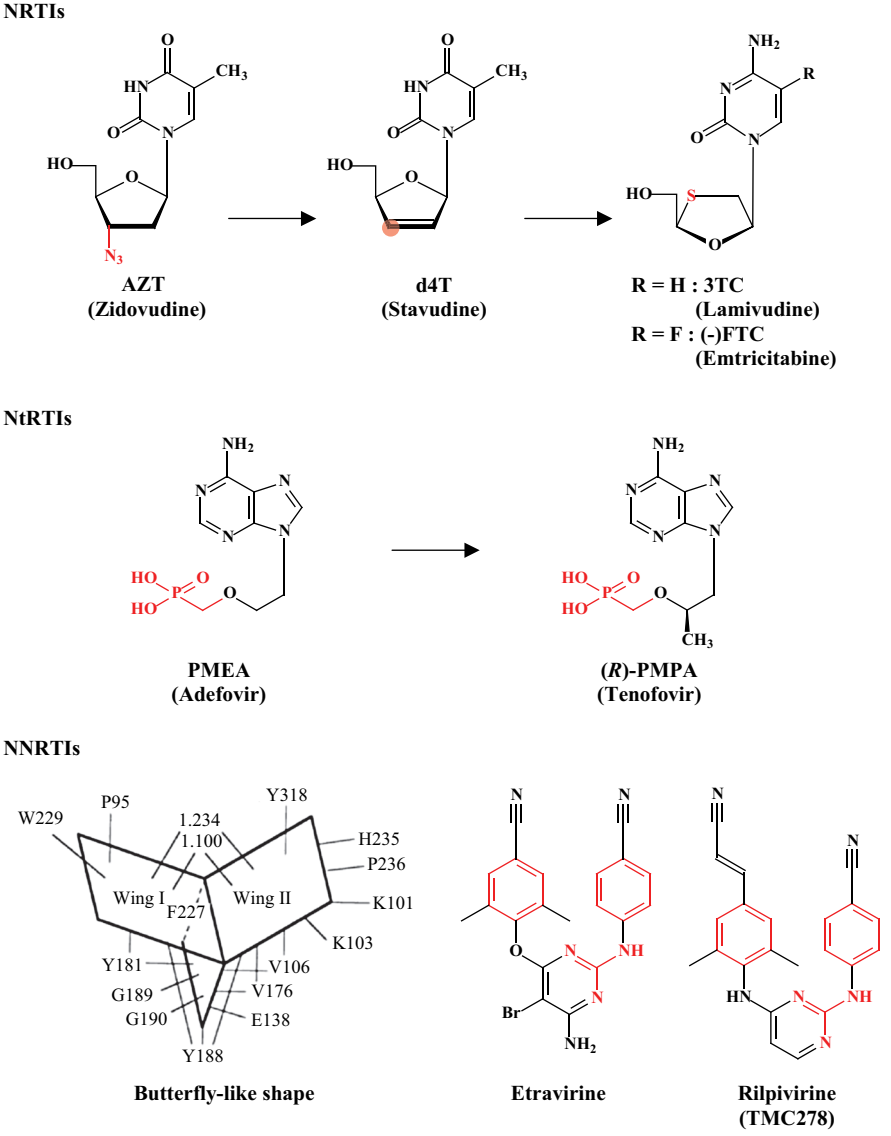
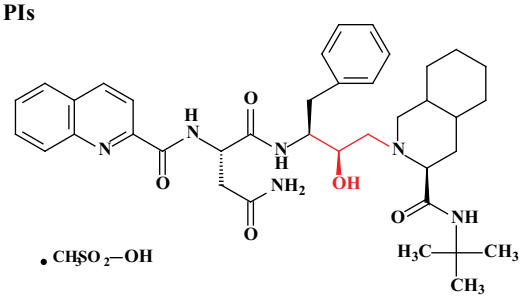
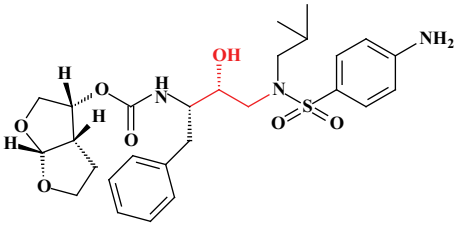


Figure 1.2 Pharmacophores in antiretrovirus agents.

PIs

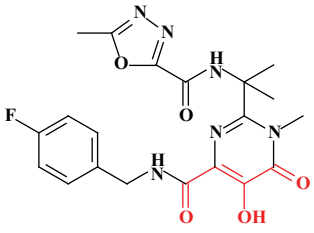


Saquinavir

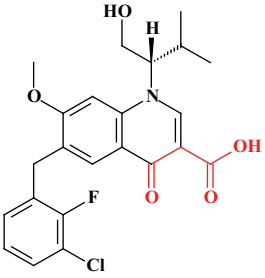


Darunavir
(TMC-114)

INIs

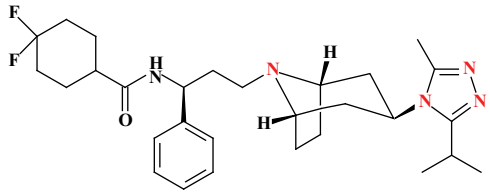


Raltegravir
(MK-0518)

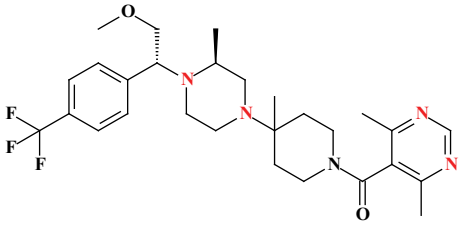


Elvitegravir
(GS-9137)

CRIs (R5 strains)



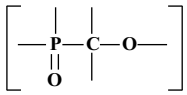
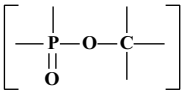
Maraviroc



Vicriviroc
(SCH-D)

Figure 1.2 (*Continued*)

FTC) [57, 58], correspond to the (−)- or L-enantiomeric form (whereas the first four have the natural D-form).

NtRTIs should be clearly distinguished from the NRTIs as they contain a phosphonate group  that is isosteric with the phosphate group  of the normal nucleotides.

To this class of compounds belong adefovir and tenofovir (Figure 1.2, NtRTIs) [59], used in their oral prodrug forms, adefovir dipivoxil and tenofovir disoproxil fumarate (TDF), in the treatment of hepatitis B virus (HBV) and HIV infections, respectively. TDF has since 2008 also been licensed for the treatment of HBV infections [60]. TDF is also commercially available, in combination with emtricitabine (Truvada®), and in combination with emtricitabine and efavirenz (Atripla®), for the treatment of HIV infections.

The HEPT and TIBO derivatives were the first nonnucleoside reverse transcriptase inhibitors to be described [61]. This class has now yielded an abundance of compounds, four of which have been formally approved (nevirapine, delavirdine, efavirenz, and etravirine) and a fifth is forthcoming (rilpivirine). All these compounds have a butterfly-like shape (Figure 1.2, NNRTIs), a term first coined by Ding *et al.* [62], and it has also been shown by crystallographic analysis [63].

All protease inhibitors (PIs) that have been licensed for clinical use (from saquinavir to darunavir, Figure 1.2, PIs) [54], with the exception of tipranavir, are built upon the hydroxyethylene scaffold [$-CH(OH)-CH_2-$], which can be considered peptidomimetic and thus imitates the peptide linkage that has to be cleaved by the viral protease during the viral protein maturation process. PIs are generally used in combination with other antiretroviral classes. Given their common scaffold they may be expected to give similar potency, side effects, and resistance profiles.

Of the fusion inhibitors (FIs), the first and still the only FI used in the treatment of HIV-1 infections is enfuvirtide (structure as given in Ref. [64]), a 36-amino acid peptide, for which proof of concept in the clinic was provided by Kilby *et al.* [65] and the clinical efficacy further demonstrated by Lalezari *et al.* [66] and Lazzarin *et al.* [67]. Limitations to the widespread use of enfuvirtide are its parenteral administration (subcutaneous injection twice daily), the local induration it may cause, and the cost.

Of the coreceptor inhibitors, none is likely to be available soon for the treatment of X4 HIV infections (instead, the CXCR4 antagonist AMD3100 has been developed, and recently licensed, as a stem cell mobilizer for autologous transplantation in patients with hematological malignancies such as non-Hodgkin's lymphoma or multiple myeloma [68]). Several CCR5 antagonists have been described for the treatment of R5 HIV infections [69]: only one (maraviroc) has been licensed for clinical use and a second one (vicriviroc) is forthcoming. It is difficult to discern what these compounds have in common structurally, except for the presence of a number of basic nitrogens (Figure 1.2, CRIs).

In HIV integrase inhibitors, the prime structural determinant is undoubtedly the diketo acid group, which was already evident in the first "diketo acid" derivative (L-731988) that was described [32] and which subsequently [70] led via L-870810 to raltegravir (MK0518) that has been licensed for clinical use after its clinical efficacy was clearly demonstrated [71, 72]. Next in line is elvitegravir (GS-9137), a quinolone 3-carboxylic acid derivative (which can also be considered a "diketo acid" derivative) (Figure 1.2, INIs). Being also a quinolone derivative, elvitegravir could theoretically act as a transcription inhibitor, but it has been ascertained that elvitegravir, just like L-870810, acts as a genuine INI [73]. Both raltegravir and elvitegravir are assumed to interfere with the strand transfer reaction of the HIV integrase, the mutations Q148K and T66I conferring the highest resistance to both drugs [74].

1.6
Antiviral Drugs Active against Hepatitis B Virus

There are at present seven drugs approved by the US FDA for the treatment of hepatitis B virus: interferon-α-2b (Intron A), lamivudine (3TC), adefovir dipivoxil, entecavir, peginterferon-α-2a (Pegasys), telbivudine, and tenofovir disoproxil fumarate (a few others, that is, clevudine (L-FMAU), emtricitabine ((−)FTC), valtorcitabine (valLdC), amdoxovir (DAPD), and racivir, are still under development). The anti-HBV agents have been reviewed recently [75]. Two of these compounds (lamivudine and tenofovir) are also used for the treatment of HIV infections, and as both HIV and HBV depend for their replication on a virus-associated reverse transcriptase (RT), it is not surprising that some of the RT inhibitors that are active against HIV are also active against HBV, and vice versa. However, there are exceptions; that is, entecavir and telbivudine (Figure 1.3) are specific inhibitors of HBV replication. They both are assumed to interact with the viral DNA polymerase, but how they do so has not been fully explained. Entecavir, if it is incorporated into the viral DNA, has to act as an obligatory chain terminator, but this is not necessarily so for telbivudine since the latter contains a 3′-hydroxyl group, theoretically allowing further chain elongation.

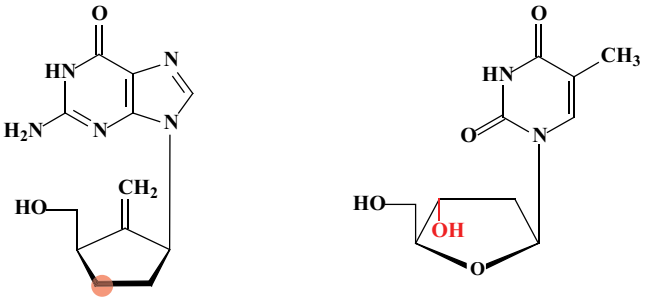


Figure 1.3 Pharmacophores in anti-HBV agents.

1.7
Antiviral Drugs Active against DNA Viruses at Large

For the majority of DNA virus infections there is no specific (formalized) treatment, including polyoma-, papilloma-, and adenovirus, the herpesviruses Epstein–Barr virus (EBV) and human herpesvirus type 6 (HHV-6), and the whole family of poxviridae (including the orthopoxviruses variola, vaccinia, monkeypox, and cowpox), the parapoxviruses (i.e., orf), and molluscviruses (i.e., molluscum contagiosum virus). Cidofovir, which has been formally licensed only for the treatment of CMV retinitis in AIDS patients, could be used "off label" for the treatment of other herpesvirus infections as well as polyoma-, papilloma-, adeno-, and poxvirus infections. The problem with cidofovir and all other acyclic nucleoside phosphonates, however, is that they have poor, if any, oral bioavailability, and to overcome this problem, alkoxyalkyl (i.e.,hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE)) esters of cidofovir have been synthesized with high efficacy in the oral treatment of various (experimental) orthopoxvirus infections in mice [76, 77], as reviewed by Hostetler [78].

The parent compound of the acyclic nucleoside phosphonates is (*S*)-HPMPA (Figure 1.4) that was first described [24] in 1986. Then followed (*S*)-HPMPC [79] and, more recently, (*R*)-HPMPO-DAPy [80] and (*S*)-HPMP-5-azaC [81], and the HDP and ODE prodrugs of (*S*)-HPMP-5-azaC [82]. (*R*)-HPMPO-DAPy (Figure 1.4) proved more effective than postexposure smallpox vaccination in a lethal model of monkeypox virus infection in cynomolgus monkeys [83], and (*S*)-HPMP-5-azaC (Figure 1.4) proved to be a more potent and more selective antiviral agent than cidofovir (*S*)-HPMPC) [84]. The new acyclic nucleoside phosphonates (*R*)-HPMPO-DAPy and (*S*)-HPMP-5-azaC, and alkoxyalkyl esters thereof, offer a wealth of potential applications in the broad field of DNA (pox, adeno, polyoma, papilloma, and herpes) virus infections, which have so far remained largely untapped.

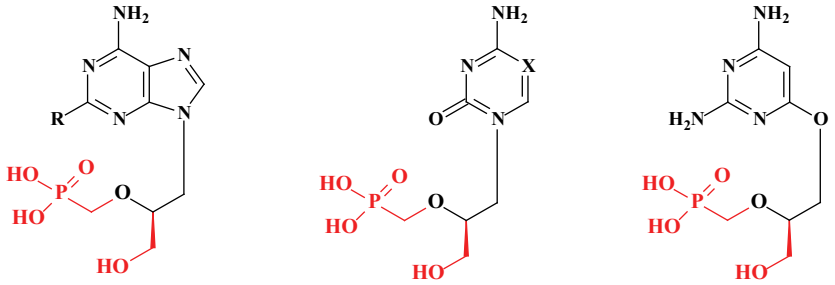


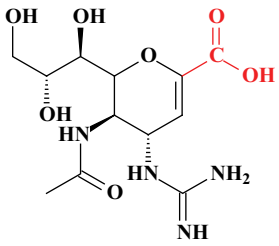
Figure 1.4 Pharmacophores in broad-spectrum anti-DNA virus agents.

1.8
Antiviral Drugs for Influenza A Virus Infections

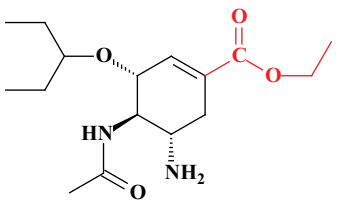
Ever since amantadine was discovered as an inhibitor of influenza A virus replication [85], it has been considered a potential strategy for the therapy and prophylaxis of influenza A virus infections [60], but amantadine has also become notorious for rapidly leading to resistance development, probably a consequence of the specificity of its interaction with the M2 protein of influenza A virus. Various other strategies have been considered in the war against influenza [86], among which are ribavirin, viramidine, siRNAs, and phosphorothioate oligonucleotides, interferon (inducers), and viral RdRp inhibitors [87]. The most fascinating [88, 89] of the RdRp inhibitors is undoubtedly T-705.

At present, the neuraminidase inhibitors are still considered the most likely candidates to be used not only to curtail the annual recurrences of seasonal influenza (A (H1N1), A (H3N2), and influenza B) but also to prevent pandemics with any influenza A virus infection, whether avian (i.e., influenza A H5N1) or any new influenza A (H1N1) strain, such as the recent "Mexican" variant.

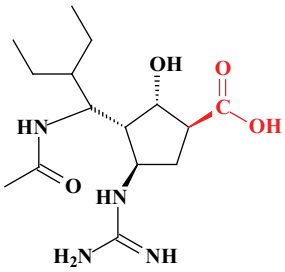
Neuraminidase inhibitors do have a very specific interaction with the viral neuraminidases (sialidase) [86] (Figure 1.5), "trapping" the newly formed virions



Zanamivir
Relenza®



Oseltamivir
Tamiflu®



Peramivir
RWJ-270201

Figure 1.5 Pharmacophores in neuraminidase (sialidase) inhibitors.

at the cell surface, thus preventing the release of these progeny influenza virions from the cells (in which they have been formed) [87]. Unfortunately, influenza A seems to readily develop resistance against neuraminidase inhibitors such as oseltamivir (Tamiflu®) [89]. On the one hand, this points to the specificity of oseltamivir as an antiviral agent and, on the other hand, it argues for a close surveillance of the possible emergence of resistance with the extended use of neuraminidase inhibitors such as oseltamivir.

1.9
Antiviral Drugs for Hepatitis C Virus

Standard care for hepatitis C nowadays consists of the administration of pegylated interferon-α-2a, in combination with ribavirin. Yet, specific anti-HCV agents are under development that are targeted at either the HCV (serine) protease or HCV RdRp. The most advanced among the HCV protease inhibitors are telaprevir [90] and boceprevir [91, 92]. The efficacy of telaprevir, in combination with pegylated interferon and ribavirin, in the treatment of hepatitis C has recently been demonstrated [93, 94]. The first HCV PI to be described and to show antiviral activity in humans was ciluprevir (BILN 2061) [34]. While ciluprevir and a successor thereof, TMC-435350 [95], do have a macrocyclic structure (not shown), telaprevir and boceprevir are built upon a (poly)peptide scaffold.

Like the HIV RT, the HCV RdRp can be targeted at both the catalytic site (by NRRIs) and the allosteric site (by NNRRIs). A characteristic of the anti-HCV activity of NRRIs is the presence of the 2′-*C*-methyl pharmacophore, as in 2′-*C*-methyladenosine, 2′-*C*-methylguanosine, 2′-*C*-methylcytidine, 7-deaza-2′-*C*-methyladenosine, and 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (Figure 1.6, NRRIs). 2-*C*-Methyl-substituted ribonucleosides are active not only against hepaciviruses such as HCV but also against pestiviruses, such as bovine viral diarrhea virus (BVDV), and flaviviruses, such as yellow fever and West Nile [35]. They act as nonobligate chain terminators of the RdRp [35]. The first NRRI to enter the clinic was valopicitabine (NM 283: 3′-valine ester of 2′-*C*-methylcytidine). It was also the first to be discontinued for further development. An exception to the rule that the nucleoside analogues active against HCV should contain a 2′-*C*-methyl group is the 4′-azidocytidine (R1479) [96], and like valopicitabine, this compound has apparently not been further developed.

Resulting from the first wave of NNRRIs were thiophene, 2-carboxylic acid, benzimidazole, and benzothiadiazine derivatives [97], further extended by various other derivatives among which was the benzofuran derivative HCV-796 [98]. The latter proved, in fact, highly active against the HCV replicon system [99], but its further development has apparently been stopped. One of the most potent anti-HCV agents (in development) acting as an NNRRI is GS-327073 (Figure 1.6, NNRRIs). It has an EC_{50} of 0.002–0.004 μM in the HCV (genotype 1b) replicon system. GS-327073 is based upon the 5-[(4-bromophenyl)methyl]-2-phenyl-5*H*-imidazo[4,5-*c*] pyridine BPIP skeleton, which was akin to VP 32947 [33] first identified as a potent

and selective inhibitor of the replication of pestiviruses such as BVDV [100]. BPIP was from the start recognized as an inhibitor of RdRp, the "finger" domain of the enzyme being its target. Further chemical modifications of the BPIP skeleton led to the identification of GS-327073 as a potent and selective NNRRI of the HCV RdRp, again with the "finger" domain being the target site [101].

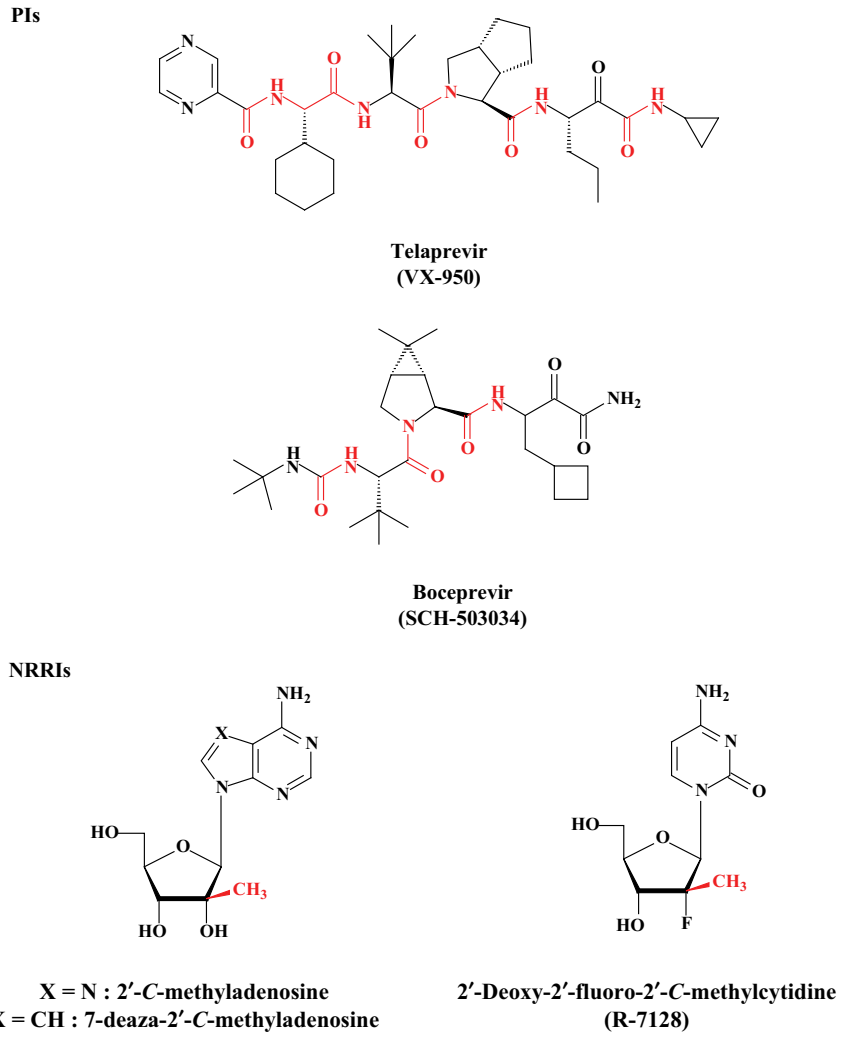
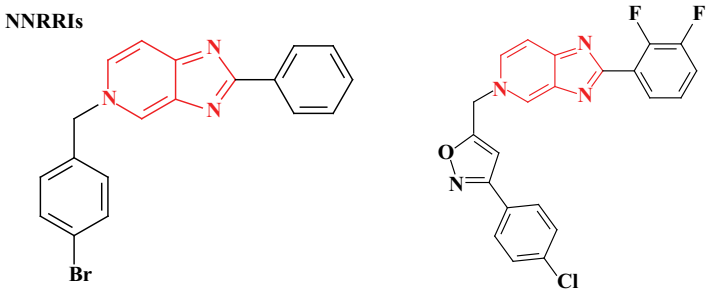


Figure 1.6 Pharmacophores in anti-HCV agents.



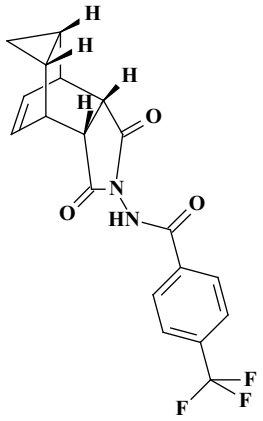
BPIP GS-327073

Figure 1.6 (*Continued*)

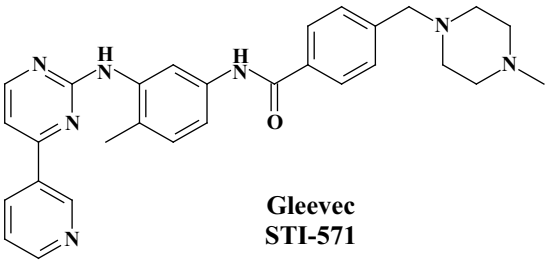
1.10
Antiviral Drugs for Poxviruses (i.e., Variola, Vaccinia, and so on)

Poxviruses (such as variola, vaccinia, cowpox, and monkeypox) are the largest of all viruses and contain the largest set of genes encoding for specific viral proteins that could be considered targets for chemotherapeutic intervention. At present, cidofovir (*S*)-HPMPC) has remained the only drug that could be used, albeit off label, both for the therapy and short-term prophylaxis of smallpox (should it, for example, occur in the context of a bioterrorist attack) and monkeypox and for the treatment of the complications of vaccinia that could arise in immunosuppressed patients inadvertently inoculated with the smallpox vaccine [60, 102]. If needed, cidofovir could be used in its oral prodrug form (i.e.,hexadecylpropyl (HDP)-cidofovir), now known as CMX001. In the mean time, a new compound ST-246 (Figure 1.7) has come along, developed by SIGA Technologies Inc., which appears to inhibit variola virus and other orthopoxvirus infections by inhibiting the F13L phospholipase involved in extracellular virus production [36, 60, 103]. ST-246 acts synergistically with CMX001 [104], which throws open interesting prospects for this drug combination in the treatment of orthopoxvirus infections. Most important would be to know whether ST-246 is efficacious against smallpox, or the complications of smallpox vaccination such as eczema vaccinatum. A recent case of severe eczema vaccinatum in a household contact of a smallpox vaccinee illustrates the importance of the complications of smallpox vaccination and the possible impact ST-246 may have in such case(s) [60, 105].

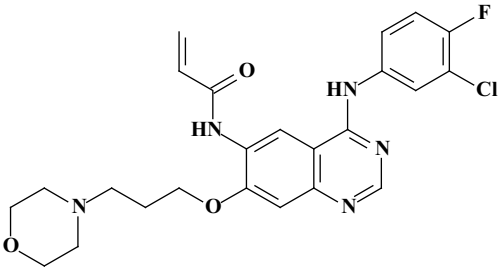
In addition to specifically viral protein-targeted agents (such as ST-246), a number of compounds that interfere with cellular signal transduction, by inhibiting protein tyrosine kinases, such as STI-571 (Gleevec) [37] and 4-anilinoquinazoline CI-1033 [38], have been reported to strongly inhibit poxvirus (i.e., vaccinia virus) infections *in vivo*. These observations point to the options still available to treat poxvirus infections *in vivo*.



ST-246
[4-Trifluoromethyl-*N*-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2-(1*H*-yl)benzamide



Gleevec
STI-571



4-Anilinoquinazoline
CI-1033

Figure 1.7 Inhibitors of poxvirus replication.

1.11
Further Options to Treat Virus Infections

Other, still, investigational strategies for the treatment of HIV and HCV infections have been described recently [98]. The farthest developed in the clinic (phase II) is a nonimmunosuppressive derivative of cyclosporin A, Debio-025 (structure as shown in Ref. [106]), a cyclophilin binding agent that has potent activity against both HCV [107] and HIV-1 [108]. Debio-025 has shown potent anti-HCV activity in patients coinfected with HCV and HIV-1 [109].

For the treatment of poliovirus, and other enterovirus, and rhinovirus infections, a large variety of antipicornavirus agents have been described [106], the last in the series being a protein 3A inhibitor, TTP-8307 [110]. None of these compounds, however, has been developed from a clinical viewpoint. Likewise, increasingly significant attempts have been undertaken to find specific inhibitors for flavivirus (such as dengue virus) and other hemorrhagic fever virus infections [89], and for these virus infections, a "druggable" candidate compound is still eagerly awaited.

While the search for new therapeutic options to treat influenza virus infections has been continuously spurred by the emergence of new virus strains with pandemic "allures," relatively little effort has been made to find or develop new therapeutics for respiratory syncytial virus (RSV) infections. Of significant potential in this regard might be a benzodiazepine, RSV604 (structure as shown in Ref. [111]), which seems to be targeted at the RSV nucleocapsid protein and has proceeded to phase II clinical trials [111].

1.12
Conclusions

In addition to the some 50 antivirals that have been formally approved, now exactly 50 years after the first antiviral drug (IDU) was synthesized, the number of potential antiviral drug candidates is steadily growing [112]. Most of the antiviral drug development efforts have been focused on HIV, followed by HCV and HBV, and influenza virus coming next because of its capriolic incidence. Also, hemorrhagic fever virus (and related encephalitis) infections, because of their global impact, should and have received accrued attention from a therapeutic viewpoint.

Other virus infections, such as herpes simplex and polio, have received relatively little attention because it has been felt they are sufficiently contained by established procedures, acyclovir therapy and vaccination, respectively. The methodology to design new antiviral drug strategies has gradually shifted from "serendipitous" screening to "rational" structure-based drug design, although in most instances this rational approach boiled down to the sheer chemical modification of a known scaffold or building on further from a known pharmacophore.

Surprisingly, the combination drug strategy that has been diligently worked out for HIV, primarily to prevent HIV drug resistance development, has not (yet) been exploited or even explored for other viruses such as HBV, HSV, or influenza. For

HCV, as for HIV, it is believed that it will be necessary to combine different drugs acting by different mechanisms, but before this could be done, the individual drugs have to be identified and approved.

Most of the antiviral drugs now in, or considered for, clinical use are targeted at specific viral events, enzymes (i.e., polymerases, proteases), or processes (i.e., virus–cell fusion). The observation that protein kinase inhibitors such as Gleevec and anilinoquinazolines have antiviral activities (e.g., against poxviruses) should signal a broader applicability of these protein kinase inhibitors. Potential usefulness in the treatment of virus infections may also extend to various other protein kinase inhibitors (such as flavopiridol and rapamycin) [113].

Acknowledgment

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References

1 Prusoff, W.H. (1959) Synthesis and biological activities of iododeoxyuridine, an analog of thymidine. *Biochimica et Biophysica Acta*, **32**, 295–296.

2 Popovic, M., Sarin, P.S., Robert-Gurroff, M., Kalyanaraman, V.S., Mann, D., Minowada, J., and Gallo, R.C. (1983) Isolation and transmission of human retrovirus (human T-cell leukemia virus). *Science*, **219**, 856–859.

3 Barré-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*, **220**, 868–871.

4 Hamre, D., Bernstein, J., and Donovick, R. (1950) Activity of *p*-aminobenzaldehyde, 3-thiosemicarbazone on vaccinia virus in the chick embryo and in the mouse. *Proceedings of the Society for Experimental Biology and Medicine*, **73**, 275–278.

5 Hamre, D., Brownlee, K.A., and Donovick, R. (1951) Studies on the chemotherapy of vaccinia virus. II. The activity of some thiosemicarbazones. *Journal of Immunology (Baltimore, MD: 1950)*, **67**, 305–312.

6 Thompson, R.L., Price, M.L., and Minton, S.A., Jr. (1951) Protection of mice against vaccinia virus by administration of benzaldehyde thiosemicarbazone. *Proceedings of the Society for Experimental Biology and Medicine*, **78**, 11–13.

7 Thompson, R.L., Minton, S.A., Jr., Officer, J.E., and Hitchings, G.H. (1953) Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse. *Journal of Immunology (Baltimore, MD: 1950)*, **70**, 229–234.

8 Thompson, R.L., Davis, J., Russell, P.B., and Hitchings, G.H. (1953) Effect of aliphatic oxime and isatin thiosemicarbazone on vaccinia infection in the mouse and in the rabbit. *Proceedings of the Society for Experimental Biology and Medicine*, **84**, 496–499.

9 Bauer, D.J., Kempe, C.H., and Downie, A.W. (1963) Prophylactic treatment of smallpox contacts with *N*-methylisatin β-thiosemicarbazone. *Lancet*, **ii**, 494–496.

10 Tamm, I., Folkers, K., Shunk, C.H., and Horsfall, F.L., Jr. (1954) Inhibition of influenza virus multiplication by *N*-

glycosides of benzimidazoles. *The Journal of Experimental Medicine*, **99**, 227–250.

11 Tamm, I. and Tyrrell, D.A.J. (1954) Influenza virus multiplication in the chorioallantoic membrane *in vitro*: kinetic aspects of inhibition by 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole. *The Journal of Experimental Medicine*, **100**, 541–562.

12 Tamm, I. and Nemes, M.M. (1959) Selective inhibition of poliovirus multiplication. *The Journal of Clinical Investigation*, **38**, 1047–1048.

13 Eggers, H.J. and Tamm, I. (1962) On the mechanism of selective inhibition of enterovirus multiplication by 2-(α-hydroxybenzyl)-benzimidazole. *Virology*, **18**, 426–438.

14 Eggers, H.J. and Tamm, I. (1963) Inhibition of enterovirus ribonucleic acid synthesis by 2-(α-hydroxybenzyl)-benzimidazole. *Nature*, **197**, 1327–1328.

15 Prusoff, W.H. (1960) Studies on the mechanism of action of 5-iododeoxyuridine, an analog of thymidine. *Cancer Research*, **20**, 92–95.

16 Herrmann, E.C., Jr. (1961) Plaque inhibition test for detection of specific inhibitors of DNA containing viruses. *Proceedings of the Society for Experimental Biology and Medicine*, **107**, 142–145.

17 Kaufman, H.E. (1962) Clinical cure of herpes simplex keratitis by 5-iodo-2′-deoxyuridine. *Proceedings of the Society for Experimental Biology and Medicine*, **109**, 251–252.

18 Kaufman, H.E. and Heidelberger, C. (1964) Therapeutic antiviral action of 5-trifluoromethyl-2′-deoxyuridine in herpes simplex keratitis. *Science*, **145**, 585–586.

19 Sidwell, R.W., Huffman, J.H., Khare, G.P., Allen, L.B., Witkowski, J.T., and Robins, R.K. (1972) Broad-spectrum antiviral activity of virazole: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science*, **177**, 705–706.

20 Manns, M.P., Foster, G.R., Rockstroh, J.K., Zeuzem, S., Zoulim, F., and Houghton, M. (2007) The way forward in HCV treatment: finding the right path. *Nature Reviews Drug Discovery*, **6**, 991–1000.

21 Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L., and Schaeffer, H.J. (1977) Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 5716–5720.

22 Schaeffer, H.J., Beauchamp, L., de Miranda, P., Elion, G.B., Bauer, D.J., and Collins, P. (1978) 9-(2-Hydroxyethoxymethyl) guanine activity against viruses of the herpes group. *Nature*, **272**, 583–585.

23 Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Lehrman, S.N., Gallo, R.C., Bolognesi, D., Barry, D.W., and Broder, S. (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America*, **82**, 7096–7100.

24 De Clercq, E., Holý, A., Rosenberg, I., Sakuma, T., Balzarini, J., and Maudgal, P.C. (1986) A novel selective broad-spectrum anti-DNA virus agent. *Nature*, **323**, 464–467.

25 Baba, M., Tanaka, H., De Clercq, E., Pauwels, R., Balzarini, J., Schols, D., Nakashima, H., Perno, C.-F., Walker, R.T., and Miyasaka, T. (1989) Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. *Biochemical and Biophysical Research Communications*, **165**, 1375–1381.

26 Miyasaka, T., Tanaka, H., Baba, M., Hayakawa, H., Walker, R.T., Balzarini, J., and De Clercq, E. (1989) A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine. *Journal of Medicinal Chemistry*, **32**, 2507–2509.

27 Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E., and Janssen, P.A.J. (1990) Potent and selective inhibition of HIV-1

replication *in vitro* by a novel series of TIBO derivatives. *Nature*, **343**, 470–474.

28 De Clercq, E., Yamamoto, N., Pauwels, R., Baba, M., Schols, D., Nakashima, H., Balzarini, J., Debyser, Z., Murrer, B.A., Schwartz, D., Thornton, D., Bridger, G., Fricker, S., Henson, G., Abrams, M., and Picker, D. (1992) Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 5286–5290.

29 Wild, C., Greenwell, T., and Matthews, T. (1993) A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell–cell fusion. *AIDS Research and Human Retroviruses*, **9**, 1051–1052.

30 von Itzstein, M., Wu, W.Y., Kok, G.B., Pegg, M.S., Dyason, J.C., Jin, B., Van Phan, T., Smythe, M.L., White, H.F., Oliver, S.W., Colman, P.M., Varghese, J.N., Ryan, D.M., Woods, J.M., Bethell, R.C., Hotham, V.J., Cameron, J., and Penn, C.R. (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature*, **363**, 418–423.

31 Spector, F.C., Liang, L., Giordano, H., Sivaraja, M., and Peterson, M.G. (1998) Inhibition of herpes simplex virus replication by a 2-amino thiazole via interactions with the helicase component of the UL5–UL8–UL52 complex. *Journal of Virology*, **72**, 6979–6987.

32 Hazuda, D.J., Felock, P., Witmer, M., Wolfe, A., Stillmock, K., Grobler, J.A., Espeseth, A., Gabryelski, L., Schleif, W., Blau, C., and Miller, M.D. (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science*, **287**, 646–650.

33 Baginski, S.G., Pevear, D.C., Seipel, M., Sun, S.C., Benetatos, C.A., Chunduru, S.K., Rice, C.M., and Collett, M.S. (2000) Mechanism of action of a pestivirus antiviral compound. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7981–7986.

34 Lamarre, D., Anderson, P.C., Bailey, M., Beaulieu, P., Bolger, G., Bonneau, P., Bös, M., Cameron, D.R., Cartier, M., Cordingley, M.G., Faucher, A.M., Goudreau, N., Kawai, S.H., Kukolj, G., Lagacé, L., LaPlante, S.R., Narjes, H., Poupart, M.A., Rancourt, J., Sentjens, R.E., St George, R., Simoneau, B., Steinmann, G., Thibeault, D., Tsantrizos, Y.S., Weldon, S.M., Yong, C.L., and Llinàs-Brunet, M. (2003) An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature*, **426**, 186–189.

35 Migliaccio, G., Tomassini, J.E., Carroll, S.S., Tomei, L., Altamura, S., Bhat, B., Bartholomew, L., Bosserman, M.R., Ceccacci, A., Colwell, L.F., Cortese, R., De Francesco, R., Eldrup, A.B., Getty, K.L., Hou, X.S., LaFemina, R.L., Ludmerer, S.W., MacCoss, M., McMasters, D.R., Stahlhut, M.W., Olsen, D.B., Hazuda, D.J., and Flores, O.A. (2003) Characterization of resistance to non-obligate chain-terminating ribonucleoside analogs that inhibit hepatitis C virus replication *in vitro*. *The Journal of Biological Chemistry*, **278**, 49164–49170.

36 Yang, G., Pevear, D.C., Davies, M.H., Collett, M.S., Bailey, T., Rippen, S., Barone, L., Burns, C., Rhodes, G., Tohan, S., Huggins, J.W., Baker, R.O., Buller, R.L., Touchette, E., Waller, K., Schriewer, J., Neyts, J., De Clercq, E., Jones, K., Hruby, D., and Jordan, R. (2005) An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge. *Journal of Virology*, **79**, 13139–13149.

37 Reeves, P.M., Bommarious, B., Lebeis, S., McNulty, S., Christensen, J., Swimm, A., Chahroudi, A., Chavan, R., Feinberg, M.B., Veach, D., Bornmann, W., Sherman, M., and Kalman, D. (2005) Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. *Nature Medicine*, **11**, 731–739.

38 Yang, H., Kim, S.K., Kim, M., Reche, P.A., Morehead, T.J., Damon, I.K., Welsh, R.M., and Reinherz, E.L. (2005) Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction. *The Journal of Clinical Investigation*, **115**, 379–387.

39 De Clercq, E. (1980) Antiviral and antitumor activities of 5-substituted 2′-

deoxyuridines. *Methods and Findings in Experimental and Clinical Pharmacology*, **2**, 253–267.

40 De Clercq, E., Descamps, J., De Somer, P., Barr, P.J., Jones, A.S., and Walker, R.T. (1979) (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine: a potent and selective anti-herpes agent. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 2947–2951.

41 De Clercq, E. (2004) Discovery and development of BVDU (brivudin) as a therapeutic for the treatment of herpes zoster. *Biochemical Pharmacology*, **68**, 2301–2315.

42 McGuigan, C., Barucki, H., Carangio, A., Blewett, S., Andrei, G., Snoeck, R., De Clercq, E., Balzarini, J., and Erichsen, J.T. (2000) Highly potent and selective inhibition of varicella-zoster virus by bicyclic furopyrimidine nucleosides bearing an aryl side chain. *Journal of Medicinal Chemistry*, **43**, 4993–4997.

43 De Clercq, E. (2003) Highly potent and selective inhibition of varicella-zoster virus replication by bicyclic furo[2,3-*d*] pyrimidine nucleoside analogues. *Medicinal Research Reviews*, **23**, 253–274.

44 Streeter, D.G., Witkowski, J.T., Khare, G.P., Sidwell, R.W., Bauer, R.J., Robins, R.K., and Simon, L.N. (1973) Mechanism of action of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proceedings of the National Academy of Sciences of the United States of America*, **70**, 1174–1178.

45 Leyssen, P., Balzarini, J., De Clercq, E., and Neyts, J. (2005) The predominant mechanism by which ribavirin exerts its antiviral activity *in vitro* against flaviviruses and paramyxoviruses is mediated by inhibition of IMP dehydrogenase. *Journal of Virology*, **79**, 1943–1947.

46 Leyssen, P., De Clercq, E., and Neyts, J. (2008) Molecular strategies to inhibit the replication of RNA viruses. *Antiviral Research*, **78**, 9–25.

47 Biswas, S. and Field, H.J. (2008) Herpes simplex virus helicase–primase inhibitors: recent findings from the study of drug resistance mutations. *Antiviral Chemistry & Chemotherapy*, **19**, 1–6.

48 Crute, J.J., Grygon, C.A., Hargrave, K.D., Simoneau, B., Faucher, A.M., Bolger, G., Kibler, P., Liuzzi, M., and Cordingley, M.G. (2002) Herpes simplex virus helicase–primase inhibitors are active in animal models of human disease. *Nature Medicine*, **8**, 386–391.

49 Kleymann, G., Fischer, R., Betz, U.A., Hendrix, M., Bender, W., Schneider, U., Handke, G., Eckenberg, P., Hewlett, G., Pevzner, V., Baumeister, J., Weber, O., Henninger, K., Keldenich, J., Jensen, A., Kolb, J., Bach, U., Popp, A., Mäben, J., Frappa, I., Haebich, D., Lockhoff, O., and Rübsamen-Waigmann, H. (2002) New helicase–primase inhibitors as drug candidates for the treatment of herpes simplex disease. *Nature Medicine*, **8**, 392–398.

50 Betz, U.A., Fischer, R., Kleymann, G., Hendrix, M., and Rübsamen-Waigmann, H. (2002) Potent *in vivo* antiviral activity of the herpes simplex virus primase–helicase inhibitor BAY 57–1293. *Antimicrobial Agents and Chemotherapy*, **46**, 1766–1772.

51 Biswas, S., Jennens, L., and Field, H.J. (2007) The helicase primase inhibitor, BAY 57-1293 shows potent therapeutic antiviral activity superior to famciclovir in BALB/c mice infected with herpes simplex virus type 1. *Antiviral Research*, **75**, 30–35.

52 Biswas, S., Smith, C., and Field, H.J. (2007) Detection of HSV-1 variants highly resistant to the helicase–primase inhibitor BAY 57-1293 at high frequency in 2 of 10 recent clinical isolates of HSV-1. *The Journal of Antimicrobial Chemotherapy*, **60**, 274–279.

53 Biswas, S., Kleymann, G., Swift, M., Tiley, L.S., Lyall, J., Aguirre-Hernández, J., and Field, H.J. (2008) A single drug-resistance mutation in HSV-1 UL52 primase points to a difference between two helicase–primase inhibitors in their mode of interaction with the antiviral target. *The Journal of Antimicrobial Chemotherapy*, **61**, 1044–1047.

54 De Clercq, E. (2009) Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. *International Journal of Antimicrobial Agents*, **33**, 307–320.

55 Soudeyns, H., Yao, X.I., Gao, Q., Belleau, B., Kraus, J.L., Nguyen-Ba, N., Spira, B., and Wainberg, M.A. (1991) Anti-human immunodeficiency virus type 1 activity and *in vitro* toxicity of 2′-deoxy-3′-thiacytidine (BCH-189), a novel heterocyclic nucleoside analog. *Antimicrobial Agents and Chemotherapy*, **35**, 1386–1390.

56 Daluge, S.M., Good, S.S., Faletto, M.B., Miller, W.H., St. Clair, M.H., Boone, L.R., Tisdale, M., Parry, N.R., Reardon, J.E., Dornsife, R.E., Averett, D.R., and Krenitsky, T.A. (1997) 1592U89, a novel carbocyclic nucleoside analog with potent, selective anti-human immunodeficiency virus activity. *Antimicrobial Agents and Chemotherapy*, **41**, 1082–1093.

57 Hoong, L.K., Strange, L.E., Liotta, D.C., Koszalka, G.W., Burns, C.L., and Schinazi, R.F. (1992) Enzyme-mediated enantioselective preparation of pure enantiomers of the antiviral agent 2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC) and related compounds. *The Journal of Organic Chemistry*, **57**, 5563–5565.

58 Schinazi, R.F., Boudinot, F.D., Ibrahim, S.S., Manning, C., McClure, H.M., and Liotta, D.C. (1992) Pharmacokinetics and metabolism of racemic 2′,3′-dideoxy-5-fluoro-3′-thiacytidine in rhesus monkeys. *Antimicrobial Agents and Chemotherapy*, **36**, 2432–2438.

59 De Clercq, E. and Holý, A. (2005) Acyclic nucleoside phosphonates: a key class of antiviral drugs. *Nature Reviews Drug Discovery*, **4**, 928–940.

60 De Clercq, E. (2009) Another ten stories in antiviral drug discovery (partC): "old" and "new" antivirals, strategies, and perspectives. *Medicinal Research Reviews*, **29**, 611–645.

61 De Clercq, E. (2004) Non-nucleoside reverse transcriptase inhibitors (NNRTIs): past, present and future. *Chemistry Biodiversity*, **1**, 44–64.

62 Ding, J., Das, K., Moereels, H., Koymans, L., Andries, K., Janssen, P.A., Hughes, S.H., and Arnold, E. (1995) Structure of HIV-1 RT/TIBO R 86183 complex reveals similarity in the binding of diverse nonnucleoside inhibitors. *Nature Structural Biology*, **2**, 407–415.

63 Chimirri, A., Grasso, S., Monforte, A.M., Monforte, P., and Zappala, M. (1995) Thiazobenzimidazoles as non-nucleoside HIV-1 RT inhibitors. Abstracts of the II Congresso Congiunto Italiano-Spagnolo di Chimica Farmaceutica/II Congreso Conjunto Italiano-Espanol de Quimica Terapeutica, Ferrara, Italy, 30 August–3 September 1995, ML20.

64 Matthews, T., Salgo, M., Greenberg, M., Chung, J., DeMasi, R., and Bolognesi, D. (2004) Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes. *Nature Reviews Drug Discovery*, **3**, 215–225.

65 Kilby, J.M., Hopkins, S., Venetta, T.M., DiMassimo, B., Cloud, G.A., Lee, J.Y., Alldredge, L., Hunter, E., Lambert, D., Bolognesi, D., Matthews, T., Johnson, M.R., Nowak, M.A., Shaw, G.M., and Saag, M.S. (1998) Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nature Medicine*, **4**, 1302–1307.

66 Lalezari, J.P., Henry, K., O'Hearn, M., Montaner, J.S., Piliero, P.J., Trottier, B., Walmsley, S., Cohen, C., Kuritzkes, D.R., Eron J.J., Jr., Chung, J., DeMasi, R., Donatacci, L., Drobnes, C., Delehanty, J., and Salgo, M. (2003) Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *The New England Journal of Medicine*, **348**, 2175–2185.

67 Lazzarin, A., Clotet, B., Cooper, D., Reynes, J., Arastéh, K., Nelson, M., Katlama, C., Stellbrink, H.J., Delfraissy, J.F., Lange, J., Huson, L., DeMasi, R., Wat, C., Delehanty, J., Drobnes, C., and Salgo, M. (2003) Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *The New England Journal of Medicine*, **348**, 2186–2195.

68 De Clercq, E. (2009) The AMD3100 story: the path to the discovery of a stem cell mobilizer (Mozobil). *Biochemical Pharmacology*, **77**, 1655–1664.

69 Westby, M. and van der Ryst, E. (2005) CCR5 antagonists: host-targeted antivirals for the treatment of HIV infection. *Antiviral Chemistry & Chemotherapy*, **16**, 339–354.

70 Hazuda, D.J., Young, S.D., Guare, J.P., Anthony, N.J., Gomez, R.P., Wai, J.S., Vacca, J.P., Handt, L., Motzel, S.L., Klein, H.J., Dornadula, G., Danovich, R.M., Witmer, M.V., Wilson, K.A., Tussey, L., Schleif, W.A., Gabryelski, L.S., Jin, L., Miller, M.D., Casimiro, D.R., Emini, E.A., and Shiver, J.W. (2004) Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science*, **305**, 528–532.

71 Steigbigel, R.T., Cooper, D.A., Kumar, P.N., Eron, J.E., Schechter, M., Markowitz, M., Loutfy, M.R., Lennox, J.L., Gatell, J.M., Rockstroh, J.K., Katlama, C., Yeni, P., Lazzarin, A., Clotet, B., Zhao, J., Chen, J., Ryan, D.M., Rhodes, R.R., Killar, J.A., Gilde, L.R., Strohmaier, K.M., Meibohm, A.R., Miller, M.D., Hazuda, D.J., Nessly, M.L., DiNubile, M.J., Isaacs, R.D., Nguyen, B.Y., and Teppler, H. (2008) Raltegravir with optimized background therapy for resistant HIV-1 infection. *The New England Journal of Medicine*, **359**, 339–354.

72 Cooper, D.A., Steigbigel, R.T., Gatell, J.M., Rockstroh, J.K., Katlama, C., Yeni, P., Lazzarin, A., Clotet, B., Kumar, P.N., Eron, J.E., Schechter, M., Markowitz, M., Loutfy, M.R., Lennox, J.L., Zhao, J., Chen, J., Ryan, D.M., Rhodes, R.R., Killar, J.A., Gilde, L.R., Strohmaier, K.M., Meibohm, A.R., Miller, M.D., Hazuda, D.J., Nessly, M.L., DiNubile, M.J., Isaacs, R.D., Teppler, H., and Nguyen, B.Y. (2008) Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *The New England Journal of Medicine*, **359**, 355–365.

73 Daelemans, D., Lu, R., De Clercq, E., and Engelman, A. (2007) Characterization of a replication-competent, integrase-defective human immunodeficiency virus (HIV)/simian virus 40 chimera as a powerful tool for the discovery and validation of HIV integrase inhibitors. *Journal of Virology*, **81**, 4381–4385.

74 Marinello, J., Marchand, C., Mott, B.T., Bain, A., Thomas, C.J., and Pommier, Y. (2008) Comparison of raltegravir and elvitegravir on HIV-1 integrase catalytic reactions and on a series of drug-resistant integrase mutants. *Biochemistry*, **47**, 9345–9354.

75 Férir, G., Kaptein, S., Neyts, J., and De Clercq, E. (2008) Antiviral treatment of chronic hepatitis B virus infections: the past, the present and the future. *Reviews in Medical Virology*, **18**, 19–34.

76 Quenelle, D.C., Collins, D.J., Herrod, B.P., Keith, K.A., Trahan, J., Beadle, J.R., Hostetler, K.Y., and Kern, E.R. (2007) Effect of oral treatment with hexadecyloxypropyl-[(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] [(*S*)-HPMPA] or octadecyloxy-ethyl-(*S*)-HPMPA on cowpox or vaccinia virus infections in mice. *Antimicrobial Agents and Chemotherapy*, **51**, 3940–3947.

77 Beadle, J.R., Wan, W.B., Ciesla, S.L., Keith, K.A., Hartline, C., Kern, E.R., and Hostetler, K.Y. (2006) Synthesis and antiviral evaluation of alkoxyalkyl derivatives of 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine against cytomegalovirus and orthopoxviruses. *Journal of Medicinal Chemistry*, **49**, 2010–2015.

78 Hostetler, K.Y. (2009) Alkoxyalkyl prodrugs of acyclic nucleoside phosphonates enhance oral antiviral activity and reduce toxicity: current state of the art. *Antiviral Research*, **82**, A84–A88.

79 De Clercq, E., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I., and Holý, A. (1987) Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. *Antiviral Research*, **8**, 261–272.

80 Holý, A., Votruba, I., Masojídková, M., Andrei, G., Snoeck, R., Naesens, L., De Clercq, E., and Balzarini, J. (2002) 6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with antiviral activity. *Journal of Medicinal Chemistry*, **45**, 1918–1929.

81 Krečmerová, M., Holý, A., Piskala, A., Masojídková, M., Andrei, G., Naesens, L., Neyts, J., Balzarini, J., De Clercq, E., and Snoeck, R. (2007) Antiviral activity of triazine analogues of 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (cidofovir) and related compounds. *Journal of Medicinal Chemistry*, **50**, 1069–1077.

82 Krečmerová, M., Holý, A., Pohl, R., Masojídková, M., Andrei, G., Naesens, L., Neyts, J., Balzarini, J., De Clercq, E., and

Snoeck, R. (2007) Ester prodrugs of cyclic 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy) propyl]-5-azacytosine: synthesis and antiviral activity. *Journal of Medicinal Chemistry*, **50**, 5765–5772.

83 Stittelaar, K.J., Neyts, J., Naesens, L., van Amerongen, G., van Lavieren, R.F., Holý, A., De Clercq, E., Niesters, H.G., Fries, E., Maas, C., Mulder, P.G., van der Zeijst, B.A., and Osterhaus, A.D. (2006) Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. *Nature*, **439**, 745–748.

84 Naesens, L., Andrei, G., Votruba, I., Krecmerová, M., Holý, A., Neyts, J., De Clercq, E., and Snoeck, R. (2008) Intracellular metabolism of the new antiviral compound 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine. *Biochemical Pharmacology*, **76**, 997–1005.

85 Davies, W.L., Grunert, R.R., Haff, R.F., McGahen, J.W., Neumayer, E.M., Paulshock, M., Watts, J.C., Wood, T.R., Hermann, E.C., and Hoffmann, C.E. (1964) Antiviral activity of 1-adamantanamine (amantadine). *Science*, **144**, 862–863.

86 von Itzstein, M. (2007) The war against influenza: discovery and development of sialidase inhibitors. *Nature Reviews Drug Discovery*, **6**, 967–974.

87 De Clercq, E. (2006) Antiviral agents active against influenza A viruses. *Nature Reviews Drug Discovery*, **5**, 1015–1025.

88 Furuta, Y., Takahashi, K., Kuno-Maekawa, M., Sangawa, H., Uehara, S., Kozaki, K., Nomura, N., Egawa, H., and Shiraki, K. (2005) Mechanism of action of T-705 against influenza virus. *Antimicrobial Agents and Chemotherapy*, **49**, 981–986.

89 De Clercq, E. (2010) Yet another ten stories on antiviral drug discovery (part D): paradigms, paradoxes and paraductions. *Medicinal Research Reviews*, **30** (4), 667–707.

90 Lin, C., Lin, K., Luong, Y.P., Rao, B.G., Wei, Y.Y., Brennan, D.L., Fulghum, J.R., Hsiao, H.M., Ma, S., Maxwell, J.P., Cottrell, K.M., Perni, R.B., Gates, C.A., and Kwong, A.D. (2004) *In vitro* resistance studies of hepatitis C virus serine protease inhibitors, VX-950 and BILN 2061: structural analysis indicates different resistance mechanisms. *The Journal of Biological Chemistry*, **279**, 17508–17514.

91 Venkatraman, S., Bogen, S.L., Arasappan, A., Bennett, F., Chen, K., Jao, E., Liu, Y.T., Lovey, R., Hendrata, S., Huang, Y., Pan, W., Parekh, T., Pinto, P., Popov, V., Pike, R., Ruan, S., Santhanam, B., Vibulbhan, B., Wu, W., Yang, W., Kong, J., Liang, X., Wong, J., Liu, R., Butkiewicz, N., Chase, R., Hart, A., Agrawal, S., Ingravallo, P., Pichardo, J., Kong, R., Baroudy, B., Malcolm, B., Guo, Z., Prongay, A., Madison, V., Broske, L., Cui, X., Cheng, K.C., Hsieh, Y., Brisson, J.M., Prelusky, D., Korfmacher, W., White, R., Bogdanowich-Knipp, S., Pavlovsky, A., Bradley, P., Saksena, A.K., Ganguly, A., Piwinski, J., Girijavallabhan, V., and Njoroge, F.G. (2006) Discovery of (1*R*,5*S*)-*N*-[3-amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[(1,1-dimethylethyl)amino] carbonyl]-amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-aza-bicyclo [3.1.0]hexan-2(*S*)-carboxamide (SCH 503034), a selective, potent, orally bioavailable hepatitis C virus NS3 protease inhibitor: a potential therapeutic agent for the treatment of hepatitis C infection. *Journal of Medicinal Chemistry*, **49**, 6074–6086.

92 Malcolm, B.A., Liu, R., Lahser, F., Agrawal, S., Belanger, B., Butkiewicz, N., Chase, R., Gheyas, F., Hart, A., Hesk, D., Ingravallo, P., Jiang, C., Kong, R., Lu, J., Pichardo, J., Prongay, A., Skelton, A., Tong, X., Venkatraman, S., Xia, E., Girijavallabhan, V., and Njoroge, F.G. (2006) SCH 503034, a mechanism-based inhibitor of hepatitis C virus NS3 protease, suppresses polyprotein maturation and enhances the antiviral activity of alpha interferon in replicon cells. *Antimicrobial Agents and Chemotherapy*, **50**, 1013–1020.

93 McHutchison, J.G., Everson, G.T., Gordon, S.C., Jacobson, I.M., Sulkowski, M., Kauffman, R., McNair, L., Alam, J., and Muir, A.J. (2009) Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *The New*

England Journal of Medicine, **360**, 1827–1838.

94 Hézode, C., Forestier, N., Dusheiko, G., Ferenci, P., Pol, S., Goeser, T., Bronowicki, J.P., Bourlière, M., Gharakhanian, S., Bengtsson, L., McNair, L., George, S., Kieffer, T., Kwong, A., Kauffman, R.S., Alam, J., Pawlotsky, J.M., and Zeuzem, S. (2009) Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *The New England Journal of Medicine*, **360** 1839–1850.

95 Raboisson, P., de Kock, H., Rosenquist, A., Nilsson, M., Salvador-Oden, L., Lin, T.I., Roue, N., Ivanov, V., Wähling, H., Wickström, K., Hamelink, E., Edlund, M., Vrang, L., Vendeville, S., Van de Vreken, W., McGowan, D., Tahri, A., Hu, L., Boutton, C., Lenz, O., Delouvroy, F., Pille, G., Surleraux, D., Wigerinck, P., Samuelsson, B., and Simmen, K. (2008) Structure–activity relationship study on a novel series of cyclopentane-containing macrocyclic inhibitors of the hepatitis C virus NS3/4A protease leading to the discovery of TMC435350. *Bioorganic & Medicinal Chemistry Letters*, **18**, 4853–4858.

96 Klumpp, K., Lévêque, V., Le Pogam, S., Ma, H., Jiang, W.R., Kang, H., Granycome, C., Singer, M., Laxton, C., Hang, J.Q., Sarma, K., Smith, D.B., Heindl, D., Hobbs, C.J., Merrett, J.H., Symons, J., Cammack, N., Martin, J.A., Devos, R., and Nájera, I. (2006) The novel nucleoside analog R1479 (4′-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture. *The Journal of Biological Chemistry*, **281**, 3793–3799.

97 De Francesco, R. and Migliaccio, G. (2005) Challenges and successes in developing new therapies for hepatitis C. *Nature*, **436**, 953–960.

98 De Clercq, E. (2007) The design of drugs for HIV and HCV. *Nature Reviews Drug Discovery*, **6**, 1001–1018.

99 Paeshuyse, J., Vliegen, I., Coelmont, L., Leyssen, P., Tabarrini, O., Herdewijn, P., Mittendorfer, H., Easmon, J., Cecchetti, V., Bartenschlager, R., Puerstinger, G., and Neyts, J. (2008) Comparative *in vitro* anti-hepatitis C virus activities of a selected series of polymerase, protease, and helicase inhibitors. *Antimicrobial Agents and Chemotherapy*, **52**, 3433–3437.

100 Paeshuyse, J., Leyssen, P., Mabery, E., Boddeker, N., Vrancken, R., Froeyen, M., Ansari, I.H., Dutartre, H., Rozenski, J., Gil, L.H., Letellier, C., Lanford, R., Canard, B., Koenen, F., Kerkhofs, P., Donis, R.O., Herdewijn, P., Watson, J., De Clercq, E., Puerstinger, G., and Neyts, J. (2006) A novel, highly selective inhibitor of pestivirus replication that targets the viral RNA-dependent RNA polymerase. *Journal of Virology*, **80**, 149–160.

101 Vliegen, I., Paeshuyse, J., De Burghgraeve, T., Lehman, L.S., Paulson, M., Shih, I.H., Mabery, E., Boddeker, N., De Clercq, E., Reiser, H., Oare, D., Lee, W.A., Zhong, W., Bondy, S., Pürstinger, G., and Neyts, J. (2009) Substituted imidazopyridines as potent inhibitors of HCV replication. *Journal of Hepatology*, **50**, 999–1009.

102 De Clercq, E. (2002) Cidofovir in the treatment of poxvirus infections. *Antiviral Research*, **55**, 1–13.

103 Bailey, T.R., Rippin, S.R., Opsitnick, E., Burns, C.J., Pevear, D.C., Collett, M.S., Rhodes, G., Tohan, S., Huggins, J.W., Baker, R.O., Kern, E.R., Keith, K.A., Dai, D., Yang, G., Hruby, D., and Jordan, R. (2007) N-(3,3a,4,4a,5,5a,6,6a-Octahydro-1,3-dioxo-4,6-ethenocycloprop[*f*] isoindol-2-(1*H*)-yl)carboxamides: identification of novel orthopoxvirus egress inhibitors. *Journal of Medicinal Chemistry*, **50**, 1442–1444.

104 Quenelle, D.C., Prichard, M.N., Keith, K.A., Hruby, D.E., Jordan, R., Painter, G.R., Robertson, A., and Kern, E.R. (2007) Synergistic efficacy of the combination of ST-246 with CMX001 against orthopoxviruses. *Antimicrobial Agents and Chemotherapy*, **51**, 4118–4124.

105 Vora, S., Damon, I., Fulginiti, V., Weber, S.G., Kahana, M., Stein, S.L., Gerber, S.I., Garcia-Houchins, S., Lederman, E., Hruby, D., Collins, L., Scott, D., Thompson, K., Barson, J.V., Regnery, R., Hughes, C., Daum, R.S., Li, Y., Zhao, H., Smith, S., Braden, Z., Karem, K., Olson, V., Davidson, W., Trindade, G., Bolken, T.,

Jordan, R., Tien, D., and Marcinak, J. (2008) Severe eczema vaccinatum in a household contact of a smallpox vaccinee. *Clinical Infectious Diseases*, **46**, 1555–1561.

106 De Clercq, E. (2009) Antiviral drug discovery: ten more compounds, and ten more stories (part B). *Medicinal Research Reviews*, **29**, 571–610.

107 Paeshuyse, J., Kaul, A., De Clercq, E., Rosenwirth, B., Dumont, J.-M., Scalfaro, P., Bartenschlager, R., and Neyts, J. (2006) The non-immunosuppressive cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication *in vitro*. *Hepatology (Baltimore, MD)*, **43**, 761–770.

108 Ptak, R.G., Gallay, P.A., Jochmans, D., Halestrap, A.P., Ruegg, U.T., Pallansch, L.A., Bobardt, M.D., de Béthune, M.-P., Neyts, J., De Clercq, E., Dumont, J.-M., Scalfaro, P., Besseghir, K., Wenger, R.M., and Rosenwirth, B. (2008) Inhibition of human immunodeficiency virus type 1 replication in human cells by Debio-025, a novel cyclophilin binding agent. *Antimicrobial Agents and Chemotherapy*, **52**, 1302–1317.

109 Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S., Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., Aeschlimann, C., Grosgurin, P., Nicolas-Métral, V., Dumont, J.-M., Porchet, H., Crabbé, R., and Scalfaro, P. (2008) The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. *Hepatology (Baltimore, MD)*, **47**, 817–826.

110 De Palma, A.M., Thibaut, H.J., van der Linden, L., Lanke, K., Heggermont, W., Ireland, S., Andrews, R., Arimilli, M., Al-Tel, T.H., De Clercq, E., van Kuppeveld, F., and Neyts, J. (2009) Mutations in the nonstructural protein 3A confer resistance to the novel enterovirus replication inhibitor TTP-8307. *Antimicrobial Agents and Chemotherapy*, **53**, 1850–1857.

111 Chapman, J., Abbott, E., Alber, D.G., Baxter, R.C., Bithell, S.K., Henderson, E.A., Carter, M.C., Chambers, P., Chubb, A., Cockerill, G.S., Collins, P.L., Dowdell, V.C., Keegan, S.J., Kelsey, R.D., Lockyer, M.J., Luongo, C., Najarro, P., Pickles, R.J., Simmonds, M., Taylor, D., Tyms, S., Wilson, L.J., and Powell, K.L. (2007) RSV604, a novel inhibitor of respiratory syncytial virus replication. *Antimicrobial Agents and Chemotherapy*, **51**, 3346–3353.

112 De Clercq, E. (2008) Emerging antiviral drugs. *Expert Opinion on Emerging Drugs*, **13**, 393–416.

113 Cohen, P. (2002) Protein kinases: the major drug targets of the twenty-first century? *Nature Reviews Drug Discovery*, **1**, 309–315.