

Highlights in Antiviral Drug Research: Antivirals at the Horizon

Erik De Clercq

Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000, Leuven, Belgium

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Abstract: This review highlights ten “hot topics” in current antiviral research: (i) new nucleoside derivatives (i.e., PSI-352938) showing high potential as a direct antiviral against hepatitis C virus (HCV); (ii) cyclopropavir, which should be further pursued for treatment of human cytomegalovirus (HCMV) infections; (iii) *North*-methanocarbothymidine (*N*-MCT), with a *N*-locked conformation, showing promising activity against both α - and γ -herpesviruses; (iv) CMX001, an orally bioavailable prodrug of cidofovir with broad-spectrum activity against DNA viruses, including polyoma, adeno, herpes, and pox; (v) favipiravir, which is primarily pursued for the treatment of influenza virus infections, but also inhibits the replication of other RNA viruses, particularly (-)RNA viruses such as arena, bunya, and hanta; (vi) newly emerging antiarenaviral compounds which should be more effective (and less toxic) than the ubiquitously used ribavirin; (vii) antipicornavirus agents in clinical development (pleconaril, BTA-798, and V-073); (viii) natural products receiving increased attention as potential antiviral drugs; (ix) antivirals such as U0126 targeted at specific cellular kinase pathways [i.e., mitogen extracellular kinase (MEK)], showing activity against influenza and other viruses; and (x) two structurally unrelated compounds (i.e., LJ-001 and dUY11) with broad-spectrum activity against virtually all enveloped RNA and DNA viruses. © 2012 Wiley Periodicals, Inc. *Med. Res. Rev.*, 33, No. 6, 1215–1248, 2013

Key words: PSI-352938; cyclopropavir; *N*-MCT; CMX001; favipiravir; antiarenaviral; antipicornaviral; U0126; LJ-001; dUY11; natural products

1. INTRODUCTION

The search for new antivirals has proceeded unabatedly. In previous reviews on “stories on antiviral drug discovery,” I have reviewed various subjects in which I was personally involved.^{1–5} In this review, hopefully the first of a new series, I will address “hot topics” in areas of antiviral research in which over the past few years significant progress has been made requiring due attention. Most of the compounds covered are still in the preclinical stage. Compounds that have successfully completed phase II and III clinical trials and progressing to approval (or have already been approved) are not subject of this review. Instead, this article is based on

Correspondence to: Erik De Clercq, Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. E-mail: erik.declercq@rega.kuleuven.ac.be

compounds in the pipeline that offer attractive perspectives as future antiviral drugs. In this sense, the present survey is a little arbitrary in the selection of the topics, which depends at least in part on my own prejudices and a number of other factors, not at least my own acquaintance with the subject.

An important impetus for initiating this review was based on the 24th ICAR Abstract Issue (Antiviral Research, vol. 90: A21–A78, 2011) covering the presentations at the 24th ICAR (International Conference on Antiviral Research), held in Sofia (Bulgaria) on May 8–11, 2011. Unfortunately, I could not attend the Conference, but guided by a certain devotion, I went through the Abstract Issue and spotted a number of interesting leads which I would like to reflect on in the present article: (i) nucleoside analogues, targeted at hepatitis C virus (HCV) NS5B RNA polymerase, such as PSI-352938;⁶ (ii) cyclopropavir for the treatment of human cytomegalovirus (HCMV) infections;⁷ (iii) *North*-methanocarbathymidine (*N*-MCT) for the treatment of α -herpesvirus [herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2)] and γ -herpesvirus [Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) infection];⁸ (iv) CMX001 (1-*O*-hexadecyloxypropyl cidofovir) for the treatment of a broad variety of DNA virus infections;⁹ (v) favipiravir (T-705) for the treatment of influenza virus infections and various other RNA virus infections;¹⁰ (vi) the increasing number of compounds found effective against arenaviruses;^{10,11} (vii) new picornavirus (i.e., rhinovirus) inhibitors;^{12,13} (viii) natural products with antiviral activity such as aglycoristocetin derivatives¹⁴ and tricin (4',5,7-trihydroxy-3',5'-dimethoxyflavone);¹⁵ (ix) mitogen extracellular kinase (MEK) inhibitors, such as U0126, acting at a cellular target, and effective against influenza and other viruses;¹⁶ and (x) two unique sets of compounds which should be effective against virtually all enveloped DNA and RNA viruses.¹⁷

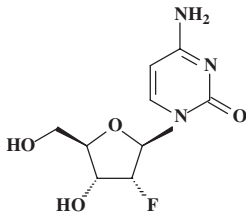
Taken together, compounds (i) through (x) cover all major viral pathogens: polyoma-, adeno-, herpes-, pox-, picorna-, flavi-, arena-, myxo-, bunya-, and retroviruses, and particularly HSV, HCMV, hemorrhagic fever, influenza, and human immunodeficiency virus (HIV).

2. NUCLEOSIDE ANALOGUES TARGETED AT THE HCV NS5B POLYMERASE

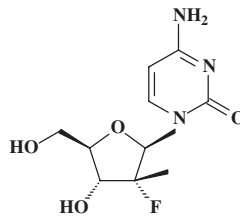
2'-Deoxy-2'-fluorocytidine (FdC) could be considered as the first nucleoside analogue (Fig. 1) found to inhibit the hepatitis C virus (HCV) replicon in cell culture, its 5'-triphosphate inhibiting the NS5B polymerase.¹⁸ Introduction of a methyl group at the C-2' position, as in β -D-2'-fluoro-2'-*C*-methylcytidine (Fig. 1), conferred a similar potency as FdC in the HCV replicon assay.¹⁹ Although the 2'-*C*-methylcytidine (Fig. 1) demonstrated somewhat lesser activity against HCV than 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine,¹⁹ the prodrug of 2'-*C*-methylcytidine, its 3'-*O*-valine ester, NM283 (valopicitabine) (Fig. 1) was developed (but later dropped) for phase II clinical trials.²⁰

Introduction of a 4'-azido group (Fig. 1), as in R1479,^{21,22} RO-9187, RO-0622,²³ 4'-azido-2'-deoxy-2',2'-difluorocytidine, and 4'-azido-2'-deoxy-2'-fluoroarabincytidine significantly increased the potency in the HCV replicon system [i.e., with 50% effective concentration (EC₅₀) values of 66 and 24 nM for the latter two compounds].²⁴ The 2',3',5'-triisobutyrate ester prodrug of 4'-azido-2'-*C*-methylcytidine (RG1626) (Fig. 1) has been (but is no longer) in development as inhibitor of HCV, according to Reddy et al.²⁵

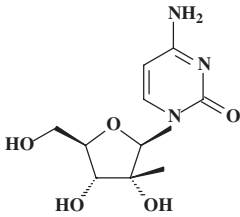
According to Reddy et al.,²⁵ RG7128 (Fig. 1) is currently in phase IIb clinical trials. RG7128 is the 3',5'-diisobutyrate ester prodrug of PSI-6130 (Fig. 1); in a 4-week combination study with the current standard of care (soc) for HCV infection (i.e., combination of pegylated interferon- α and ribavirin), RG7128 demonstrated efficacy in genotype 1, 2, and 3 patients, and, therefore, represents the first direct acting antiviral to show pan-genotype HCV coverage in the clinic.



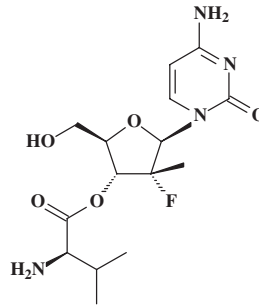
2'-Deoxy-2'-fluorocytidine



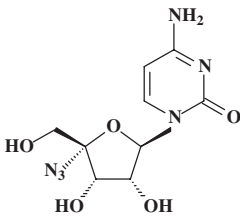
2'-Deoxy-2'-fluoro-2'-C-methylcytidine



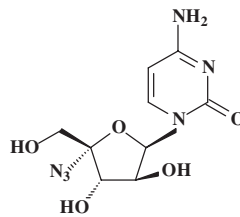
2'-C-methylcytidine



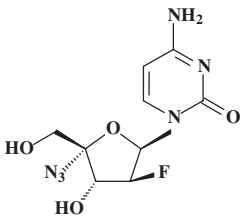
3'-O-L-Valine ester of 2'-C-methylcytidine
Valopicitabine (NM283)



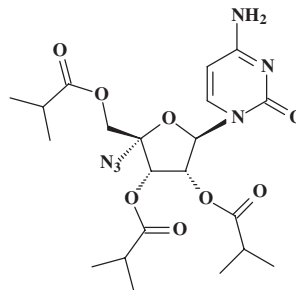
R1479



RO-9187



RO-0622



RG1626

Figure 1. Structures of nucleoside analogues and their prodrugs active against HCV NS5B polymerase.

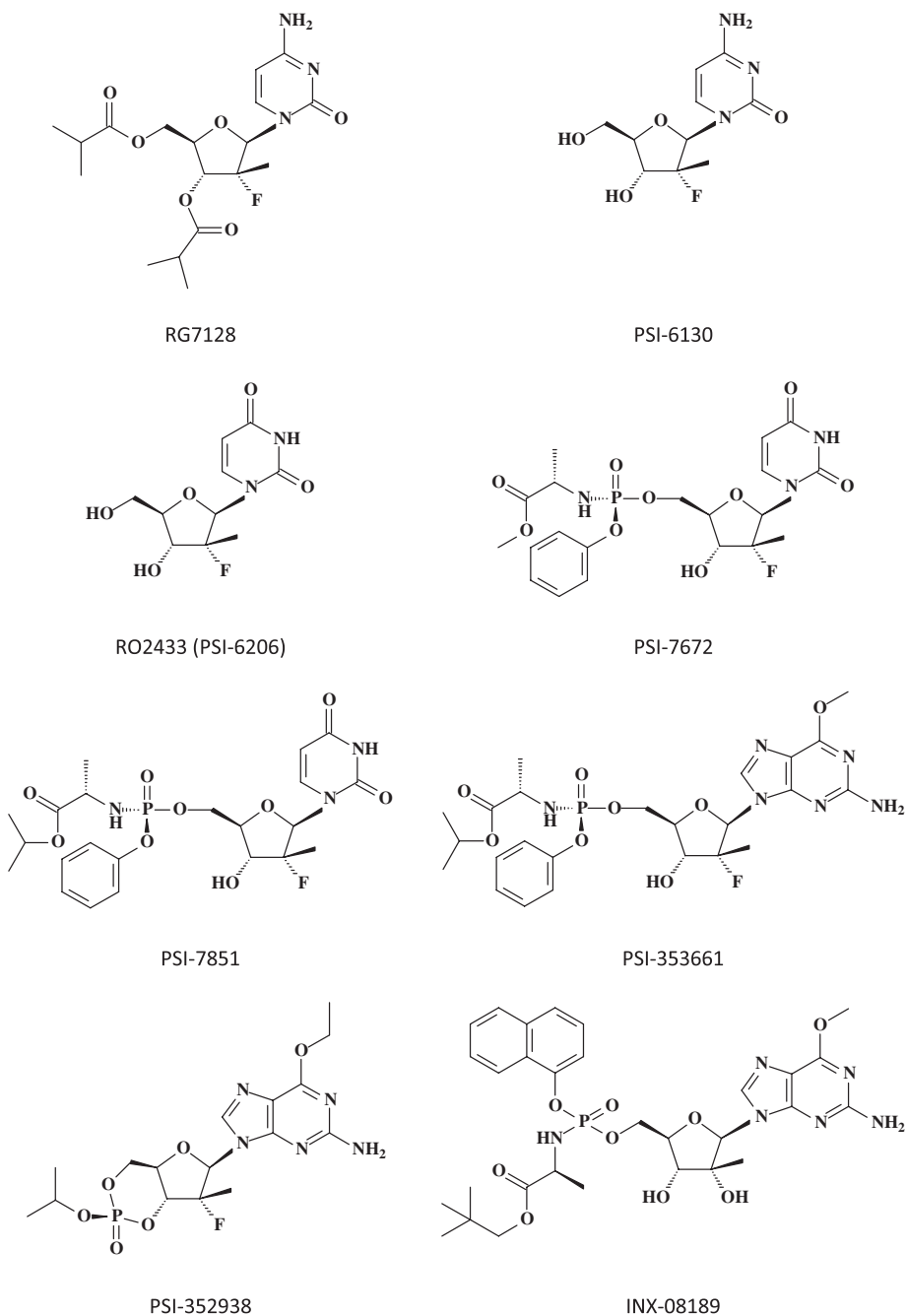


Figure 1. Continued.

PSI-6130 (β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine) is a potent inhibitor of HCV replication in the HCV replicon system [for an efficient, diastereoselective synthesis of PSI-6130, see Wang et al.²⁶]. To be active at the NS5B RNA polymerase level, PSI-6130 must be phosphorylated successively to its 5-mono-, di-, and triphosphate, the final, active metabolite which acts as a chain terminator of the NS5B RNA polymerase.²⁷ However, in addition to the

5'-triphosphate of PSI-6130, the 5'-triphosphate of its uridine counterpart, β -D-2'-deoxy-2'-fluoro-2'-C-methyluridine (RO2433) (Fig. 1), is also formed,²⁸ and this second metabolite of PSI-6130 is also a potent inhibitor of the HSV NS5B RNA polymerase.²⁹ Deamination of PSI-6130 occurs at the 5'-monophosphate level.²⁹ RO2433 (Fig. 1) itself is inactive in the HCV replicon system, but its phosphoramidate prodrug PSI-7672 (Fig. 1) is active in this system, where it is released as its 5'-monophosphate which can then be further phosphorylated intracellularly to the 5'-di- and 5'-triphosphate.²⁹

From R02433 (a uridine homolog, which was renamed PSI-6206), another phosphoramidate prodrug, PSI-7851 (Fig. 1) was prepared, which proved to be pan-genotype inhibitor of HCV replication, with, however, lesser activity against the S282T replicon mutant (while the S96T/N142T mutation remained fully susceptible to PSI-7851).³⁰ Inside the cell, PSI-7851 is converted to the 5'-monophosphate of PSI-6206, and in this sense, PSI-7851 can be considered as a prodrug of PSI-7411.³¹ PSI-7851 is, in fact, a mixture of two diastereoisomers, PSI-7976 (*Rp* diastereomer) and PSI-7977 (*Sp* diastereomer), the latter being the more active inhibitor of HCV RNA replication in the replicon system.^{31,32} PSI-7977 is currently being evaluated in phase II clinical trials.³³

From 2'-deoxy-2'-fluoro-2'-C-methylguanosine-5'-monophosphate a phosphoramidate prodrug was prepared, PSI-353661 (Fig. 1), which proved highly active against genotypes 1a, 1b, and 2a HCV RNA replication in the replicon system, genotype 1a and 2a infectious virus production, and HCV replicons harboring the NS5B S282T or S96T/N142T mutations.³³ PSI-353661 [(*S*)-2-[(*S*)-[(1*R*,4*R*,5*R*)-5-(2-amino-6-methoxy-purin-9-yl)-4-(*R*)-fluoro-3-hydroxy-4-methyl-tetrahydro-furan-2-yl-methoxy]-phenoxy-phosphonylamino]-propionic acid isopropyl ester] is the more active isomer of a mixture (PSI-352879) of two diastereoisomers.

Recently, the 2'-deoxy-2'- α -fluoro-2'- β -C-methyl 3',5'-cyclic phosphate prodrug PSI-352938 (Fig. 1) was described. Based on its antipan-genotype HCV activity (EC_{50} for the replicons in the 0.1–0.2 μ M range) also including activity against S282T or S96T/N142T mutations, ability to produce high intracellular 5'-triphosphate levels both in vitro and in vivo, the synthetic accessibility of a single diastereomer,³⁴ PSI-352938 was selected for further development.^{25,35} The compound is currently in phase I clinical trials.

Supportive of continued development as a clinical candidate for the treatment of HCV infection is INX-08189 or (2*S*)-neopentyl 2-(2*R*,3*R*,4*R*)-5-(2-amino-6-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl(methoxy)(naphthalen-1-yloxy)(phosphorylamino) propanoate, an aryl-phosphoramidate of 6-O-methyl-2'-C-methyl guanosine; its EC_{50} for 1a, 1b, and 2a HCV replicons is around 0.01 μ M.³⁶ The separated diastereomers of INX-08189 were shown to have similar activity in the replicon assay. INX-08189 has completed investigational new drug (IND) enabling studies and has progressed to human clinical trials for the treatment of chronic HCV infection.³⁷

3. CYCLOPROPAVIR

Cyclopropavir (Fig. 2) can be viewed as structurally related to acyclovir, ganciclovir, and penciclovir in that the acyclic side chain of the latter has been replaced by a methylenecyclopropane. Its antiviral properties have been known since a decade or so.³⁸ Cyclopropavir has proven to be effective against herpesviruses.^{39,40} It has proven particularly effective in animal models for cytomegalovirus (CMV) infection,⁴¹ including SCID-hu (where SCID is severe combined immunodeficient) mouse models for HCMV.⁴² In addition, cyclopropavir has also been found effective in vitro against human herpesvirus type 6 (HHV-6), like HCMV, a β -herpesvirus.⁴³

Currently available drugs for the treatment of HCMV infections are ganciclovir, cidofovir, foscarnet, and valganciclovir (the valine ester of ganciclovir). Esterification of ganciclovir with

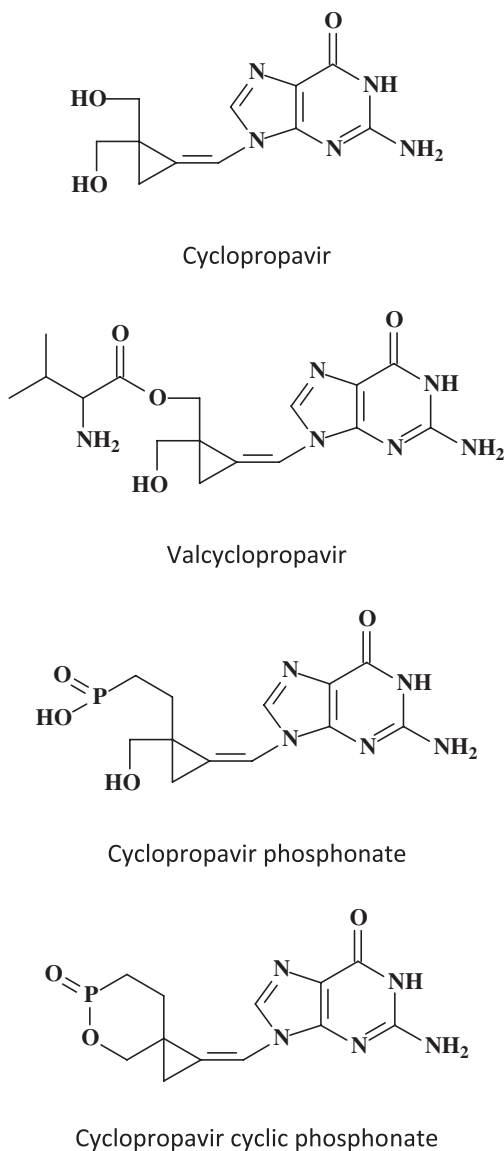


Figure 2. Structures of cyclopropavir, valcyclopropavir, and the cyclic phosphonate of cyclopropavir.

L-valine increased its oral bioavailability, as was previously also shown for acyclovir (valacyclovir) and would be later shown for the valine esters of 2'-deoxy-L-cytidine (valtorcitabine) and 2'-C-methylcytidine (valopicitabine).²⁰ Also, the L-valine ester of cyclopropavir (valcyclopropavir) (Fig. 2) has been constructed.⁴⁴ In mice, oral bioavailability of valcyclopropavir was 95%.

Against all those HCMV strains against which cyclopropavir was compared with ganciclovir, cyclopropavir displayed an EC₅₀ that was five- to tenfold lower than that of ganciclovir.⁴⁵ This was also the case for UL97 mutations that affected cyclopropavir and ganciclovir susceptibility. In fact, purified pUL97 phosphorylated cyclopropavir (to its monophosphate) 45-fold more extensively than ganciclovir.⁴⁶ This phosphorylation is stereoselective.

Cyclopropavir monophosphate is converted successively to its diphosphate and its triphosphate (the latter is the active metabolite interacting with the HCMV pUL54 DNA polymerase) by a single cellular enzyme, guanosine monophosphate kinase (GMPK), once the monophosphate is formed by a virally encoded kinase.⁴⁷

While new and safe anti-HCMV drugs are eagerly awaited, the fact that cyclopropavir has excellent activity against HCMV, combined with its specific phosphorylation by the viral enzyme pUL97 would seem to justify further development of the compound and its valine ester (valcyclopropavir), and eventually that of its phosphonate and cyclic phosphonate as well.⁴⁸ It is curious, in this regard, that while both the phosphonate and cyclic phosphonate (Fig. 2) were equally active against HCMV, only the phosphonate, but not the cyclic phosphonate, was active against the γ -herpesvirus, EBV.⁴⁸

4. NORTH-METHANOCARBATHYMIDINE (N-MCT)

N-methanocarbathymidine (*N*-MCT) with a pseudosugar rigidly fixed in the Northern conformation (1*R*,2*S*,4*S*,5*S*)-1-(hydroxymethyl)-2-hydroxy-4-(5-methyl-2,4(1*H*,3*H*)-dioxypyrimidin-1-yl)bicyclo[3.1.0]hexane (Fig. 3) was first synthesized by Marquez et al. in 1996.⁴⁹ It was found to exhibit potent antiviral activity against HSV-1 and HSV-2. This was further corroborated in subsequent studies.^{50–52} *N*-MCT appeared to be phosphorylated to the mono- and diphosphate by the HSV-encoded thymidine kinase, and to inhibit the viral DNA polymerase through its triphosphate metabolite.⁵⁰ Kinases would prefer substrates that adopt the *S* sugar conformation, whereas cellular DNA polymerases almost exclusively incorporated the triphosphate of the locked *N* conformer, notwithstanding the presence of higher triphosphate levels of the *S*-conformer *S*-MCT (Fig. 3).⁵³

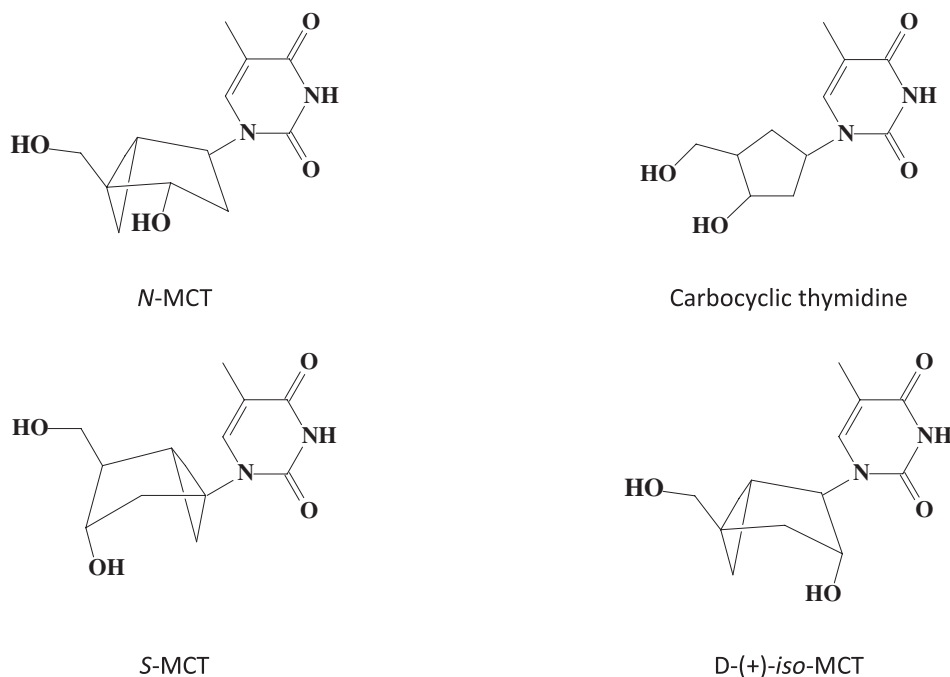


Figure 3. Structures of carbocyclic thymidine, *North*-methanocarbathymidine (*N*-MCT), *South*-methanocarbathymidine (*S*-MCT), and *D*-(+)-*iso*-methanocarbathymidine (*D*-(+)-*iso*-MCT).

Antiviral activity against vaccinia virus was first shown with carbocyclic thymidine (Fig. 3).⁵⁴ *N*-MCT was found to be highly effective against orthopoxvirus infections in vivo (mice),^{55,56} although the lung, nasal, brain virus reductions it achieved for vaccinia virus infection (1HD strain) were not nearly to the same extent as for cidofovir.⁵⁷

Vaccinia virus lacking the F2L gene encoding functional deoxyuridine triphosphatase (dUTPase, that catalyzes the conversion of dUTP to dUMP) continued to replicate well in vitro and in vivo, but proved hypersensitive to the inhibitory effect of *N*-MCT.⁵⁸

As to its activity spectrum, *N*-MCT is not only active against the α -herpesviruses HSV-1, HSV-2, but also against the γ -herpesviruses EBV⁵⁵ and KSHV.⁵⁹ Apparently, *N*-MCT inhibits lytic KSHV DNA synthesis through its triphosphate metabolite produced in KSHV-infected cells expressing a virally encoded thymidine kinase.⁵⁹

Recently, a “greener” enantioselective synthesis of *N*-MCT from 2-deoxy-D-ribose has been reported⁶⁰ and a new MCT distinct from *N*-MCT, namely D-(+)-*iso*-MCT (Fig. 3), has been described as a high-affinity substrate for HSV-1 thymidine kinase.⁶¹ *N*-MCT shows potent anti-HIV activity in human osteosarcoma (HOS) cells modified so as to contain, and express, the HSV-1-encoded thymidine kinase. Possible anti-HIV activity of D-(+)-*iso*-MCT may have been masked by cytotoxicity.⁶¹

N-MCT represents an interesting conformational concept.⁶² Its therapeutic utility, however, remains to be demonstrated. As there are, at present, no therapeutic options for EBV and KSHV infections, these infections may well represent unique opportunities for the clinical potential of *N*-MCT to be further explored.

5. CMX001 (HDP-CDV)

CMX001 is the 1-O-hexadecyloxypropyl (HDP) prodrug of the acyclic nucleoside phosphonate cidofovir (CDV), representing an oral version of cidofovir with reduced (nephro)toxicity.⁶³ The active form of HDP-CDV (CMX001) is cidofovir (Fig. 4), which explains why, in principle, CMX001 should possess an activity spectrum similar to that of cidofovir, thus, encompassing DNA viruses, herpes-, adeno-, polyoma-, and poxviruses. For all these indications, CMX001 could, given its oral bioavailability and safer (nephro)toxicity profile, replace cidofovir in future therapeutic regimens.

Against disseminated or central nervous system (CNS) HSV infections, firm Chimerix (CMX) appears to be superior to acyclovir.⁶⁴ Orally administered HDP-CDV is four-

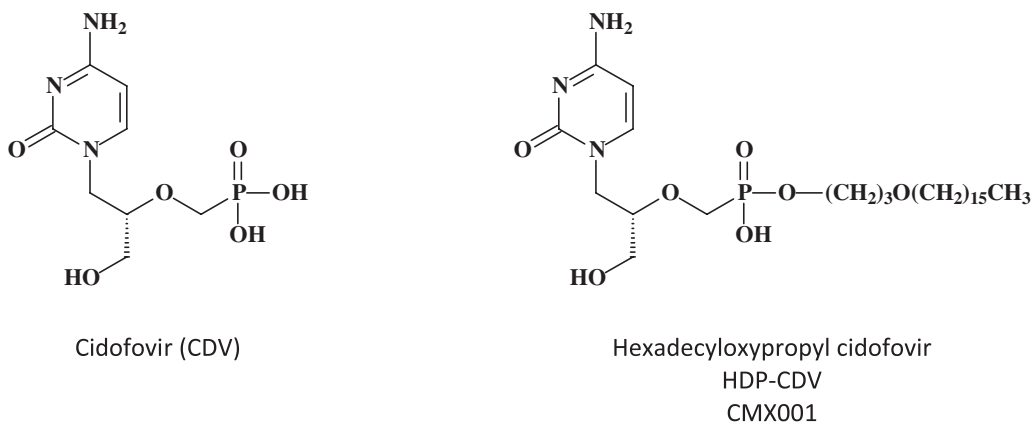


Figure 4. Structures of cidofovir (CDV) and HDP-CDV (CMX001).

eightfold more active, on a molar basis, than intraperitoneally administered cidofovir against HCMV infection in SCID/hu mice.⁶⁵ HDP-cidofovir exhibits multiple-log enhancement of antiviral activity against both HCMV and HSV replication in vitro,⁶⁶ and oral treatment with HDP-CDV is as effective as parenteral CDV for the treatment of murine CMV infections.⁶⁷ As long awaited, oral HDP-CDV improves the outcome of CMV infection in a congenital model for CMV infection in pregnant guinea pigs.⁶⁸

Ether lipid esters of cidofovir, such as HDP-CDV, are much more potent than cidofovir against adenovirus replication in vitro.⁶⁹ HDP-CDV (CMX001) suppressed adenovirus-induced mortality in immunosuppressed hamsters, a powerful model to evaluate the efficacy of anti-adenovirus agents.⁷⁰ A first case of the successful eradication of disseminated adenovirus infection by CMX001 has been recently reported in a severely immunosuppressed pediatric stem cell transplant recipient.⁷¹

In line of earlier observations on the inhibitory effects of cidofovir against murine and primate polyomaviruses,⁷² cidofovir was shown to inhibit polyomavirus BK replication in human renal tubular cells.⁷³ Either lipid esters of cidofovir such as HDP-CDV were then shown to inhibit polyomavirus BK replication in vitro at a 3 log₁₀-fold lower concentration than cidofovir.⁷⁴ CMX001 proved highly effective in inhibiting polyomavirus BK replication in primary human renal tubular epithelial cells.⁷⁵ This points to the potential of CMX001 in the treatment of BK virus nephropathy that is seen in 1–10% of kidney transplant recipients.

The JC polyomavirus infects human oligodendrocytes leading to the development of progressive multifocal leukoencephalopathy. CMX001 was shown to suppress polyomavirus JC in human fetal brain SVG cell cultures⁷⁶ [the SVG cell line is derived from primary human brain cells transfected with Simian virus 40 (SV40) and expressing SV40 T antigen in these cells]. CMX001 was recently shown to inhibit polyomavirus JC replication in human brain progenitor-derived astrocytes,⁷⁷ and a case of progressive multifocal leukoencephalopathy (accompanied by idiopathic CD4⁺ lymphocytopenia) responded successfully to treatment with CMX001.⁷⁸

Alkoxyalkyl esters of cidofovir, including HDP-CDV, have been most intensively pursued for inhibition of orthopoxvirus replication.⁷⁹ They were first proven active in vitro against vaccinia virus and cowpox,⁸⁰ before their in vivo activity against the same viruses was demonstrated.⁸¹ Their efficacy was demonstrated in a lethal mousepox model (based on lethal, aerosol ectromelia virus infection in A/NCR mice).⁸² In an improved model for evaluating antipoxvirus therapies, based on the use of C57BL/6 mice infected with mousepox (ectromelia) virus, CMX001 proved more efficacious than in A/NCR mice.⁸³

Ectromelia virus infection of mice serves as a model to support the licensure of anti-orthopoxvirus therapeutics based on the “animal efficacy rule” because of the genetic similarity of ectromelia virus to variola and monkeypox viruses.⁸⁴ In the lethal mousepox model, complete protection against mortality was achieved when administration of CMX001 was delayed until as late as 5 days postinfection.⁸⁵ A single dose of 25 mg/kg of CMX001 administered 4 or 5 days postinfection sufficed to be effective in the mousepox model.⁸⁶

CMX001 is also efficacious in the treatment of monkeypox virus infection in STAT1-deficient C57BL/6 mice.⁸⁷ Against the highly virulent, interleukin-4 expressing ectromelia virus recombinant, CMX001 may afford the highest efficacy when combined with another antiviral drug, ST-246.⁸⁸ The pre- and postexposure prophylactic efficacy of CMX001 has also been demonstrated in rabbits infected with rabbitpox virus.^{89,90}

Compared with CDV, CMX001 would have the advantages that it could be administered orally (whereas CDV needs to be administered intravenously) and, unlike CDV, CMX001 may not lead to nephrotoxicity.⁹¹ Furthermore, various additional alkoxyalkyl esters of CDV have been described, that is, 1-O-octadecyl-2-O-benzyl-*sn*-glycero-3-CDV (ODBG-CDV),^{92,93} which may be worth further exploring for potential advantages over HDP-CDV (CMX001).

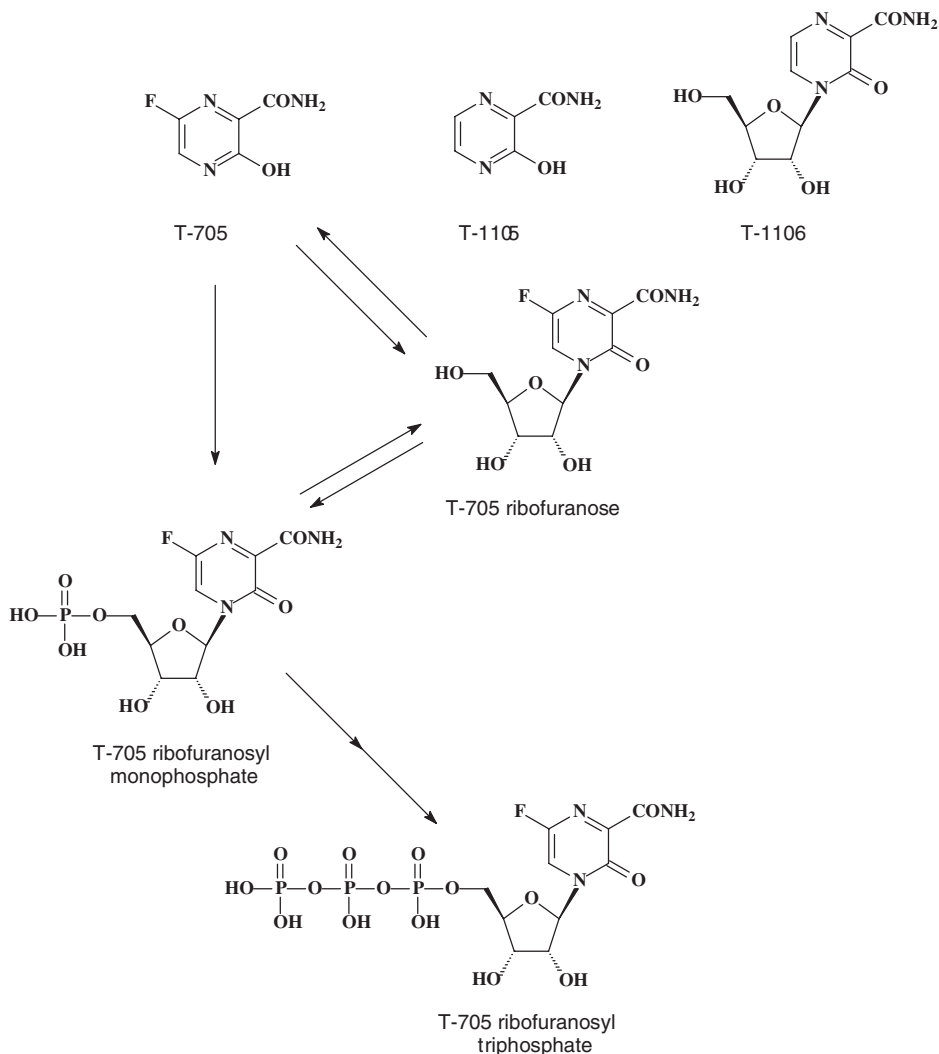


Figure 5. Structures of T-705, T-1105, and T-1106, and of T-705 metabolites.

6. FAVIPIRAVIR (T-705)

Favipiravir (T-705) (Fig. 5) is currently in clinical trials in Japan (phase III) and the United States (phase II) for the treatment of influenza virus infections (as mentioned by Buys et al.⁹⁴). The *in vitro* and *in vivo* activities of T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) were first reported in 2002 by Furuta et al.⁹⁵ The compound showed potent inhibitory activity against influenza A, B, and C viruses, some activity against picorna- and paramyxoviruses, but no activity whatsoever against DNA viruses. From its structure, it was immediately clear that the mode of action of T-705 had to be different from that of the M2 ion channel inhibitors amantadine and rimantadine, as well as that of the neuraminidase inhibitors zanamivir and oseltamivir.

This mechanism of action was further addressed by Furuta et al.⁹⁶ Within the cells, T-705 would be converted to T-705 ribofuranosyl monophosphate by a purine (adenine,

hypoxanthine/guanine) phosphoribosyl transferase. Two phosphorylations would then generate T-705-4-ribofuranosyl-5'-triphosphate (T-705 RTP), the active metabolite of T-705.⁹⁶ That the latter is indeed the active metabolite of T-705 was also shown in cells infected with the highly pathogenic influenza A (H5N1) virus.^{97,98}

Meanwhile, Sidwell et al.⁹⁹ had shown that T-705, when orally administered at dosages from 30 to 300 mg/kg (once or twice daily), prevented death due to a lethal avian influenza A (H5N1) virus infection in mice. In vitro, favipiravir proved antivirally active against influenza A (H1N1) virus strains that were resistant to antiviral drugs such as oseltamivir,¹⁰⁰ and, in vivo, synergistic effects were obtained with favipiravir combined with oseltamivir against influenza A (H5N1) infection.¹⁰¹

The carboxamide group present in favipiravir (T-705) is reminiscent of the carboxamide present in ribavirin. This carboxamide group is also present in T-1105 and T-1106, two compounds that are structurally related to T-705 (Fig. 5). All the compounds share, with ribavirin, a broad-spectrum activity against various RNA viruses. Thus, T-1105 shows activity against foot-and-mouth disease virus (FMDV) and, as it can be administered through food, it could be a powerful tool to control foot-and-mouth disease in pigs.¹⁰² T-1106 has proven efficacious against Yellow fever virus (YFV) in a hamster model of YFV infection¹⁰³ and is also active against bovine viral diarrhoea virus (BVDV),¹⁰² another flavivirus that could be considered as a surrogate virus for hepatitis C virus (HCV).

T-705 is also efficacious in the YFV hamster model, although the dose of T-705 required for efficacy in hamsters is higher than that of T-1106 required for efficacy. Yet, T-705 improved the disease parameters in YFV-infected hamsters, which may indicate its potential utility in the treatment of YFV infection in humans.¹⁰⁴

In rodents (mice or hamsters), orally administered T-705 is also effective against West Nile virus (WNV),¹⁰⁵ another flavivirus related to YFV. Whether T-705 or any of its analogues T-1105 or T-1106 would be effective against two other mosquito borne flaviviruses, dengue virus and Japanese encephalitis virus, remains an intriguing possibility worth exploring. Western equine encephalitis virus (WEEV), an alphavirus belonging to the broad family of the flaviviridae, would seem to respond to T-705 treatment.¹⁰⁶

The antiviral activity spectrum of T-705 extends to arenaviruses: that is, Junin virus (JUNV), Pichinde virus, Tacaribe virus, Machupovirus (MACV), and Guanarito virus (GTOV) replication in cell culture could be inhibited by T-705, and as previously noted for its inhibitory effect on influenza virus, the antiarenavirus activity of T-705 could be reversed by the addition of purine, but not pyrimidine nucleosides.^{107,108} T-705 also proved efficacious against Pichinde virus infection in hamsters, even when treatment was begun after the animals fell ill, the day before the animals began to succumb to the disease.¹⁰⁹ Thus, for the treatment of late stage arenaviral hemorrhagic fever, T-705 could be considered as an alternative option to ribavirin. This would seem very important for severe arenavirus infections, such as Lassa fever, in humans.

Besides arenavirus infections, bunyaviruses, that is, Punta Toro, La Crosse, Rift Valley fever, and sandfly fever virus, have also proven sensitive to inhibition by T-705 in vitro, and, for Punta Toro virus, also in vivo (mice and hamsters).¹⁰⁷ In hamsters infected with Punta Toro virus, T-1106 was more efficacious than T-705, but in mice, T-705 was the more effective.¹¹⁰

Of the bunyaviridae, the hantaviruses Dobrava and Maporal were found to be sensitive to the inhibitory effects of T-705.⁹⁴ Maporal virus is phylogenetically similar to Andes virus, the principal cause of hantavirus cardiopulmonary syndrome (HCPS) in Argentina. It would now seem mandatory to further explore the efficacy of T-705 in the suitable hantavirus models in hamsters and/or mice.

In conclusion, T-705 is an intriguing new antiviral compound that should be further explored not only for its clinical potential in the prevention/therapy of influenza virus infections,

but also for its broad activity spectrum against (–) and (+) RNA strand viruses, its target of action (RNA-dependent RNA polymerase for all these RNA viruses ?), and its structure-activity relationship and pharmacodynamics relative to that of related structural analogues such as T-1105 and T-1106.

7. ARENAVIRUS INHIBITORS

Since McCormick's pioneering paper in 1986¹¹¹ on the effective therapy of Lassa fever with ribavirin, ribavirin has remained the only antiviral drug available for the treatment of arenavirus infections. The inhibitory effect of ribavirin on arenaviruses might, at least partially, be attributed to lethal mutagenesis.¹¹² In recent years, antiarenaviral drug development has received increasing attention,^{113,114} one of the new antiarenaviral drug candidates being favipiravir (T-705) (see preceding section).

The Tacaribe arenavirus infection model has been employed to explore the antiviral potential of novel aristeromycin analogues,¹¹⁵ and new imidazo[2,1-b]thiazole carbohydrate derivatives have been found to inhibit the replication of the Argentine hemorrhagic fever virus Junin.¹¹⁶ Small interfering (si)RNAs targeting the conserved RNA termini of Lassa fever virus¹¹⁷ offer therapeutic potential and so do interferon- α and interferon- γ ,¹¹⁸ whereas antimicrobial cationic peptides were found active against JUNV as well as HSV-1 and HSV-2.¹¹⁹

Lassa fever virus is restricted by the bone marrow stromal antigen 2 (BST-2), also called tetherin, which besides inhibiting the release of HIV-1, also inhibits the egress of arenaviruses.¹²⁰ Tetherin could thus be considered an innate immunity strategy to suppress arenavirus replication.

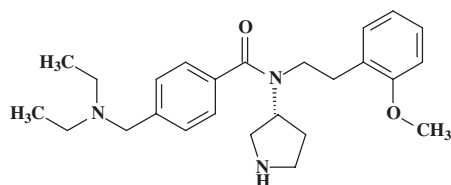
The Lassa fever viral nucleoprotein (NP) is endowed with several functions (i.e., a 3'-5' exoribonuclease at the C-domain, involved in suppressing interferon induction, and a m7GpppN cap-binding site at the N-domain, protecting the cap against cap snatching), which may serve as potential targets for chemotherapeutic intervention.¹²¹

One of the most fascinating targets for novel antiarenavirus strategies is the arenavirus envelope glycoprotein complex (GPC) processing by the cellular site 1 protease (S1P), which is strictly required for the production of infectious progeny and cell-to-cell virus propagation.¹²² The small molecule PF-429242 (Fig. 6) was recently reported to be a potent S1P inhibitor *in vitro* (cell-based assay).^{123,124} This correlated with the compound's potent antiviral activity against Lassa fever virus in cell culture.¹²⁵

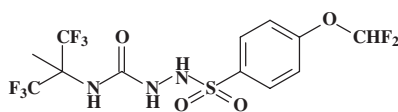
Of the small molecular weight inhibitors targeted at arenaviral entry, in particular the viral glycoprotein GP2, the first to be announced chosen for drug development was ST-294 (Fig. 6).¹²⁶ The arenavirus GP is synthesized as a single polypeptide that undergoes posttranslational processing to yield the mature virion glycoproteins GP1 and GP2. GP1 is involved in receptor binding, whereas GP2 is similar to the fusion proteins of other enveloped viruses such as retroviruses, paramyxoviruses, and filoviruses. A series of small molecules, including 17C8 (Fig. 6) have been identified to be targeted at the arenavirus GP.¹²⁷

These small molecule entry inhibitors (including ST-294 and ST-193) interact with the envelope GPC of arenaviruses so as to stabilize the complex against pH-induced activation of membrane fusion in the endosome.¹²⁸ Both ST-294 and ST-193 (Fig. 6) inhibit the pH-induced dissociation of the receptor-binding GP1 subunit from GPC. ST-294 and ST-193 thus stabilize the GPC against pH-acidification which would otherwise initiate the fusion process.¹²⁹

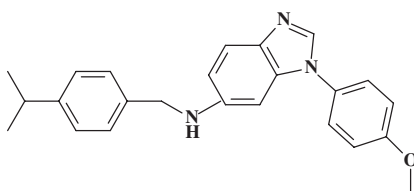
The antiviral potency of ST-193 (Fig. 6) against Lassa virus and other arenavirus pseudotypes is within the range of 0.2–12 nM. The sensitivity to ST-193 is dictated by a segment



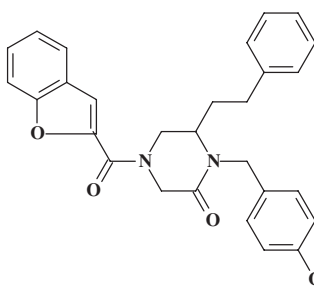
PF-429242



ST-294



ST-193



17C8

Figure 6. Arenavirus inhibitors targeted at viral entry.

of about 30 amino acids within the GP2 subunit. This region includes the carboxy-terminal region of the ectodomain of the transmembrane domain of the envelope protein.¹³⁰

The small molecule arenavirus inhibitor ST-193 was compared with ribavirin in a guinea pig model for Lassa virus infection, and found to increase the survival rate from 0% (control, ribavirin) to 62.5% (ST-193).

What now remains to be established is how ST-193 compares with other arenavirus inhibitors such as favipiravir (T-705) and PF-429242, both in terms of efficacy and safety, and whether its efficacy can be extrapolated from Lassa to other arenavirus infections such as Junin, Machupo, Sabia, and Guanarito.

8. PLECONARIL AND ANTIPICORNAVIRUS AGENTS REVISITED

Although picornaviruses encompass a number of important human pathogens, including the enteroviruses polio, Coxsackie A and B, and echo, and rhinoviruses, there is still no single antipicornavirus agent approved for clinical use. Yet, a wealth of compounds has been shown to inhibit picornaviruses, including, especially for Coxsackie B virus, a number of natural products (see Section 8). Prominent among the currently envisaged antipicornavirus therapies¹³¹ are the original Winthrop compounds (disoxaril derivatives), which engage in a specific binding to the viral capsid.

Pleconaril (Fig. 7), the prototype of this class of compounds,¹³² had been shown to inhibit the replication of various entero- and rhinoviruses¹³³ and had demonstrated tentative efficacy against potentially life-threatening enterovirus infections,¹³⁴ before, in 2002, it was rejected by the US FDA for the treatment of common cold.¹³⁵ Several double-blind placebo-controlled trials with pleconaril in infants with enterovirus meningitis,¹³⁶ and adults with common cold¹³⁷ were conducted, and Pevear et al.¹³⁸ showed that the efficacy of pleconaril in reducing the duration and severity of common cold symptoms (when it was administered within 24 hr of symptom onset) was related to the virus susceptibility to pleconaril. De Palma et al.¹³⁹ in their review mentioned that a phase II double-blind, placebo-controlled trial to evaluate the effects of pleconaril nasal spray on common cold symptoms and asthma exacerbations following rhinovirus exposure was completed in 2007, but that results of this trial have not yet been divulged.

Pleconaril has in the meantime been found to shorten the course of illness, compared to placebo, in patients with enteroviral meningitis, but the benefit appeared to be modest after adjusting for confounding variables.¹⁴⁰ Pleconaril did not have any effect on viral replication in a common variable immunodeficiency (CVID) patient with parechovirus-associated

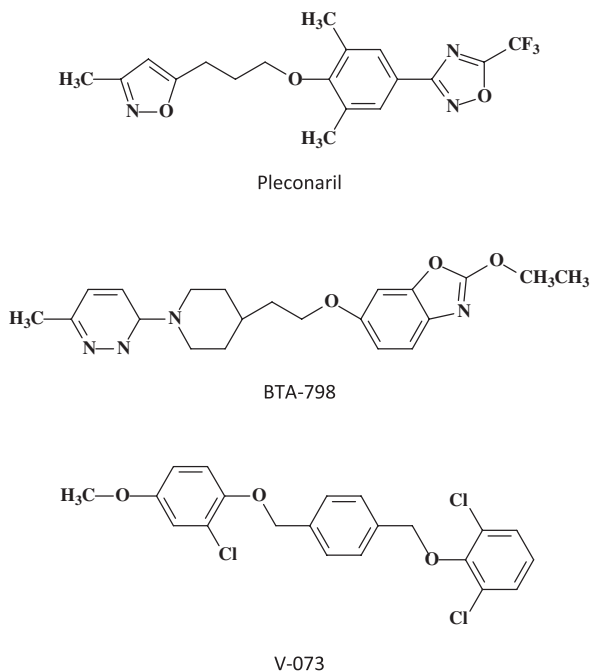


Figure 7. Structures of pleconaril, BTA-798, and V-073.

enteropathy.¹⁴¹ New pleconaril derivatives have been reported to be active against pleconaril-resistant Coxsackie B virus.¹⁴²

Meanwhile, new benzimidazole derivatives have been synthesized and found active against Coxsackie B3 virus,¹⁴³ and a small interfering (si)RNA has been shown to block Coxsackie B virus replication.¹⁴⁴ Synergistic activity against Coxsackie B3 virus was obtained if the siRNA was combined with the soluble Coxsackievirus-adenovirus receptor.¹⁴⁵ A new class of compounds, which be structurally described as 9-arylpurines, was recently described to inhibit a variety of enteroviruses, that is, Coxsackie A16, A21, A24, Coxsackie B3, and echovirus 9, at low micromolar concentrations.¹⁴⁶

In earlier papers dating from 2003 to 2004, we have demonstrated that Coxsackie B3 virus-induced myocarditis in mice can be inhibited by mycophenolate mofetil,¹⁴⁷ 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile,¹⁴⁸ and the interferon inducer ampligen [poly(I).poly(C₁₂,U)].¹⁴⁹

Three antipicornavirus agents are currently in clinical development: pleconaril, BTA-798, and V-073¹⁵⁰ (Fig. 7). Pleconaril is under development as an oral formulation for the treatment of rhinovirus infections in high-risk patients with chronic lung diseases.^{139,151,152} Oral BTA-798 is in phase II trial for symptomatic human rhinovirus infection in asthmatic adults.¹³⁹ V-073 is under further scrutiny for the treatment of poliovirus infections. Their EC₅₀ values are, respectively, for BTA-798 0.02 μM against human rhinovirus type 14 and 0.2 μM against enterovirus 71, 0.06 μM for pleconaril against human rhinovirus type 14, and 0.026 μM for V-073 against poliovirus type 1.¹⁵⁰ Pleconaril, BTA-798 and V-073 behave as capsid binders. They could, if required, be combined to provide an additive to slightly synergistic antiviral effect.¹⁵⁰

As reviewed recently,¹⁵³ antivirals directed against human rhinoviruses could be used to treat the common cold, but also be employed therapeutically or prophylactically to prevent asthma and chronic obstructive pulmonary disease (COPD) exacerbations in high-risk patients.

9. NATURAL PRODUCTS WITH ANTIVIRAL ACTIVITY

Natural compounds, primarily those originating from plants, have received increasing attention for their antiviral potential. Typical examples are constituents of *Ardisia chimensis*¹⁵⁴ and caffeoylquinic acids from *Schefflera heptaphylla*,¹⁵⁵ both originating from Southern China and found active particularly against Coxsackie B3 virus.

Norsesquiterpenoids isolated from the roots of *Phyllanthus emblica* showed, again, some activity against Coxsackie B3 virus¹⁵⁶ and triterpenoids isolated from *S. heptaphylla* (Fig. 8) were accredited with broader activity against Coxsackie B3, influenza A, respiratory syncytial virus (RSV), and HSV-1.¹⁵⁷

Calycosin-7-O-beta-D-glucopyranoside (Fig. 8), the main isoflavonoid isolated from *Astragalus membranaceus*, also showed activity against Coxsackie B3 virus in vitro, and would improve the survival rate of mice infected with Coxsackie B3 virus.¹⁵⁸

Flavans 7-O-galloyltricitifavan and 7,4'-di-O-galloyltricitifavan isolated from the leaves of *Pithecellobium clypearia* would show activity against the same array of viruses (Coxsackie B3, influenza A, RSV, and HSV-1) as the triterpenoids mentioned above.¹⁵⁹

Homoisoflavonoids 3-benzyl-4-chromones (Fig. 8) showed activity against Coxsackie virus B1, B3, B4, A9, and echovirus 30, but not against poliovirus.¹⁶⁰

The flavone 4',5,7-trihydroxy-3',5'-dimethoxyflavone (tricin), isolated from the bamboo *Sasa albo-marginata* was found effective against HCMV at an EC₅₀ of 0.17 μg/mL which means a stronger antiviral activity than ganciclovir.¹⁶¹ Tricin has also been accredited with

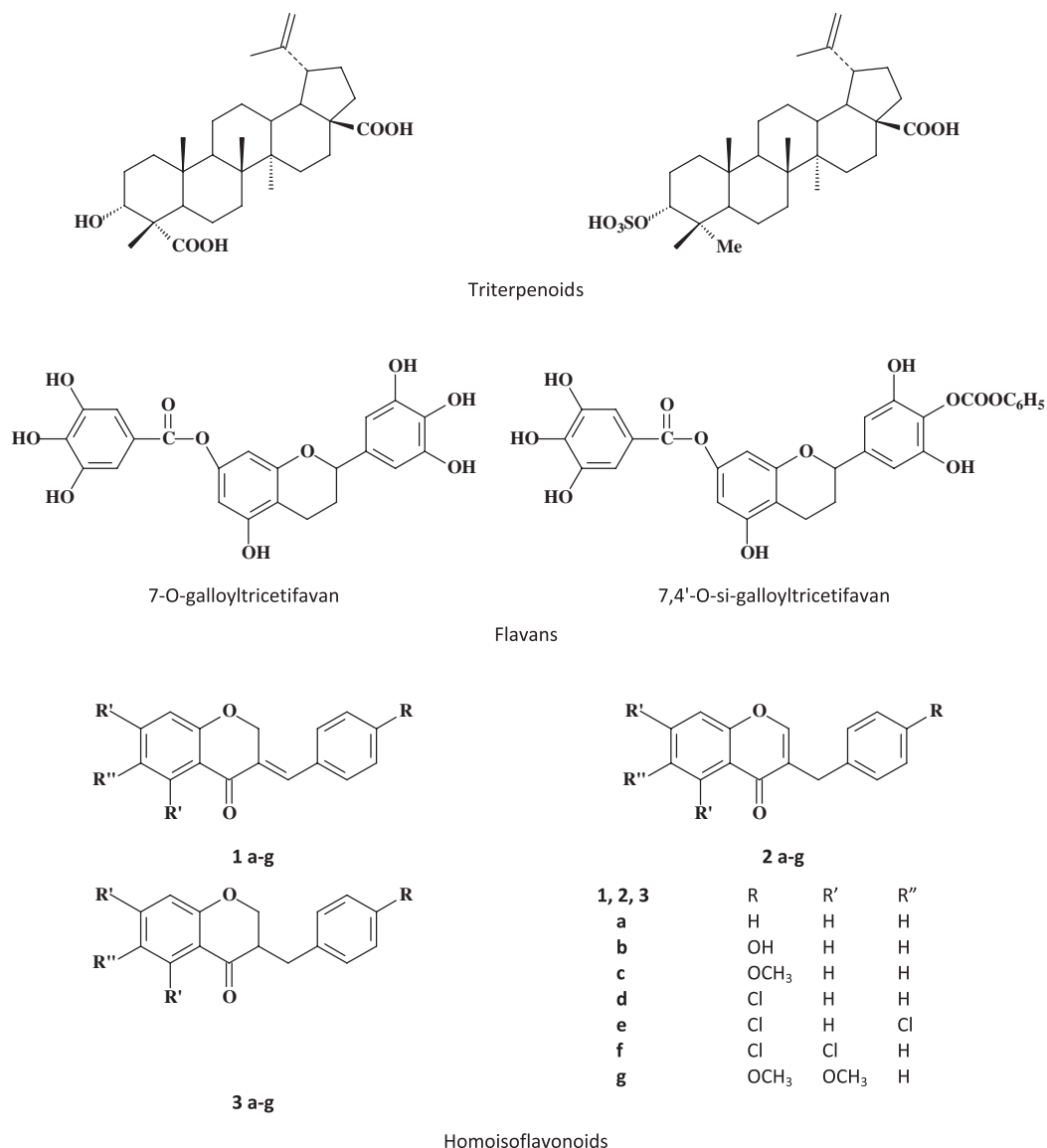


Figure 8. Structures of natural products.

antiinfluenza virus activity.¹⁶² To increase the oral bioavailability of tricetin, it has been conjugated with alanine-glutamic acid. The prodrug of tricetin (tricetin-alanine-glutamic acid) showed excellent oral bioavailability upon oral administration in rats.¹⁶³

The isoflavone genistein (Fig. 8) was originally isolated from fermentation broth of *Pseudomonas* sp.¹⁶⁴ and initially described as a tyrosine-specific protein kinase inhibitor.¹⁶⁵ Only recently, genistein has been shown to inhibit arenavirus infection,¹⁶⁶ putatively by inhibiting arenavirus entry which occurs through a cholesterol-dependent clathrin-mediated endocytic mechanism.¹⁶⁷ Genistein has been found to increase the survival rate of hamsters infected with the arenavirus Pirital virus, a surrogate model for hemorrhagic fever causing arenaviruses.¹⁶⁸

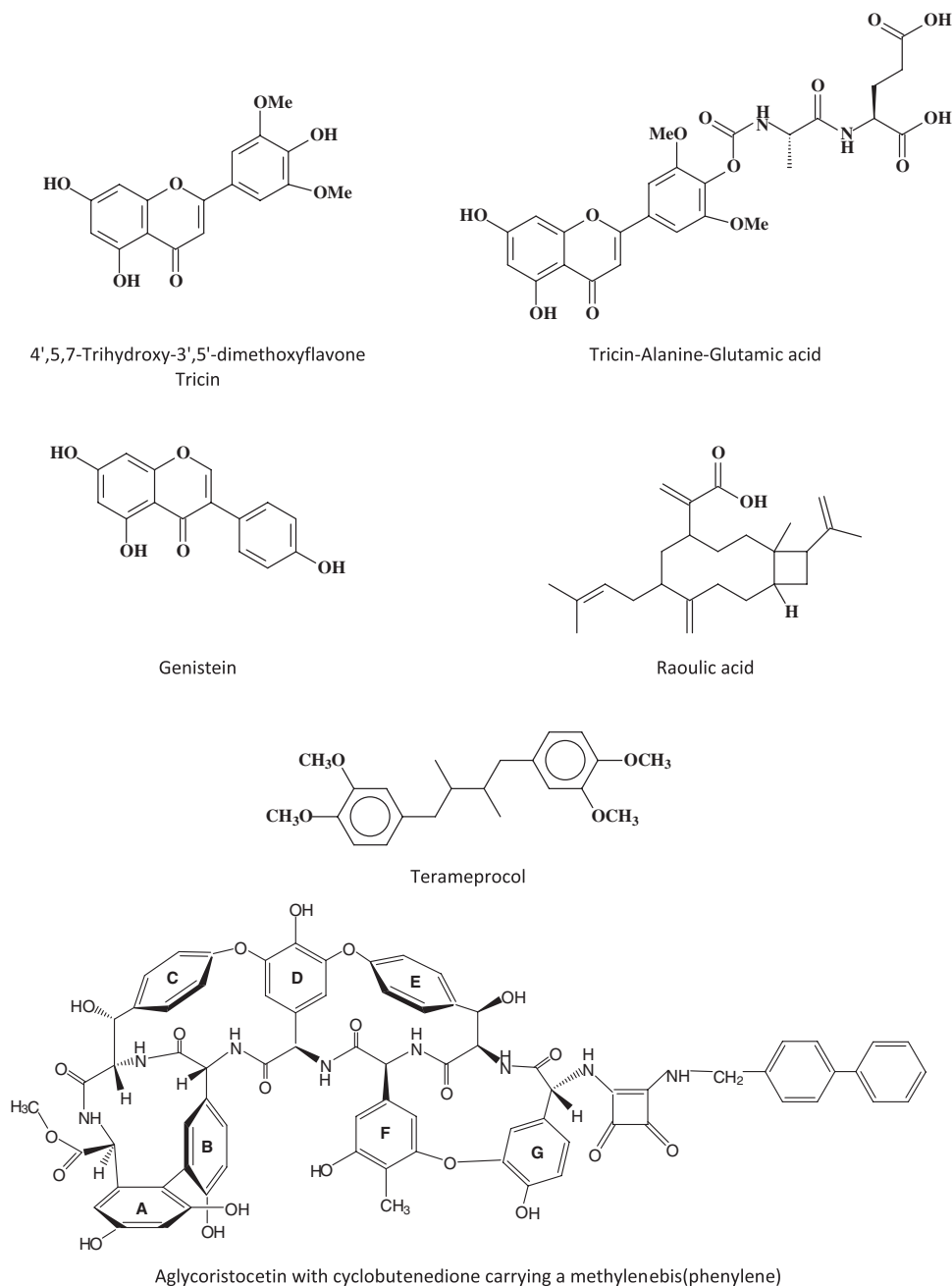


Figure 8. Continued.

Raoulic acid (Fig. 8) is the principal ingredient of *Raoulia australis*. It was shown to inhibit picornaviruses, that is, rhinovirus 2 (HRV2), rhinovirus 3 (HRV3), Cocksackie B3, Cocksackie B4, and enterovirus 71 at EC_{50} values in the range of 0.1–0.4 $\mu\text{g}/\text{mL}$, that is, at lower concentrations than those at which the aforementioned triterpenoids and flavans showed antiviral activity.¹⁶⁹ However, raoulic acid did not show activity against influenza A or B.¹⁶⁹

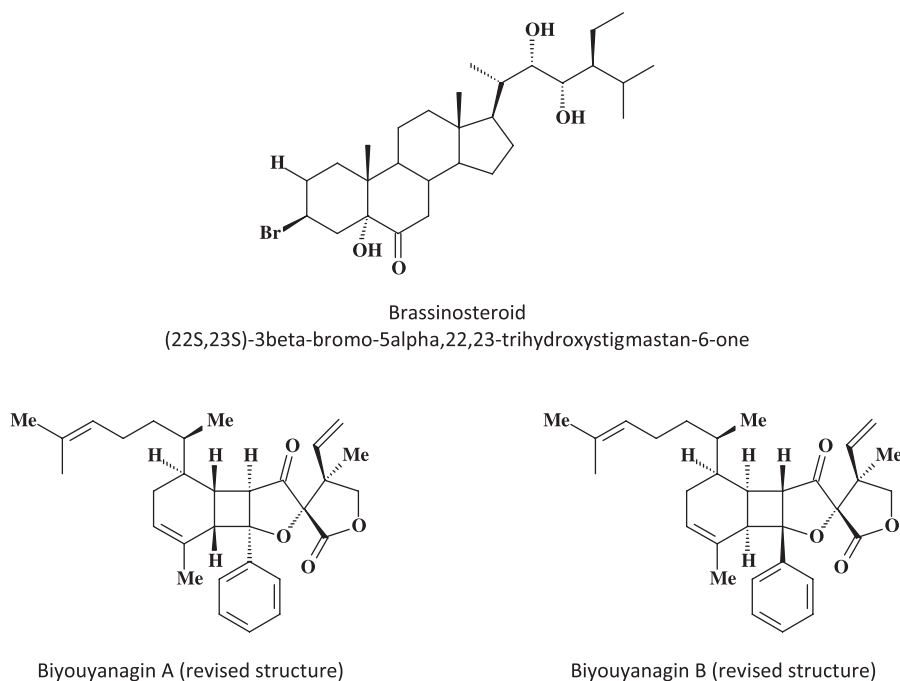


Figure 8. Continued.

Terameprocol is a methylated derivative of nordihydroguaiaretic acid, a phenolic antioxidant extracted from the creosote bush *Larrea tridentate*.¹⁷⁰ Terameprocol (Fig. 8) has been found to inhibit the growth of poxviruses, that is, cowpox and vaccinia, by preventing the spread of virus particles from cell to cell.¹⁷⁰ Nigericin (also known as antibiotic K-178, helexin C, azalomycin M, antibiotic X-464, and polyetherin A) has also been shown to inhibit poxvirus replication.¹⁷¹ It had been previously reported to inhibit poliovirus and influenza virus replication.^{172,173}

Aglycoristocetin derivatives with a cyclobutenedione carrying hydrophobic chains such as methylene bis(phenylene) (Fig. 8) inhibit influenza A and B virus infections, probably by interference with the viral entry process.¹⁷⁴ These types of compounds (derivatives of glycopeptide antibiotics) have been previously shown to inhibit the replication of retro- and coronaviruses and this inhibitory effect was also attributed to interference with virus entry.^{175,176}

Brassinosteroids, that is (22S,23S)-3 β -bromo-5 α ,22,23-trihydroxy stigmastan-6-one (Fig. 8), represent naturally occurring polyhydroxy steroidal plant hormones modulating the growth and differentiation of plant cells. Their antiviral activity has been well documented.^{177,178} Brassinosteroids are particularly active against arenaviruses such as Tacaribe, Pichinde, and Junin.¹⁷⁹

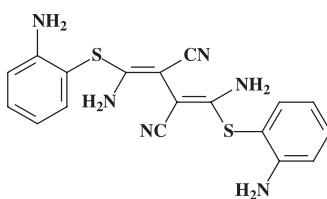
Biyouyanagins A and B were originally obtained from *Hypericum chinense* and shown to be active against HIV.¹⁸⁰ These compounds possessing antiarenavirus and anti-HIV properties have been recently obtained by total synthesis, and their originally assigned structures were revised (Fig. 8).¹⁸¹

In conclusion, a wealth of natural products has been reported to possess antiviral properties. In the majority of these cases the chemical structure was well identified but the full antiviral

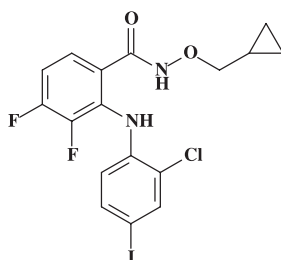
activity spectrum of the compounds still needs to be evaluated, their mode of action elucidated, and, most importantly, their therapeutic value delineated.

10. MEK INHIBITORS

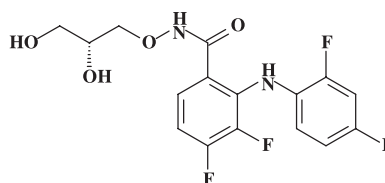
U0126 (Fig. 9) is the prototype of the MEK (*mitogen-activated protein/extracellular signal-regulated kinase*) inhibitors acting at the tiered serine/threonine kinase Raf/MEK/extracellular regulated kinase (ERK) signaling pathway, able to suppress the propagation of the pandemic H1N1 influenza virus and highly pathogenic avian influenza virus in vitro and in



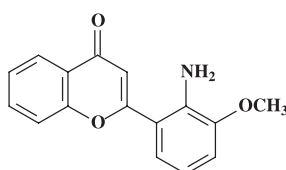
U0126



PD 184352



PD 0325901



PD 098059

Figure 9. Structures of MEK inhibitors U0126, PD 184352, PD 0325901, and PD 098059.

vivo.¹⁸² Among the MEK inhibitors, PD 0325901 and PD 184352 (Fig. 9) have been used in clinical trials against cancer.^{183,184} They are also inhibitory to influenza virus infection in vitro.¹⁸² MEK inhibitors such as U0126 not only reduce virus titers in vitro and in vivo, but also reduce proinflammatory cytokine expression.¹⁸⁵

MEK inhibitors, such as U0126, have also been shown to suppress influenza B virus propagation.¹⁸⁶ Most importantly, to date this happened without the emergence of any resistant virus variants, demonstrating that influenza viruses cannot easily adapt to interference with cellular functions.

Influenza virus infections require the induction of a variety of cytokines including those that are regulated by transcription factors of the activating protein-1 (AP-1) family and the NK (Jun-N-terminal kinase) pathway.¹⁸⁷ These different protein kinase pathways may ultimately lead to RANTES production in influenza virus-infected human bronchial epithelial cells.¹⁸⁸

The Raf/MEK/ERK cascade is the prototype of mitogen-activated protein (MAP) kinase cascades: inhibition of Raf-signaling results in nuclear retention of viral ribonucleoprotein complexes (RNPs), and concomitant inhibition of virus production. Signaling through the mitogenic cascade seems to be essential for influenza virus production.¹⁸⁹

The Raf, MEK, and ERK pathway not only plays an important role in the replication of influenza A and B virus, but also in the replication of HIV,^{190,191} Coxsackie virus B3,¹⁹² coronavirus,¹⁹³ and HSV.¹⁹⁴ MEK inhibitors such as U0126 should therefore impair the propagation of these viruses, as has been specifically shown for U0126 against HSV-2¹⁹⁴ and X4 HIV-1.¹⁹¹

The Raf/MEK/ERK signaling cascade is activated upon infection with Borna disease virus (BDV), a noncytolytic highly neurotropic single-stranded RNA virus, the only known member of the Bornaviridae (Mononegavirales) and, again, the MEK inhibitor U0126 was found to block spread of BDV in cultures cells.¹⁹⁵

The pathogenesis of hemorrhages in dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) is poorly understood. The hemorrhages may be related to the induction of the plasminogen activator inhibitor type 1 (PAI-1) via activation of the MEK/ERK pathway, the MEK inhibitor U0126 almost completely suppressed PAI-1 expression,¹⁹⁶ and may therefore be assumed to suppress hemorrhages in DHF/DSS.

The MEK/ERK pathway is also associated with the MAP kinases (MAPKs), which may contribute to the visna virus-induced processes leading to neurodegenerative pathology. Treatment of visna virus-infected cells with PD 98059 (Fig. 9), which had since long been recognized as a specific inhibitor of MAPK,^{197,198} abolished visna virus replication,¹⁹⁹ attesting as to the potential of PD 98059 to prevent the neuropathology of visna virus.

The replication of HCMV depends on a number of protein kinase pathways. Sorafenib is a multitargeted tyrosine kinase inhibitor registered for anticancer treatment (Nexavar[®], Bayer and Onyx Pharmaceuticals). Through the MAPK signaling pathway, sorafenib may also interfere with the replication of HCMV.²⁰⁰ Imatinib may suppress HCMV replication through inactivation of the platelet-derived growth factor- α receptor (PDGFR), which is a critical receptor required for HCMV infection.²⁰¹

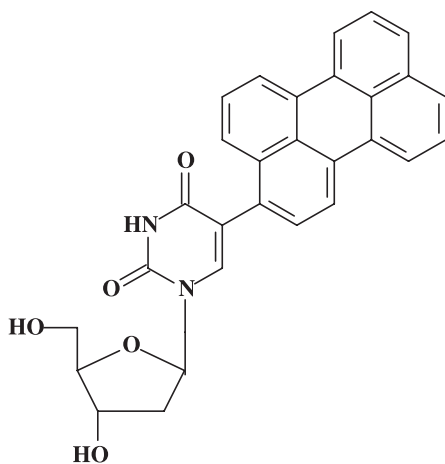
Various protein kinase inhibitors containing a quinazoline moiety such as gefitinib (Iressa[®], AstraZeneca and Teva) exert anti-HCMV activity in vitro and in vivo (gefitinib also inhibits the HCMV kinase UL97).²⁰² Furthermore, HCMV replication depends on the MEK/ERK pathway, and could therefore be suppressed by MEK inhibitors such as PD 98059.²⁰³

In conclusion, MEK inhibitors may exhibit a broad-spectrum activity against a multitude of viruses, including influenza, herpes simplex, HIV and other retroviruses, dengue, corona, and HCMV. Although the antiviral effects could not be considered as highly specific, the advantage

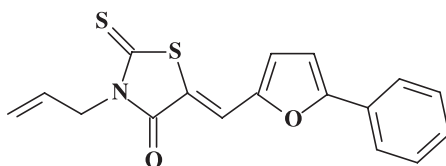
of MEK inhibitors, in view of their action targeted at a cellular process, is that they are unlikely to lead to the (rapid) emergence of drug resistance.

11. BROAD-SPECTRUM ANTIVIRAL AGENTS TARGETING ENTRY OF ENVELOPED VIRUSES

In the February 16, 2010, issue of the Proc Natl Acad Sci (USA) appeared a paper on a broad-spectrum antiviral agent LJ-001 targeting entry of envelope viruses,²⁰⁴ followed in the October 5, 2010, issue of the same Journal by a remarkably similar antiviral activity of a structurally unrelated inhibitor dUY11.²⁰⁵ LJ-001 is a rhodanine derivative and dUY11 is a rigid amphipathic fusion inhibitor (RAFI) derived from 2'-deoxyuridine (Fig. 10). They both possess a rigid and planar hydrophobic moiety. With their hydrophobic moiety, LJ-001 and dUY11 would intercalate into the lipid bilayer of the viral envelope, thereby affecting the virus-cell fusion process. Both LJ-001 and dUY11 should, in principle, be active against all enveloped viruses. For two important human pathogens, HCV and HSV (-1 and -2), this activity was demonstrated, but for several others (i.e., yellow fever, dengue, Japanese encephalitis), this was not. In fact, the compounds should be compared side by side for their spectrum of activity. Admittedly, they should not easily lead to drug resistance development, but other issues should be further addressed, that is, in vivo activity and selectivity, biodistribution, drug formulation,



dUY11



LJ-001

Figure 10. Structures of dUY11 and LJ-001.

and pharmacodynamics, before their therapeutic value could be assessed. LJ-001 and dUY11 herald a new approach or strategy to combat enveloped virus infections, but the question can be raised whether they are druggable as well.

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Erik De Clercq, M.D., Ph.D., has been Chairman of the Department of Microbiology and Immunology of the Medical School at the Katholieke Universiteit Leuven (K.U. Leuven) as well as Chairman of the Board of the Rega Institute for Medical Research, until September 2006 when he was forced to retire. He is a member the Belgian (Flemish) Royal Academy of Medicine, a member of the Academia Europaea, and Fellow of the American Association for the Advancement of Science. He has also been the titular of the Prof. P. De Somer Chair for Microbiology. He has been teaching the courses of Cell Biology, Biochemistry, and Microbiology at the K.U. Leuven (and Kortrijk) Medical School until September 2006, and continued to teach part of the Biochemistry course at the K.U. Leuven Campus Kortrijk until 2009. Since 2008, he has been teaching the course of “Biochemistry at the Service of Medicine” at the Faculty of Sciences at the University of South Bohemia (Česke Budějovice, Czech Republic). Professor De Clercq received in 1996 the Hoechst Marion Roussel (now called “Aventis”) award (American Society for Microbiology), in 2000 the Maisin Prize for Biomedical Sciences (National Science Foundation, Belgium), and in 2010 the IS3NA John A. Montgomery Award and the Dr. Paul Janssen Award for Biomedical Research for his pioneering efforts in the field of antiviral research. He is a honorary doctor of several Universities [i.e., Ghent (Belgium), Athens (Greece), Ferrara (Italy), Shandong (Jinan, China), Charles University (Prague, Czech Republic), Česke Budějovice (Czech Republic), Tours (France), and Hull (United Kingdom)]. In 2008, he was elected European Inventor of the Year (Life time achievement award) and in 2011 Alumnus of the Year 2011 (Faculty of Medicine, K.U. Leuven, Belgium). His scientific interests are in the antiviral chemotherapy field, and, in particular, the development of new antiviral agents for various viral infections, including herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B virus (HBV), human papilloma virus (HPV), and hepatitis C virus (HCV). He has (co)-discovered a number of antiviral drugs, currently used in the treatment of HSV infections (valaciclovir, Valtrex[®], Zelitrex[®]), VZV infections (brivudin, Zostex[®], Brivirac[®], Zerpex[®]), CMV infections (cidofovir, Vistide[®]), HBV infections (adefovir dipivoxil, Hepsera[®]), and HIV infections (AIDS) (tenofovir disoproxil fumarate, marketed as Viread[®], and, in combination with emtricitabine, as Truvada[®]; and in combination with both emtricitabine and efavirenz, as Atripla[®]). The combination of Truvada[®] with rilpivirine (Edurant[®]) has been approved in 2011 (Complera[®], Eviplera[®]) for the treatment of HIV infections (AIDS). Viread[®] has since 2008 been approved for the treatment of HBV infections (chronic hepatitis B).