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STATUS PRESENS OF ANTIVIRAL DRUGS AND STRATEGIES: PART II: RNA VIRUSES (EXCEPT RETROVIRUSES)

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ABSTRACT

More than 40 compounds have been formally licensed for clinical use as antiviral drugs, and half of these are used for the treatment of HIV infections. The others have been approved for the therapy of herpesvirus (HSV, VZV, CMV), hepadnavirus (HBV), hepacivirus (HCV) and myxovirus (influenza, RSV) infections. New compounds are in clinical development or under preclinical evaluation, and, again, half of these are targeting HIV infections. Yet, quite a number of important viral pathogens (i.e. HPV, HCV, hemorrhagic fever viruses) remain in need of effective and/or improved antiviral therapies.

I. INTRODUCTION

There are at present a forty some antiviral drugs that have been formally licensed for clinical in the treatment of viral infections.[31] These are mainly used in the treatment of infections caused by human immunodeficiency virus (HIV), hepatitis B virus (HBV), herpesviruses [herpes simplex virus (HSV), varicella-zoster virus (VZV),

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cytomegalovirus (CMV)], orthomyxoviruses (influenza), paramyxoviruses [respiratory syncytial virus (RSV)], and hepaciviruses [hepatitis C virus (HCV)]. As these are the viruses that are most in demand of antiviral therapy, they have prompted the search for new antiviral strategies and drugs directed towards either the same molecular targets as the approved antiviral drugs or to other targets.

Most of the newly described antiviral compounds (that are currently in development) are targeted at HIV, HBV or HCV. Some are targeted at HSV, VZV or CMV, but, there are, in addition, many other important viral pathogens for which medical intervention, either prophylactic or therapeutic, is highly needed, and, these are, among the DNA viruses, the papillomaviruses [human papilloma virus (HPV)], adenoviruses, poxviruses (variola, vaccinia, monkeypox, ...) and the herpesviruses Epstein–Barr (EBV) and human herpesvirus type 6 (HHV-6), and, among the RNA viruses, enteroviruses (i.e. Coxsackie B and Echo), coronaviruses [i.e. severe acute respiratory syndrome (SARS)-associated coronavirus], flaviviruses (i.e. Dengue, Yellow fever) and other RNA viruses associated with hemorrhagic fever [arenaviruses (i.e. Lassa fever), bunyaviruses (i.e. Rift Valley fever, Crimean-Congo fever) and filoviruses (i.e. Ebola and Marburg)].

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

II. PICORNAVIRUSES (ENTERO- AND RHINOVIRUSES)

Among the enteroviruses, polio and hepatitis A can be efficiently controlled by vaccination: for polio both a life attenuated and an inactivated ("killed") virus vaccine, whereas for hepatitis A an inactivated virus vaccine is available. The other enteroviruses (Coxsackie A and B and echoviruses) and the rhinoviruses need to be approached by chemotherapeutic agents. No single antiviral drug has ever been licensed for clinical use against entero- or rhinovirus infections. The most extensively studied for its potential against enteroviruses has

been pleconaril. This compound binds to a hydrophobic pocket beneath the "canyon floor" of the VP1 capsid protein of picornaviruses,[93] thereby "freezing" the viral capsid and preventing its dissociation (uncoating) from the viral RNA genome. The clinical efficacy of pleconaril has been evaluated in experimentally induced enterovirus (Coxsackie A21) respiratory infections in adult volunteers[145] and, on a compassionate basis, against potentially life-threatening enterovirus infections.[142] Pleconaril has also been shown to reduce the duration and severity of picornavirus-associated viral respiratory illnesses in adolescents and adults.[66,67] Pleconaril would also shorten the course of enteroviral meningitis (as compared to placebo recipients), albeit only modestly in patients with more severe disease.[37]

For the prevention and/or treatment of rhinovirus infections ("common colds") inhibitors of the human rhinovirus (HRV) 3C protease have been extensively investigated. Ruprintrivir is an irreversible 3C protease inhibitor,[43] which, upon intranasal administration in human volunteers, appeared to be safe and well tolerated.[75] In experimentally induced rhinovirus colds in healthy volunteers, ruprintrivir prophylaxis reduced the proportion of subjects with positive viral cultures but did not decrease the frequency of colds.[68] Another, irreversible inhibitor of HRV 3C protease, here referred to as a pyrrolidinyl pentenoic acid ethyl ester [compound 3[44] or compound 1[130]] offers the advantage to be orally bioavailable: in healthy volunteers, single oral doses of this compound appeared to be safe and well tolerated, although the compound is currently not progressing toward clinical development.[130]

Despite extensive research efforts that have led to the discovery of many potent antiviral agents, no drug today has been approved for the treatment or prevention of rhinovirus-associated illnesses. There are several reasons to consider when trying to understand this situation.[129] In the majority of individuals, rhinovirus-induced colds are mild and self-limiting. This alone dictates that potential drugs must be very safe and have a high risk/benefit ratio. The agent must have limited side effects and have no or low risk of resistance development and, furthermore, must be administered with low frequency (e.g. less than three times a day). To date no agent has been able to achieve these criteria and demonstrate appropriate clinical efficacy.[129]

In great need of antiviral treatment are the often severe complications of Coxsackie B virus infections, such as myocarditis which may lead to idiopathic dilated cardiomyopathy. In mice, Coxsackie B3 virus-induced myocarditis is inhibited by the immunosuppressive agent mycophenolic acid (MPA) mofetil.[124] This beneficial outcome must apparently result from the immunosuppressive effect of MPA

(through inhibition of IMP dehydrogenase and, hence, GTP supply), since MPA did not reduce the infectious virus titers in the myocard. A more pronounced inhibitory effect on Coxsackie B3 virus-induced myocarditis, accompanied by a marked reduction in the virus titers in the heart, was obtained with the interferon inducer poly(I)·poly(C) and poly(I)·poly(C_{12}U) (also known as Ampligen), and to a lesser extent with (pegylated) interferon-α2b.[125] Combination of an inhibitor of viral replication (such as Ampligen) with an immunosuppressant (such as MPA mofetil) could be an ideal treatment strategy for viral myocarditis, whether due to Coxsackie B or other viruses. How to implement such as treatment regimen in the clinical setting should be further addressed.

To investigate whether RNA interference (RNAi) can protect against Coxsackie virus B3 infection, several Coxsackievirus B3-specific small interfering RNAs (siRNAs) targeting distinct regions of the viral genome were evaluated, the most effective one (in inhibiting virus replication) being that targeting the viral protease 2A.[200] A primordial requirement for being effective was a perfect sequence match in the central region of the target.[200] As shown for poliovirus,[54] the virus may readily escape from RNA interference (RNAi) through unique point mutations (i.e. resulting in G:U mismatches) within the targeted regions; however, the emergence of resistant virus could be prevented by using a pool of siRNAs to simultaneously target multiple sites in the viral genome.[54]

III. ALPHA- AND FLAVIVIRUSES (YELLOW FEVER, DENGUE, WEST NILE, . . .)

No antivirals are currently available for the treatment of alpha- or flavivirus infections (although there is a live virus vaccine routinely used for the prophylaxis of Yellow fever), and the prospects for an effective therapy of flavivirus infections do not seem encouraging.[96] Antiviral compounds such as ribavirin have only weak activity against flaviviruses. Greater hope may be vested in interferon and interferon inducers. Based on infection of hamsters with the murine Modoc virus, an experimental flavivirus encephalitis model has been developed, which is reminiscent of Japanese encephalitis virus infection in humans.[97] In a related model with Modoc virus in SCID mice, both interferon-α2b (whether pegylated or not) and interferon inducers [poly(I)·poly(C) and Ampligen) were shown to significantly delay virus-induced morbidity (paralysis) and mortality (due to progressive

encephalitis).[98] Ribavirin did not provide any beneficial effect in this model, whether given alone or in combination with interferon.

Recently, new inhibitors of flavivirus infection have been identified through high-throughput screening of a compound library. In particular, triaryl pyrazoline {[5-(4-chloro-phenyl)-3-thiophen-2-yl-4,5-dihydro-pyrazol-1-yl]-phenyl-methanone} was found to inhibit the *in vitro* replication of a number of flaviviruses (i.e. West Nile, Dengue, Yellow fever, St. Louis encephalitis) as well as some other viruses such as Western equine encephalitis virus (an alphavirus), mouse hepatitis virus (a coronavirus) and vesicular stomatitis virus (a rhabdovirus).[134] The triaryl pyrazoline would be specifically targeted at viral RNA synthesis.[134]

RNA interference (RNAi) as demonstrated for hepadna-, picorna-, hepaci-, corona-, myxo- and retroviruses may also be further explored as an antiviral approach to control alpha- and flaviviruses, for example, at the level of their vector mosquitoes. RNA interference acts as a natural antiviral response to infection of *Anopheles gambiae* by the alphavirus O'nyong-O'nyong[82] and for two mosquito-borne viruses, Semliki Forest virus (SFV) and Dengue virus (serotype 1), it has been shown that their replication in mosquito cells could be specifically blocked by si (double-stranded) RNA.[13] This indicates that the use of specific siRNAs may inhibit virus replication in the insect host and thus prevent disease transmission.

IV. HEPACIVIRUSES (HCV)

Current, approved therapy for chronic hepatitis C consists of pegylated interferon-α2 (180 µg, parenterally, once weekly) combined with ribavirin (1000 or 1200 orally, daily).[49,112] This treatment regimen is associated with a sustained viral response in at least 50% of the patients infected with HCV genotype 1, and of 80% in patients infected with another genotype (2, 3 or 4) of HCV. Duration of treatment is 48 weeks (or longer) for patients infected with HCV genotype 1, but may be reduced to 24 weeks for patients infected with another genotype.

Although interferon is generally acting as an immunomodulatory agent (i.e. in the treatment of hepatitis B) and ribavirin is an antiviral agent, when the two agents are used in combination against hepatitis C, they appear to act the other way around.[31] Interferon appears to be targeted at the phosphoprotein encoded by the non-structural NS5A gene of the HCV genome.[166] thus achieving its antiviral effect, whereas

ribavirin, akin to MPA, primarily acts as an inhibitor of IMP dehydrogenase, thus reducing the biosynthesis of GTP. Ribavirin has recently been shown to modulate T-cell reactivity to HCV, i.e., by suppressing IL-10 production.[140]

The combination of peginterferon α-2a with ribavirin has also been advocated for the therapy of HCV infection in patients with HIV coinfection.[178] However, it should not be forgotten that, should these patients be treated (for their HIV infection) with azidothymidine (zidovudine, ZDV), the latter may be antagonized by ribavirin.[180] Therefore it was re-assuring to note that ribavirin (at 800 mg/day) administered in combination with peginterferon α-2a did not significantly affect the intracellular phosphorylation or plasma pharmacokinetics of ZDV (or other pyrimidine dideoxynucleosides such as 3TC or d4T) in HIV/HCV-co-infected patients.[141]

In addition to peginterferon α-2a and peginterferon α-2b, other interferons are in clinical development, e.g. albuferon-α (IFN-α-2b fused to human serum albumin) which allows dosing at intervals of 2–4 weeks compared with one week for the peginterferons.[30] Consensus interferon (i.e. alfacon-1), when combined with ribavirin, has been shown to achieve a higher sustained response rate in naïve patients with chronic hepatitis C as compared to standard IFN-α and ribavirin.[30] Recently, a novel IFN-α variant (GEA 007.1) has been described which would have a better inhibitory activity than the standard IFN-α2b in the HCV replicon system, due to a more potent activation of the JAK-STAT signaling pathway.[48]

In the combination with peginterferon, ribavirin may be advantageously replaced by viramidine (taribavirin), its amidine analogue (which is converted, mainly in hepatocytes, by adenosine deaminase, to ribavirin), as the latter has a reduced uptake by, and, therefore, lesser toxicity for red blood cells, as compared to ribavirin.[186] Viramidine would give less anemia as compared with ribavirin. Phase III studies with viramidine combined with peginterferon, as compared to ribavirin combined with peginterferon, are eagerly awaited to assess which one to choose, ribavirin or viramidine.

Taking into account the duration of the combined interferon plus ribavirin treatment, the therewith associated side effects and costs, and the partial responses observed with this treatment regimen, fierce attempts have been made, rightfully, to develop more selective anti-HCV agents, targeted at specific viral proteins such as the NS3.4A serine protease and RNA helicase, and the NS5B RNA replicase (RNA-dependent RNA polymerase). Also the HCV p7 protein, which forms an

ion channel and can be blocked by long-alkyl-chain iminosugar derivatives, has been considered as a potential target for antiviral therapy.[131]

Proof-of-principle that compounds targeted at the NS3.4A protease could reduce plasma concentrations of HCV RNA has already been delivered with BILN 2061 administered orally for no longer than 2 days in patients infected with HCV genotype 1.[89] BILN 2061 (Ciluprevir), for which an efficient large-scale synthetic procedure has been described recently,[196] was able to reduce HCV RNA levels by 2–3 $\log_{10}$ in patients infected with HCV genotype 1, after 2 days of treatment,[72] but in patients infected with HCV genotypes 2 or 3, it proved less effective, apparently due to a lower affinity of BILN 2061 for the HCV protease of genotypes 2 and 3, as compared to genotype 1.[138] Replacement of five residues at positions 78, 79, 80, 122, and 132 could account for most of the reduced sensitivity of genotype 2b protease,[169] while replacement of residue 168 alone,[169] or in combination with substitution of residues at positions 123 and 132,[177] could account for the reduced sensitivity of genotype 3a. Apparently, the rigidity of BILN 2061, while conferring greater potency against genotype 1, rendered it more sensitive to variations near its binding site at the NS3.4A protease.[177] Despite the robust antiviral response observed with BILN 2061 in genotype 1 HCV-infected individuals, further clinical development of this compound was halted because of (cardio)toxicity issues in animals.

Another NS3.4A protease inhibitor, which differs in its *in vitro* resistance profile from BILN 2061, is VX-950 (telaprivir).[102] While substitution of Ala156 with either valine (A156V) or threonine (A156T) led to cross-resistance between BILN 2061 and VX-950 [but also reduced fitness (or replication capacity) in a transient replicon cell system,[103] the major BILN 2061-resistant mutants (D168V and D168A) were fully susceptible to VX-950, and, *vice versa*, the dominant VX-950-resistant mutant (A156S) remained sensitive to BILN 2061.[102,103] Thus, VX-950 and BILN 2061 must elicit resistance to HCV protease (NS3.4A) by different mechanisms.[103]

VX-950 (750 mg every 8 hours) was found to achieve, at the end of a 14 day-treatment, a main reduction of HCV RNA of 4.4 $\log_{10}$. Out of all patients receiving VX-950, 26/28 showed a >3 log decline of HCV RNA.[137] [In some patients dosed with VX-950, the virus became undetectable at day 14 of dosing.] The overall preclinical profile of VX-950 supports its candidacy as a novel oral therapy against hepatitis C.[132] The combination of VX-950 and interferon-α was found to be additive to synergistic in reducing HCV RNA in replicon cells, and this combination also suppressed the emergence of *in vitro* resistance mutations

against VX-950 in replicon cells.[104] Following promising results with VX-950 in phase I clinical trials, the compound has now progressed to phase II trials where it is evaluated in combination with peginterferon-α with or without ribavirin.

In addition to VX-950, other, 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid-based macrocyclic inhibitors of HCV NS3.4A protease[20] as well as SCH 503034, a mechanism-based inhibitor of HCV NS3.4A protease,[179,109] are in preclinical development. In fact, the latter was found to act synergistically with α-interferon in suppressing HCV replicon synthesis.[109] SCH 503034 [compound **70** in Venkatraman et al.[179]] demonstrated good oral bioavailability in rats and dogs[179] and has been advanced to clinical trials in humans for the treatment of HCV infections. SCH 503034 at the highest dose used 400 mg three times daily for 14 days achieved a 1.5 $\log_{10}$ decline in viremia[201] SCH 503034 is now being evaluated in combination with pegylated interferon-α, with or without ribavirin in ongoing 48-week phase II studies.

The HCV protease inhibitor SCH 503034 selected for a number of mutations, i.e. T54A, V170A, A156S and A156T; the A156T mutation conferred the highest level of resistance to SCH 503034 but also led to the greatest reduction in fitness.[176] The A156T mutation also conferred high level resistance to SCH6 (SCH 446211), a novel ketoamide (structurally related to SCH 503034) inhibitor of the NS3.4A protease.[198] A novel mutation, R109K, was identified which conferred moderate resistance only to SCH6. Unlike R109K which had minimal impact on NS3.4A enzymatic activity, A156T significantly reduced the enzymatic activity, polyprotein processing and replication fitness.[198] However, three separate second-site mutations, P89L, Q86R and G162R were capable of partially reversing A156T-associated fitness without significantly reducing resistance to the protease inhibitor.[198]

In addition to the NS3.4A protease, the NS5B RNA replicase has also been perceived as an attractive target for the development of HCV inhibitors. Highly potent and selective antiviral agents (i.e. VP32947) [*N*-propyl-*N*-[2-(2*H*-1,2,4-triazino[5,6-b]indol-3-ylthio)ethyl]-1-propanamine][5] -and compound 1453 [1-[2-diethylamino)ethyl]-6-(1*H*-imidazol-1-yl)-1,3-dihydro-2*H*-benzimidazol-2-one][165] targeted at the viral RNA replicase have been described to inhibit the replication of bovine viral diarrhoea virus (BVDV), a pestivirus which could be considered as a surrogate virus for HCV[5,165] We have recently described two novel series of compounds [prototypes: 5-[(4-bromophenyl)methyl]-2-phenyl-5*H*-imidazo[4,5-c]pyridine (BPIP)][126] and ethyl-2-methylimidazo[1,2-a]pyrrolo[2,3-c]pyridin-8-carboxylate AG110,[128] which act as "non-

nucleoside" RNA replicase inhibitors (NNRRIs) and effect a highly potent and selective inhibition of the replication of BVDV.[126,128] From the BPIP class of compounds,[135] new congeners have been derived that act equally efficiently against HCV replication.[136] In future treatment strategies for HCV infections, these NNRRIs may likely to be combined with "nucleoside" RNA replicase inhibitors (NRRIs), in analogy with the strategy followed for the treatment of HIV infections, where NRTIs are combined with NNRTIs (see *infra*).

The sole NRRI which has already proceeded to phase I/II clinical trials for the therapy of hepatitis C is the 3′-*O*-valine ester of 2′-*C*-methylcytidine (NM-283, valopicitabine)I, which can be administered by the oral route[33,2] and shows enhanced antiviral efficacy if combined with peginterferon (valopicitabine is currently in phase II combination trials with pegylated interferon-α). It would be wise not to add ribavirin to this combination, as it has been shown that ribavirin antagonizes the *in vitro* anti-HCV activity of 2′-*C*-methylcytidine, the active compound of valopicitabine.[28]

HCV replicons that are resistant to 2′-*C*-methylcytidine (and 2′-*C*-methylpurine nucleosides) can be readily isolated *in vitro*, and resistance is the result of the S282T mutation in the NS5B gene. As a consequence of the S282T mutation, 2′-*C*-methyl CTP is no longer incorporated during the initiation step of RNA synthesis. In addition, the presence of the S282T mutation also compromises incorporation of the natural nucleotides, which may translate in decreased viral fitness.[45]

In addition to 2′-*C*-methylcytidine, several other ribonucleoside analogues (NRRIs) have been reported to inhibit HCV replication (as reviewed by):[174] 2′-*O*-methylcytidine,[14], 2′-*C*-methyladenosine,[14,171] 7-deaza-2′-*C*-methyladenosine,[122] 2′-*C*-methylguanosine,[115,46] 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (PSI-6130),[159] and 4′-azidocytidine (R1479).[86]

PSI-6130 is a specific inhibitor of HCV.[159] It has no anti-BVDV activity. The fact that PSI-6130 shows reduced activity against the RdRp S282T mutant, which is resistant to 2′-*C*-methylated nucleosides suggests that PSI-6130 is an inhibitor of HCV RNA synthesis.[159] In contrast, R1479 (4′-azidocytidine) did not show cross-resistance with 2′-*C*-methylcytidine.[91] Thus, the S282T mutation[86] did not confer resistance to R1479. *In vitro* studies mapped resistance to R1479 to amino acid substitutions S96T and S96T/N142T of the NS5B polymerase.[91]

All the aforementioned nucleoside analogues are, in principle, non-obligate chain-terminating nucleoside analogues. It would now seem interesting to prepare and evaluate the corresponding obligatory chain-terminating 3′-deoxyribonucleoside analogues for their anti-

HCV activity. This was recently done for 2′-*C*-methylcytidine and 2′-*C*-methyluridine, which were modified at the C-3′ position.[133] The newly synthesized compounds were quoted as antivirally inactive, but HCV did not figure among the viruses that were evaluated for their susceptibility to the compounds.

A new class of HCV NS5B RNA replicase inhibitors is represented by the α, γ-diketo acids (DKAs),[162,163] one of the more active DKAs being DKA compound 30. These compounds are reminiscent of the DKA type of HIV integrase inhibitors (see *infra*) and are assumed to inhibit the HCV polymerate activity *via* chelation of the active site Mg^{++} ions. In a certain sense they may be considered as "pyrophosphate mimics", thus acting as product-like inhibitors of the polymerase reaction.[174]

In recent years, a pleiade of NNRRIs has been described to act in a very similar ("allosteric") fashion with HCV NS5B RNA replicase as NNRTIs do with respect to the HIV reverse transcripase: benzimidazole-based derivatives[172] [i.e., benzimidazole 5-carboxamide derivatives,[7,88] indole-*N*-acetamide derivatives,[61,39] benzo-1,2,4-thiadiazine derivatives,[38,173] phenylalanine derivatives,[183] thiophene 2-carboxylic acid derivatives,[17,8] dihydropyranone derivatives,[107] the tetrahydropyranoindolyl acetic acid derivative HCV-371 (R-enantiomer of the racemic mixture HCV-570)[73,74] and the *N*-1-aza-4-hydroxyquinolone benzothiadiazine A-782759.[117] The allosteric binding site for some of these compounds have already been identified by crystallographic studies.[39,183,17,8] It corresponds to a narrow cleft on the protein's surface in the "thumb" domain, about 30–35 Å from the enzyme's catalytic center.[183,17]

Curiously, most of the NNRRIs that are active against the HCV NS5B polymerase contain, besides a large hydrophobic region, a carboxylic acid group (or a similar motif) that allows hydrogen bonding with main chain amide nitrogen atoms (i.e. Ser 476 and Tyr 477, as demonstrated for the phenylalanine derivative).[183] For the indole-*N*-acetamide derivatives, it has been suggested that they may displace part of the fingertip loop anchoring the fingers domain to the thumb domain.[39] Using the thiophene 2-carboxylate type of inhibitors, it has been reported that these inhibitors can only be soaked in crystal form I (which adopts a "closed" conformation that is believed to be the active form, and not in form II (which adopts an "open" conformation, and is thus in the inactive form.[8] Resistant mutations that emerged with the benzimidazole 5-carboxamide and related compounds were found at three amino acid positions in the thumb domain: Pro495 (P495S/L/A/T), Pro496 (P496S/A) and Val499 (V499A); mutation at

each of these positions conferred different levels of resistance (in decreasing order P495S/L/A/T > P496S/A > 499A.[88]

Mutations conferring resistance to both the HCV NS5B RNA replicase (i.e. H95Q, N411S, M414L, M414T or Y448H) and NS3 protease (i.e. A156V or D168V) have been identified).[117] These mutations conferred high levels of resistance to A-782759 and BILN 2061, respectively. However, the A-782759-resistant mutants remained susceptible to the NRRIs and other classes of NNRRIs, as well as interferon. In addition, the dually (A-782759- and BILN 2061-) resistant mutants displayed significantly reduced replicative ability as compared to the wild-type. These findings support a rationale for drug combinations in the therapy of HCV infections.[117]

As recently reviewed,[30] several other approaches, including ribozymes, antisense oligonucleotides, and RNA interference (RNAi) based on short interfering (si)RNAs could be envisaged to target the HCV genome. ISIS 14803, a 20-base antisense oligonucleotide complementary to the internal ribosome entry site (IRES), in a phase I clinical trial gave transient HCV reduction (1.2–1.7 $\log_{10}$) in 3/28 patients but ALT (alanyl transaminase) flares up to 10-fold in 5/28 patients.[113]

Short interfering (si)RNAs, aimed at posttranscriptional gene silencing, may be considered an attractive approach to curtail HCV infections.[87] The siRNA approach has proven to be efficacious *in vivo*, in suppressing (SARS) coronavirus infections[100] and influenza A virus infections[52,175] in monkeys[100] and mice,[52,175] respectively.

From the screening of various marketed compounds, arsenic trioxide (As_2O_3) emerged as a potent HCV inhibitor.[77] Similarly, sodium stibogluconate (SSG), along with several other antimonial compounds, including Sb_2O_3 and $SbCl_3$ were found to exert potent anti-HCV activity at concentrations that did not affect cell viability.[78] When SSG was combined with interferon-α, these two drugs acted synergistically to suppress HCV replication.[78]

Various cellular targets may also be envisaged to interfere with HCV infections. In this sense, α-glucosidase inhibitors such as deoxynojirimycin have been shown to disturb the morphogenesis of HCV-like particles and may eventually be useful to fight HCV infections as part of drug combination protocols.[18] Another cellular target for chemotherapeutic intervention is cyclophilin B (CyP B) which is critical for the efficient replication of the HCV genome. CyP B is a functional regulator of the HCV RNA polymerase.[185] Cyclosporin A which interacts with CyP B inhibits HCV replication *in vitro*.[185] We have recently demonstrated that the non-immunosuppressive cyclosporin DEBIO-025 is even 10-fold more potent an inhibitor of HCV replication.[127]

DEBIO-025 proved also additive to slightly synergistic when combined with interferon-α2a, and at concentrations of 0.5 and 1 μg/ml was able to clear the cells from their HCV replicon within three to four passages, whereas treatment with cyclosporin A at the same concentrations for seven consecutive passages did not result in clearance of the HCV replicon.[127] DEBIO-025 may form an attractive new option for the therapy of HCV infections, particularly in HCV/HIV co-infected patients,[127] and the clinical studies with DEBIO-025 have been recently initiated.

V. CORONAVIRUSES (SARS)

As for HCV, there are several proteins encoded by the SARS coronavirus which could be considered as targets for chemotherapeutic intervention: i.e. the spike (S) protein, the 3C-like main protease, the NTPase/helicase, the RNA-dependent RNA polymerase (RNA replicase), and, possibly, other viral (or cellular) protein-mediated processes.[157] The SARS coronavirus S protein mediates infection of permissive cells through interaction with its receptor, the angiotensin-converting enzyme 2 (ACE 2),[101] and monoclonal antibody to the S1 domain was found to neutralize the virus by blocking its association with the receptor ACE 2.[161]

Also the fusion of the SARS coronavirus with the cell could be considered an attractive target. To the extent that this fusion process bears resemblance to the fusogenic mechanism of HIV, i.e. with regard to heptad repeat interactions and six-helix bundle formation, it might be feasible to develop SARS coronavirus inhibitors, analogous to the HIV fusion inhibitor enfuvirtide.[105]

Following receptor binding and induced conformational changes in the spike glycoprotein, a third step would be involved in the viral entry process, namely cathepsin L proteolysis within endosomes.[154] The cathepsin-L-specific inhibitor, MDL 28170 [also known as calpain inhibitor III, or Z-Val-Phe(CHO)], at the same time inhibited cathepsin-L activity and S protein-mediated infection (at an IC_{50} of 2.5 nM and 0.1 μM, respectively). In addition to calpain inhibitor III, some other calpain inhibitors have been described as inhibitors of SARS coronavirus replication, the most selective (selectivity index >100) being calpain inhibitor VI [4-fluorophenylsulfonyl-Val-Leu(CHO)].[6]

The crystal structure of the SARS coronavirus protease has been revealed.[194,94] This offers a solid basis for the rational drug design of SARS protease inhibitors. For other potential targets such as the

NTPase/helicase and the RNA replicase (RNA-dependent RNA polymerase) such structural basis still has to be delineated.

Of a number of peptidomimetic compounds (aziridinyl peptides, ketoglutamine analogues, chymotrypsin-like protease inhibitors and peptide anilides) that have been reported as inhibitors of the SARS coronavirus main protease, the niclosamide anilide, with a $K_i = 0.03$ μM ($IC_{50} = 0.06$ μM), proved to the most potent (competitive) inhibitor.[146] There are only a few cases where the 3C-like protease inhibitors were shown to inhibit both the SARS coronavirus protease activity and virus replication in cell culture. For example, the Phe–Phe dipeptide inhibitor was found to inhibit the 3C-like protease at an IC_{50} of 1 μM and inhibited virus replication in Vero cells at an EC_{50} of 0.18 μM, while not being toxic to the host cells at a concentration of 200 μM (selectivity index: >1000).[147]

Recently, TG-0205221 was described as a potent SARS coronavirus 3C-like protease inhibitor ($K_i = 53$ nM), which reduced virus production in cell culture by 4.7 $\log_{10}$ at a concentration of 5 μM.[195] An octapeptide, specifically designed for the SARS coronavirus main protease, namely AVLQSGFR, was reported to inhibit SARS coronavirus replication in Vero cells at an EC_{50} of 0.027 μg/ml, while not being cytotoxic at 100 μg/ml, thus establishing a selectivity index of > 3700.[51] Whether this highly selective antiviral effect was actually mediated by an inhibition of the SARS coronavirus main protease was not ascertained in this study.[51]

The SARS coronavirus NTPase/helicase has been considered a potential target for the development of anti-SARS agents.[167]. Bananin and three of its derivatives (iodobananin, vanillinbananin and eubananin) were shown to inhibit both the ATPase and helicase activity of the SARS coronavirus NTPase/helicase, with IC_{50} values (for the ATPase activity) in the range of 0.5–3 μM).[168] Bananin was also found to inhibit SARS-CoV replication in fetal rhesus kidney (FRhK-4) cells at an EC_{50} of less than 10 μM and a CC_{50} of over 300 μM, thus exhibiting a selectivity index of over 30).[168] Whether the antiviral effect obtained in cell culture was causally linked to inhibition of the NTPase/helicase was not ascertained.

The SARS coronavirus RNA-dependent RNA polymerase (RdRp), because of its pivotal role in viral replication, represents another potential target for anti-SARS therapy. This enzyme does not contain a hydrophobic pocket for non-nucleoside inhibitors similar to those that have proven effective against the HCV polymerase or HIV-1 reverse transcriptase.[192] In fact, non-nucleoside HIV-1 reverse transcriptase inhibitors were shown to have no evident inhibitory effect on SARS

coronavirus RdRp activity).[21] At present, few, if any, nucleoside analogues have been recognized as specific inhibitors of the SARS coronavirus RdRp. There is N^4-hydroxycytidine, which has been accredited with both anti-HCV and anti-SARS coronavirus effects. Against SARS coronavirus it proved active at an EC_{50} of 10 μM (selectivity index $\geqslant$10)[6] Whether this antiviral effect was mediated by an inhibition of the viral RdRp was not ascertained, however.

A wide variety of "old" and "new" compounds have been reported to inhibit the *in vitro* replication of the SARS coronavirus at relatively high concentration ($\geqslant$1 μM).[31] There is no shortage of small molecules that inhibit the replication of the SARS virus within the 1–10 μM (or higher) concentration range,[188] but whether any of these molecules would be able to prevent or suppress SARS *in vivo*, remains to be determined. Typical examples of such miscellaneous compounds, often with an ill-defined mode of action but selectivity indexes up to 100, that have been reported to inhibit SARS coronavirus replication are valinomycin,[188] glycyrrhizin,[25] chloroquine,[83] niclosamide[189] and nelfinavir.[193]

Short interfering (si) RNAs have been developed that target the replicase[70] and spike (S)[202] genes of the SARS coronavirus genome, thereby silencing their expression in cell culture. Potent siRNA inhibitors of SARS coronavirus *in vitro* [i.e. the siRNA duplexes siSC2 (forward sequence: 5′-GCUCCUAAUUACACUCAACdtdt-3′) and siSC5 (forward sequence: 5′-GGAUGAGGAAGGCAAUUUAdtdt-3′), targeting the SARS coronavirus genome at S protein- and non-structural protein-12-coding regions, respectively] were further evaluated for their efficacy in a rhesus macaque SARS model),[100] and found to provide relief from SARS coronavirus infection-induced fever, diminish SARS-CoV levels and reduce acute diffuse alveolar damage. Further studies with siRNA in prophylactic and therapeutic regimens against SARS coronavirus seem warranted.

Shortly after SARS coronavirus was identified as the causative agent of SARS, interferons were shown to inhibit the replication of SARS coronavirus in cell culture *in vitro*, interferon-β being more potent than either interferon-α or -γ.[26] These observations were subsequently confirmed and extended in several other studies.[71,158,143] Interferon-β, in conjunction with interferon-γ, was found to synergistically inhibit the replication of SARS coronavirus in Vero cells.[143] Being a prophylactic rather than therapeutic agent, interferon(s) may have their highest utility in the prophylaxis or early post-exposure management of SARS. Pegylated interferon-α has been shown to reduce viral replication and excretion, viral antigen expression by type 1 pneumocytes and the

attendant pulmonary damage in cynomolgous macaques that were infected experimentally with SARS coronavirus.[58] Pegylated interferon-α is commercially available for the treatment of hepatitis C (where it is generally used in combination with ribavirin) and hepatitis B. Pegylated interferon-α as well as the other commercially available interferons (interferon-β, alfacon-1, etc.) could be considered for prevention and/or early post-exposure treatment of SARS should it re-emerge.

VI. ORTHOMYXOVIRUSES (INFLUENZA A, B)

For many years, amantadine and rimantadine have been used for the prophylaxis and therapy of influenza A virus infections, but they never gained wide acceptance, primarily because of the risk of rapid emergence of drug-resistant virus mutants. These compounds interact specifically with the matrix M2 protein, which through its function as an hydrogen ion (H^+) channel, helps in the decapsidation ("uncoating") of the influenza A virus particles. Influenza A (H1N1) viruses harboring amantadine-resistance mutations are as virulent as wild-type virus strains[1] and can be readily transmitted during antiviral pressure in the clinical setting.

In recent years, the neuraminidase inhibitors zanamivir[181] and oseltamivir[84] have become available for the therapy and/or prophylaxis of influenza A and B virus infections. Influenza has adopted a unique replication strategy by using one of its surface glycoproteins, hemagglutinin (H), to bind to the target cell receptor [which contains a terminal sialic acid (*N*-acetylneuraminic acid, NANA)], and another surface glycoprotein neuraminidase (*N*), to cleave off the terminal sialic acid, thus allowing the virus particles to leave the cells after the viral replicative cycle has been completed[31] Neuraminidase inhibitors block the release of progeny virus particles from the virus-infected cells, thus preventing virus spread to other host cells.

When used therapeutically, neuraminidase inhibitors lead to a reduction in illness by 1–2 days, a reduction in virus transmission to household or healthcare contacts, a reduction in the frequency and severity of complications (such as sinusitis and bronchitis) and a diminished use of antibiotics.[81] When used prophylactically, neuraminidase inhibitors significantly reduced the number of new influenza cases.[69] Although resistance of human influenza viruses to neuraminidase inhibitors can develop,[85] there is no evidence of naturally occurring resistance to either zanamivir or oseltamivir.[114,118] Zanamivir and oseltamivir should be effective against both influenza

A and B, and, among influenza A, the prevailing variants H_1N_1, H_3N_2 and, also, the avian "flu" H_5N_1. Zanamivir, which must be taken through (oral) inhalation, and, in particular, oseltamivir, which can be more conveniently administered as oral capsules, should be stockpiled to affront a potential influenza pandemic in the future.[184,123] With the increasing threat of the avian "flu" (H_5N_1), the need for a sufficient supply of neuraminidase inhibitors, such as oseltamivir and zanamivir, has become extremely urgent. In comparison with the neuraminidase inhibitors, the existing influenza vaccines are likely to be of limited value against newly emerging influenza virus strains.

Following zanamivir and oseltamivir, similar structure-based neuraminidase inhibitors have been developed, such as the cyclopentane derivative RWJ-270201 (peramivir)[155,152] and the pyrrolidines A-192558[182] and A-315675.[36,60] These novel neuraminidase inhibitors may themselves be considered as potential drug candidates, and, while being amenable to further optimization,[110] lead to the development of yet newer compounds with improved activity, bioavailability and/or resistance profiles.

In fact, both peramivir (RWJ-270201) and A-315675 proved effective against a panel of five zanamivir-resistant and six oseltamivir-resistant A and B influenza virus strains.[116] Oseltamivir resistance in clinical isolates of human influenza A has been associated with mutations at positions 119, 198, 274, 292 or 294 of the neuraminidase. Recently, resistance of avian influenza A H5N1 against oseltamivir was shown to be caused by the H274Y mutation.[92,34] The H274Y variant still appeared sensitive to zanamivir.[92] Prominent among the other neuraminidase inhibitor-resistant influenza A (H3N2) virus mutations (not yet demonstrated for H5N1) are E119V and R292K: whereas the R292K mutation was associated with compromised virus growth and transmissibility, the growth and transmissibility of the E119V variant were comparable to those of wild-type virus.[197]

The emergence of antiviral drug resistance during oseltamivir treatment and its association with clinical failure in immunocompromised hosts[79] and influenza A H5N1-infected patients[34] has highlighted the need for alternative therapies and the use of antiviral combinations.[63] The concept of using two or more antivirals to enhance the antiviral effects and perhaps reduce resistance emergence is decades old.[62,64,90]

Are there other antiviral agents, besides amantadine, rimantadine and the neuraminidase inhibitors, which may be considered for their potential, in the prevention and/or therapy, of influenza A virus infections, including avian influenza (H5N1)? Ribavirin has since long been recognized as a broad-spectrum antiviral agent, with particular

activity against both ortho- and paramyxoviruses.[151] While earlier observations point to the lack of oral ribavirin (at 1 g daily) in naturally occurring influenza A (H1N1) virus infection,[156] intravenous ribavirin (at 5 mg/kg/hour for 8 hours, followed by 1.5 mg/kg/hour for 2 to 6 days) may be worth exploring as a therapeutic strategy for serious influenza and parainfluenza virus infections.[65]

Recently, viramidine, the carboxamidine analogue of ribavirin was shown to have similar efficacy as ribavirin against influenza virus infections, and considering the lesser toxicity, viramidine may warrant further evaluation as a possible therapy for influenza, including H5N1.[153] Yet, other recently described compounds with specific activity against influenza A, B and C viruses are T-705 (6-fluoro-3-hydroxy-2-pyrazine carboxamide) and the 2,6-diketopiperazine flutimide, which would target the viral polymerase[50] and cap-dependent endonuclease,[170] respectively. In fact, the polymerase (PA, PB1, PB2) complex contributes to the high virulence of avian influenza H5N1, and has been considered an attractive target for novel anti-influenza virus drug development.[144]

Other approaches to curtail a potential influenza virus epidemic include carbohydrate-binding molecules, such as the defensing retrocyclin 2, which inhibit viral fusion and entry by cross-linking membrane glycoproteins, and which have been shown to inhibit influenza A virus infection,[95] as well as T- and M-tropic HIV-1 virus infections.[29] Furthermore, as has been shown for several other virus infections, i.e. HCV infections[87] and (SARS) coronavirus infections,[70,202,100] short interfering RNAs (siRNAs) specific for conserved influenza virus genes may also be expected, and have in fact been demonstrated, to protect animals against highly pathogenic avian influenza A viruses.[52,175]

VII. PARAMYXOVIRUSES (PARAINFLUENZA, MEASLES, MUMPS, RSV, HMPV, NIPAH, . . .)

Of the paramyxoviruses, parainfluenza (types 1–5) has received little attention from either a preventative or curative viewpoint; mumps and measles, like the rubellivirus rubella, are now considered to be sufficiently contained by vaccination (although, despite existence of a vaccine, over half a million of deaths per year result from measles virus, which has prompted the search for fusion inhibitors that block measles virus entry),[164] which makes RSV (respiratory syncytial virus) and hMPV (human metapneumovirus) as well as Nipah, the paramyxoviruses with the greatest need for antiviral therapy.

For RSV the only approved antiviral therapy is aerosol administration of ribavirin. In practice, however, ribavirin is rarely used owing to the technical burden of delivery by aerosol under the given circumstances (RSV bronchopneumonitis in young infants). Recently, intravenous ribavirin (together with oral corticosteroids) has proved to be a safe and cost-effective treatment for RSV infection after lung transplantation: RSV represents a risk factor for bronchiolitis obliterans syndrome, the major limiting factor for long-term survival after lung transplantation.[55]

Given the high incidence of RSV infections (which are often diagnosed as influenza), there is a high (an as yet unmet) medical need for an appropriate therapy (and prophylaxis) of RSV infections; The same holds for hMPV infections, which usually occur during the same (winter) season as RSV, mainly in young children, elderly people and immunocompromised individuals. Ribavirin certainly holds promise for the treatment of hMPV infections, as has recently been demonstrated in the mouse model for hMPV.[59]

As has been mentioned above for HCV,[87] short interfering RNAs (siRNAs) may also be applicable, if properly designed and administered (intranasally) in the treatment of respiratory virus infections:[9] using the RNA interference (RNAi) approach, Bitko et al.[9] demonstrated that respiratory syncytial virus and parainfluenza virus infections, both individual and joint, could be specifically prevented and inhibited by siRNAs instilled intranasally in the mouse, with or without transfection reagents. In the case of measles, however, the RNAi approach may either prevent or enhance viral transcription, depending on the target mRNA [nucleocapsid (N), phosphoprotein (P) and polymerase (L) mRNA *versus* the matrix (M) mRNA].[139] For avian metapneumovirus, one of the major causes of respiratory infections in poultry, siRNAs specific towards the phosphoprotein (P) led to marked inhibition of virus replication.[119]

Recently, a number of small molecules, i.e. VP-14637 and JNJ-2408068 (formerly known as R-170591), although structurally dissimilar, have been shown to fit into a small hydrophobic cavity in the inner core of the RSV fusion (F) protein, thereby interacting with the heptad repeats HR1 and HR2 domains, and to inhibit RSV fusion.[41,42,4,190] Although the therapeutic potential of these compounds in the treatment of RSV infections is presently unclear, there is no doubt that further exploration of the mechanism of interaction between these inhibitors and the F protein should facilitate the design of new RSV fusion inhibitors.[42] In cotton rats, treatment by VP-14637 (or JNJ-2408068)

small droplet aerosol for 60 min (or 15 min) significantly reduced mean lung virus titers.[190,191]

BMS-433771 was found to be a potent inhibitor of RSV replication *in vitro*:[22] it exhibited excellent potency against multiple laboratory and clinical isolates of both A and B RSV with an average EC_{50} of 20 nM. BMS-433771 inhibits fusion the (viral and cellular) lipid membranes during both the early and virus entry stage and late-stage syncytium formation.[22] BMS-433771 was shown to be orally active against RSV in BALB/c mice and cotton rats, even if administered as a single oral dose 1 hour prior to intranasal RSV inoculation.[23] It could be considered the prototype of small-molecular-weight inhibitors that target the formation of the six helical coiled-coil bundles as a prelude to virus-cell fusion, not only of RSV but also HIV.[24] In fact, starting from BMS-433771 new water-soluble (i.e. carboxylic acid-substituted benzimidazol-2-one derivatives have been synthesized with potent *in vitro* and *in vivo* activity against RSV.[199]

Peptides containing multiple copies of alternating HR1 and HR2 sequences of the terminal heptal repeats of the F-protein have been designed to inhibit F-protein-mediated fusion.[120] MBX 300 or [2,2-bis(docosyloxymethyl)propyl-5-acetoamido-3,5-dideoxyl-4,7,8,9-tetra-O-(sodium-oxysulfonyl)-D-glycero-D-galacto-2-nonulopyranosid]-onate, on the other hand, inhibits RSV attachment to the cell by targeting the G-protein.[40]

RSV attachment/fusion inhibitors targeted at either the G- or F-protein have, despite their marked *in vitro* potency and *in vivo* efficacy in the cotton rat model, not made much progress in the clinical setting, in part because of their poor pharmacokinetic properties. Therefore, new RSV inhibitors acting at a target distinct from the attachment/fusion process have been searched for. One such target is the viral nucleocapsid (N) protein which appears to be the target for the 1,4-benzodiazepines (i.e. A-60444).[15] The compound has entered phase II clinical trials (according to),[150] but data have not yet been made available.

Another target protein worth pursuing for potential anti-RSV agents is the RNA-dependent RNA polymerase L-protein, which has been identified as the point of attack for 4-amino-8-(3-{[2-(3,4-dimethoxyphenyl)ethyl]amino}propyl)-6,6-dimethyl-2-(4-methyl-3-nitrophenyl)-1*H*-imidazo[4,5-h]-isoquinoline-7,9(6*H*, 8*H*)-dione ("Compound D")[106] and 6-{4-[(biphenyl-2-ylcarbonyl)amino]benzoyl}-*N*-cyclopropyl-5,6-dihydro-4*H*-thieno[3,2-d][1]benzazepine-2-carboxamide (YM-53403).[160] Whether these compounds ("compound D" and YM-53403) have any

Table 1. The past, present and future of antiviral drugs (Part II: RNA viruses except retroviruses)

Virus	Compound		
	Approved for medical use	In clinical development	In preclinical evaluation
Picorna (Entero, Rhino)	–	Pleconaril Ruprintrivir	Pyrrolidinyl pentenoic acid (ethyl ester) Mycophenolic acid (MPA) mofetil Interferon (inducers)
Alpha and Flavi (Yellow fever, Dengue, West Nile, . . .)	–	–	Triaryl pyrazoline Interferon (inducers)
Hepaci (HCV)	Pegylated interferon-α combined with ribavirin	BILN 2061 (Ciluprevir) VX-950 (Telaprevir) NM 283 (Valopicitabine) Viramidine (Taribavirin) SCH 503034 DEBIO-025	SCH 446211 (SCH6) 2′-*C*-methylcytidine 2′-*O*-methylcytidine 2′-*C*-methyladenosine 7-Deaza-2′-*C*-methyladenosine 2′-*C*-methylguanosine 2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine 4′-Azidocytidine (R1479) DKA compd 30 Benzimidazole derivative Benzimidazole 5-carboxamide derivative

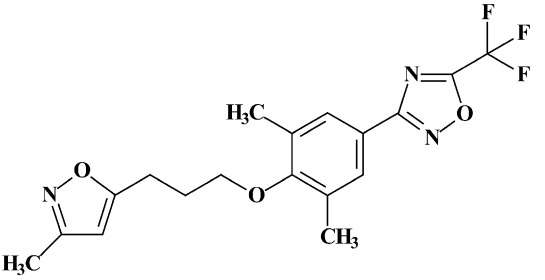
Table 1. *— Continued*

Virus	Compound		
	Approved for medical use	In clinical development	In preclinical evaluation
			Indole-*N*-acetamide derivative Benzothiadiazine derivative Phenylalanine derivative Thiophene 2-carboxylic acid derivative Dihydropyranone derivative Tetrahydropyranoindolyl acetic acid derivative HCV-371 *N*-1-Aza-4-hydroxyquinolone benzothiadiazine A-782759
Pesti (BVDV)			VP32947 Compound 1453 BPIP AG110
Corona (SARS)	–	Pegylated interferon-α (“off label”)	Calpain inhibitors (III, VI) Niclosamide anilide Phe–Phe dipeptide TG-0205221 Bananin Valinomycin Glycyrrhizin Chloroquine Niclosamide Nelfinavir

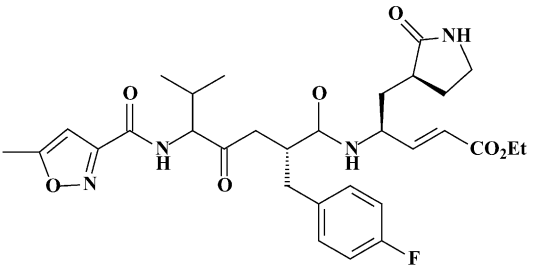
(*continued on next page*)

Table 1. *— Continued*

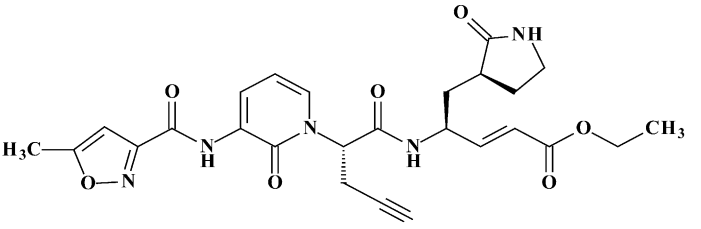
Virus	Compound		
	Approved for medical use	In clinical development	In preclinical evaluation
Orthomyxo (Influenza)	Amantadine Rimantadine Zanamivir Oseltamivir Ribavirin ('off label')	–	RWJ-270201 A-192558 A-315675 T-705 Flutimide
Paramyxo (Parainfluenza, Measles, Mumps, RSV, hMPV, ...)	Ribavirin (approved for RSV only)	–	VP-14637 JNJ-2408068 BMS-433771 MBX-300 A-60444 Compound D YM-53403 BCX 2798 & BCX-2855
Arena (Lassa, ...)	Ribavirin ("off label")	–	–
Bunya (Crimean–Congo, Rift Valley, ...)	Ribavirin ("off label")	–	–
Rhabdo (Rabies)	–	–	–
Filo (Ebola, Marburg)	–	–	3-Deazaneplancin A



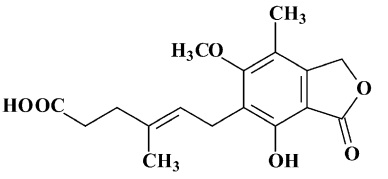
Pleconaril



Ruprintrivir

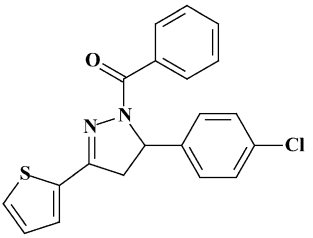


Pyrrolidinyl pentenoic acid ethyl ester

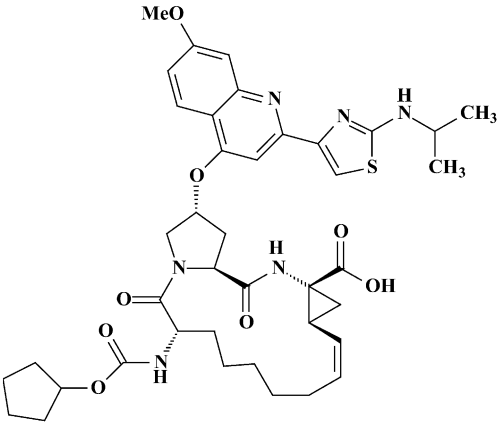


Mycophenolic acid (MPA) mofetil

Figure 1. Structural formulae of antiviral compounds.



Triaryl pyrazoline

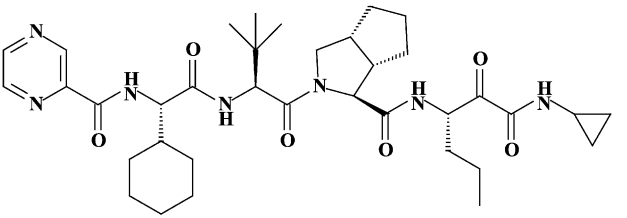


BILN 2061
Ciluprevir

Figure 1. *— Continued.*

potential in the therapy and/or prophylaxis of RSV infections remains to be further evaluated.

Although human parainfluenza viruses are important respiratory tract pathogens, especially in children, they have received little attention from either prophylactic (vaccine) or therapeutic viewpoint. Yet, they contain a unique target, the major surface glycoprotein hemagglutinin-neuraminidase (HN) that serves, at the same time, for cell attachment and virus spread. The HN inhibitors BCX 2798 and BCX 2855 were found to inhibit both functions, and to block infection with parainfluenza viruses both *in vitro* and *in vivo*[3] These compounds may limit parainfluenza virus infections in humans. Other compounds which may be further pursued for their activity against parainfluenza



VX-950
Telaprevir

Figure 1. *— Continued.*

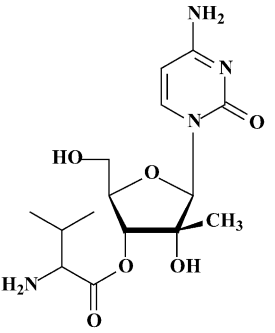
viruses, RSV, as well as influenza viruses, include flavonoids,[187] uncinosides[108] and polyoxotungstates,[148] and diffaeoylquinic acid isolated from the ethnobotanical L-deflexicalyx.[121]

VIII. ARENA-, BUNYA-, RHABDO- AND FILOVIRUSES

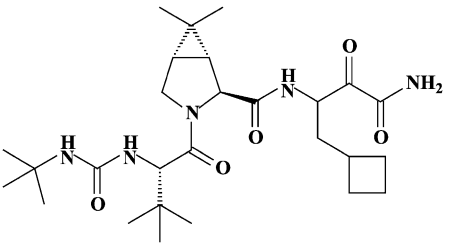
Of the 23 arenaviruses known, five are associated with viral hemorrhagic fever: Lassa, Junin, Machupo, Guanarito and Sabia.[19] Ribavirin has proven to be effective in the post-exposure prophylaxis and therapy of experimental arenavirus infections in animal models, and anecdotal reports suggest that it might also be effective in the treatment of arenavirus infections (i.e. Machupo and Sabia) in humans.[19] The most convincing evidence for the (clinical) efficacy was obtained in the case of Lassa fever, where it was found to reduce the case-fatality rate, irrespective of the time point in the illness when treatment was started[111]

Of equal significance is that in a prospective, double-blind, placebo-controlled clinical trial, intravenous ribavirin therapy was shown to reduce mortality due to Hantaan infections [HFRS (Hemorrhagic Fever with Renal Syndrome)],[76] which is not surprising as the (−)RNA bunyaviruses, and in particular, hantaviruses, are among the most sensitive RNA viruses to ribavirin *in vitro*.

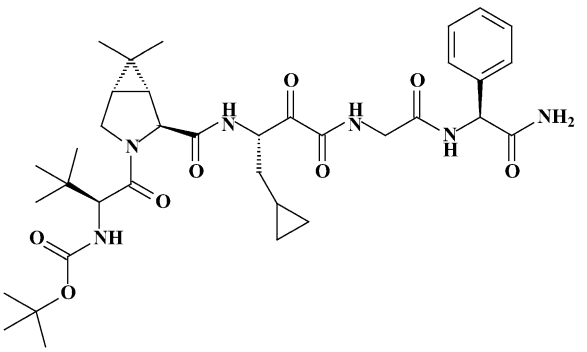
Of the bunyaviruses, one of the most feared (because it is highly infectious, easily transmitted between humans, and associated with a case-fatality rate of approximately 30%) is Crimean–Congo hemorrhagic fever virus.[27] Bunyaviruses are sensitive to ribavirin, and this has also been demonstrated in experimental animal models.[149] Also, interferon and interferon inducers have proved effective in the treatment of experimental bunyavirus infections, and, likewise, interferon-α should be considered for the treatment of arenavirus infections, as



NM283
3'-Valine ester of 2'-*C*-methylcytidine
Valopicitabine

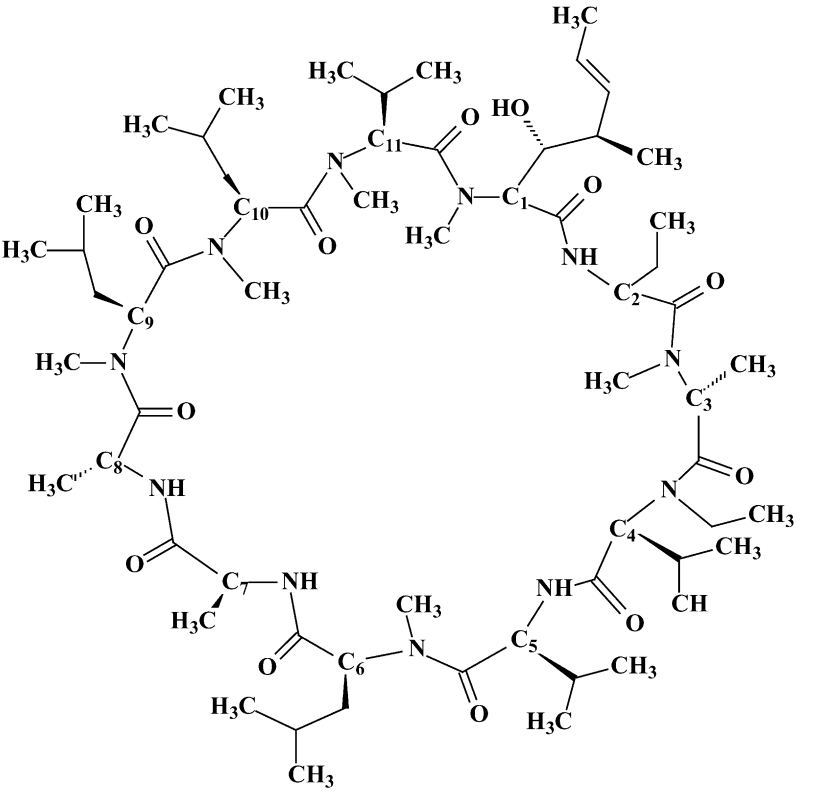


SCH 503034



SCH6 (SCH 446211)

Figure 1. *— Continued.*



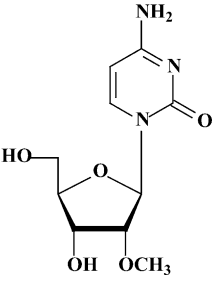
DEBIO-025

Figure 1. *— Continued.*

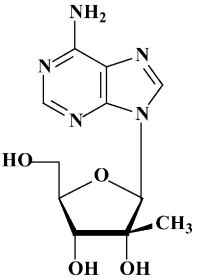
warranted by its efficacy in the therapy of Pichinde virus infection in hamsters.[56]

Of the rhabdoviruses, rabies, which is almost invariably fatal if no control measures are taken, can be contained by repeated injections of specific immunoglobulin and/or the inactivated ("killed") rabies vaccine as soon as possible after the infection. For the filovirus infections Ebola and Marburg no vaccine is (yet) available. Specific immunoglobulin or interferon-α may only be of limited value in the treatment of filovirus infections, as indicated by experimental findings in rhesus macaques infected with Ebola (Zaire) virus.[80]

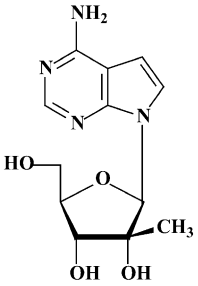
No antiviral drugs that are currently in clinical use, including ribavirin, provide meaningful protection against filoviruses *in vivo*.[10]



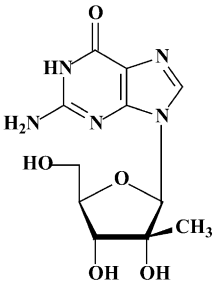
2'-*O*-methylcytidine



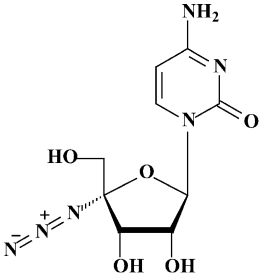
2'-*C*-methyladenosine



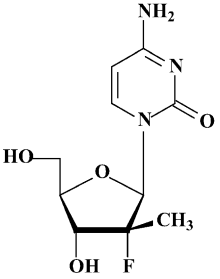
7-deaza-2'-*C*-methyladenosine



2'-*C*-methylguanosine

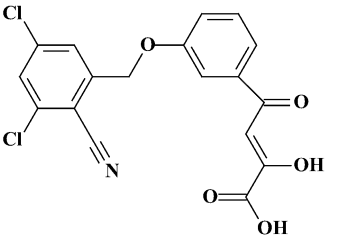


4'-Azidocytidine
(R1479)

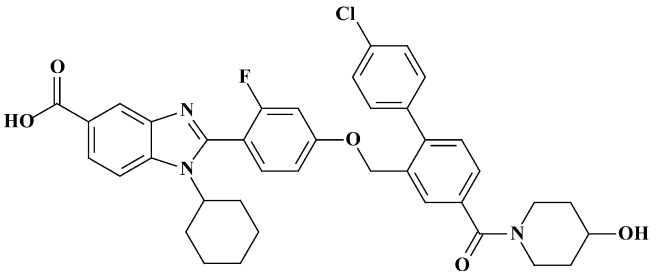


2'-Deoxy-2'-fluoro-2'-*C*-methylcytidine

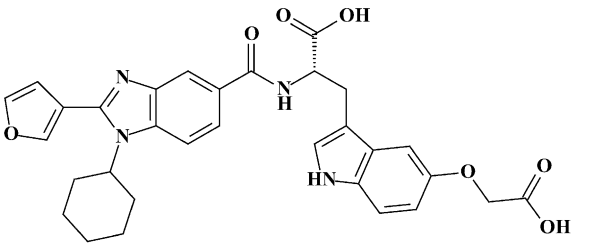
Figure 1. *— Continued.*



DKA Compound 30



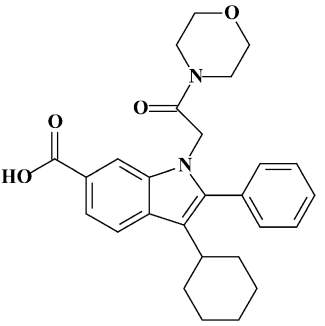
Benzimidazole-based derivative



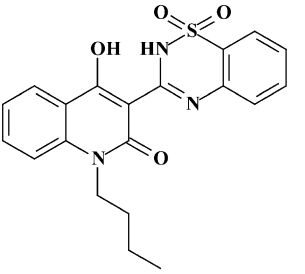
Benzimidazole 5-carboxamide derivative

Figure 1. *— Continued.*

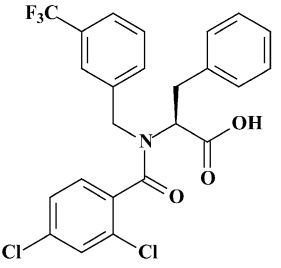
A possible therapeutic strategy may be based on the use of S-adenosylhomocysteine (SAH) hydrolase inhibitors.[31] SAH hydrolase inhibitors, such as 3-deazaneplanocin A, interfere with S-adenosyl-methionine (SAM)-dependent methylation reactions, particularly those involved in the "capping" of viral mRNA. Some viruses, such as the rhabdovirus VSV (vesicular stomatitis virus), heavily rely on mRNA



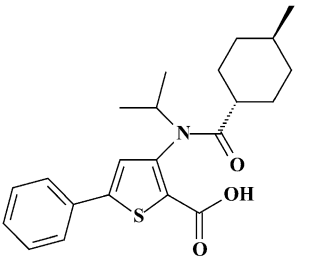
Indole-N-acetamide derivative



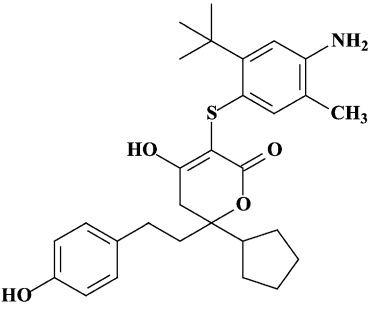
Benzothiadiazine derivative



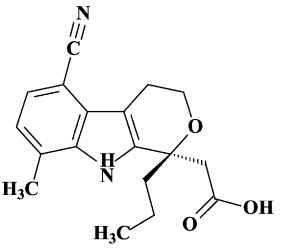
Phenylalanine derivative



Thiophene 2-carboxylic acid derivative

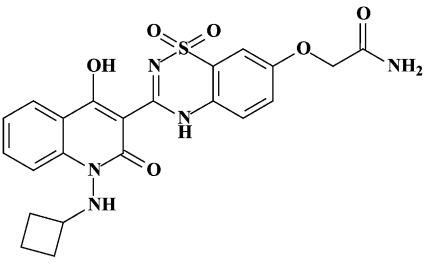


Dihydropyranone derivative

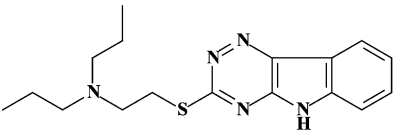


Tetrahydropyranoindolyl acetic acid derivative HCV-371

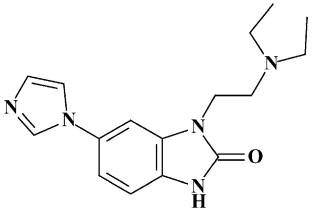
Figure 1. *— Continued.*



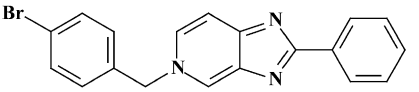
A-782759



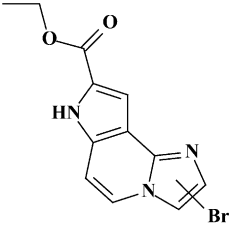
VP32947



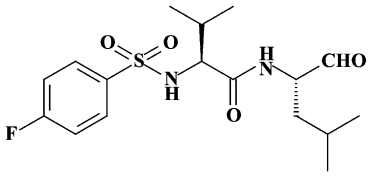
Compound 1453



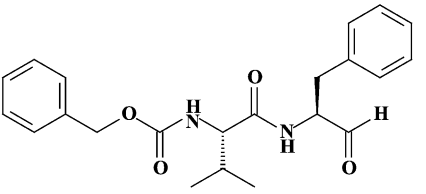
BPIP



AG110

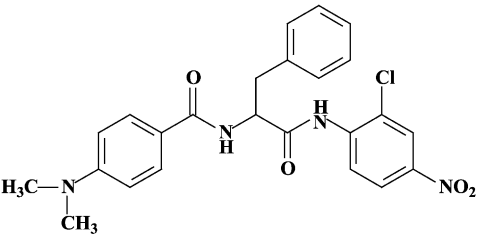


Calpain inhibitor VI

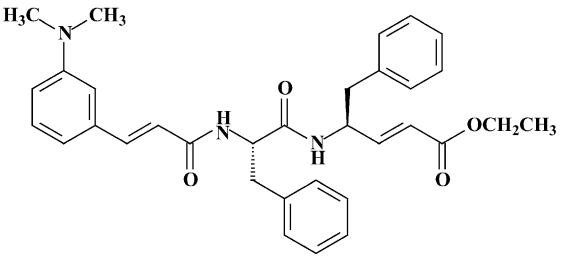


Calpain inhibitor III

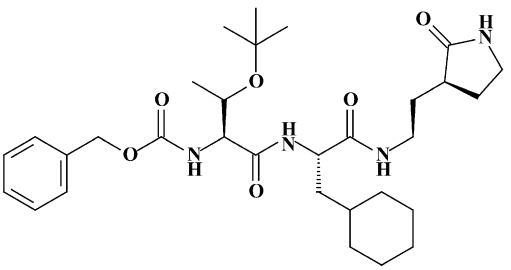
Figure 1. *— Continued.*



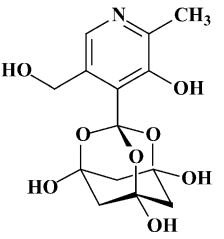
Niclosamide anilide
(JMF 1507)



Phe-Phe dipeptide



TG-0205221

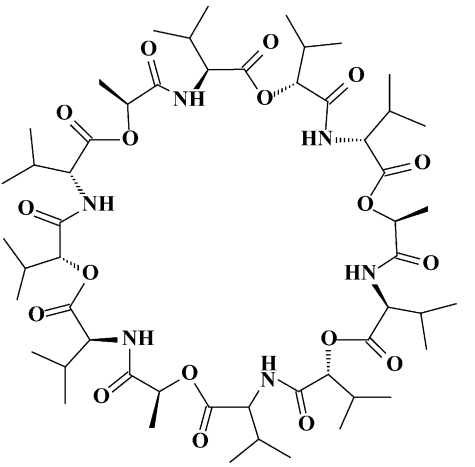


Bananin

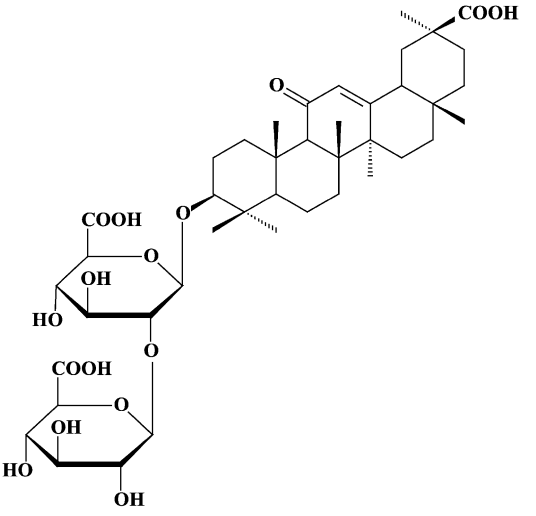
Figure 1. *— Continued.*

capping and are particularly sensitive to inhibition by SAH hydrolase inhibitors, including 3-deazaneplanocin A.[32]

As, biochemically, filo- and rhabdoviruses are quite similar in their replication machinery, both requiring 5′-capping of their mRNAs, SAH hydrolase inhibitors such as 3-deazaneplanocin A may logically be expected to be effective in the treatment of Ebola virus infections. In



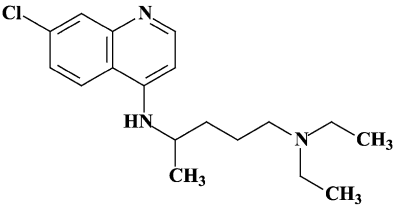
Valinomycin



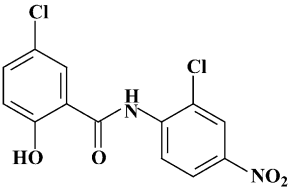
Glycyrrhizin

Figure 1. *— Continued.*

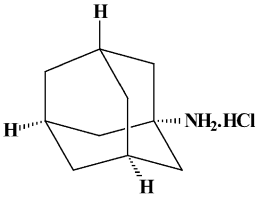
fact, when administered as a single dose of 1 mg/kg, 3-deazaneplanocin A was found to protect mice against a lethal infection with Ebola virus (Zaire strain).[11,12] This protective effect was accompanied, and proba-



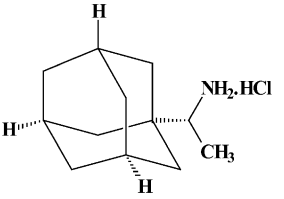
Chloroquine
(Nivaquine)
7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline



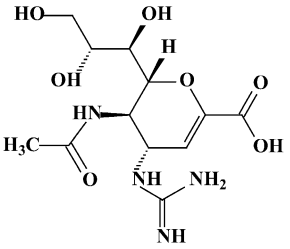
Niclosamide



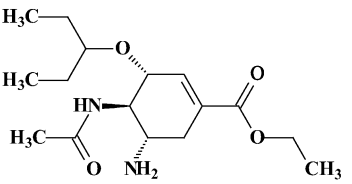
Amantadine
Symmetrel®, Mantadix®



Rimantadine
Flumadine®



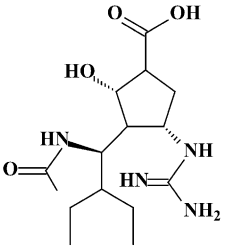
Zanamivir
Relenza®



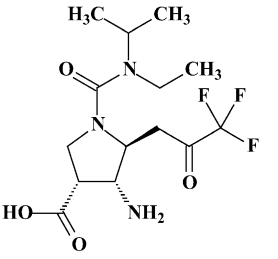
Oseltamivir
Tamiflu®

Figure 1. *— Continued.*

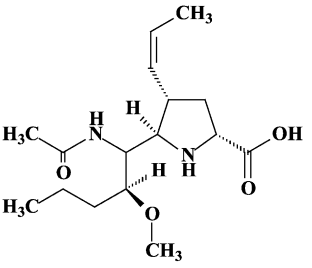
bly mediated, by the production of high concentrations of interferon in the Ebola virus-infected mice.[12] It can be hypothesized that, by blocking the 5′-capping of the nascent (+)RNA viral strands (and, hence, their maturation towards mRNAs), 3-deazaneplanocin A stimulated



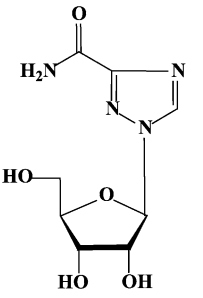
RWJ-270201
Peramivir



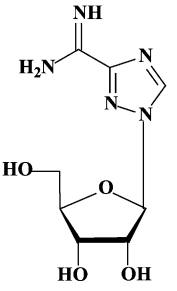
A-192558



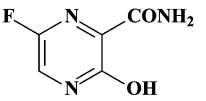
A-315675



Ribavirin



Viramidine

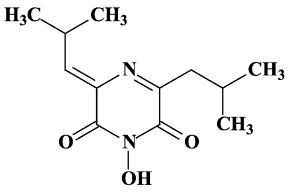


T-705

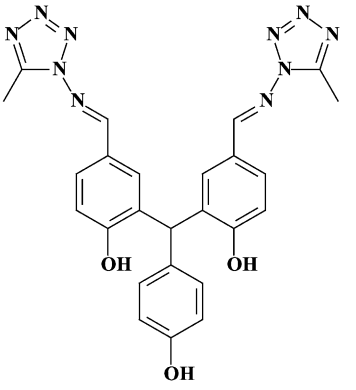
Figure 1. *— Continued.*

the formation of double-stranded (±)RNA complexes, which have since long been known as excellent inducers of interferon.[16]

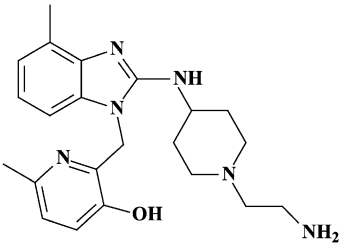
Like SAH hydrolase, IMP (inosine monophosphate) dehydrogenase is another cellular enzyme that may be envisaged as a target for antiviral agents. IMP dehydrogenase is a crucial enzyme involved in the



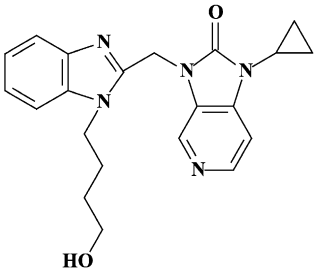
Flutimide



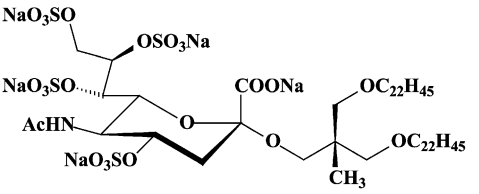
VP-14637



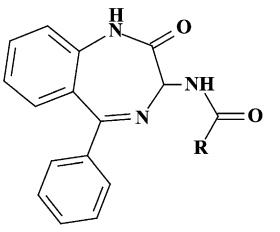
JNJ-2408068
R-170591



BMS-433771

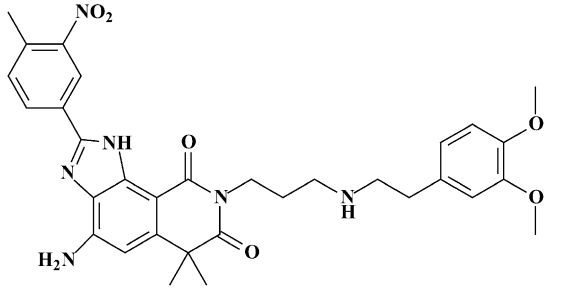


MBX-300

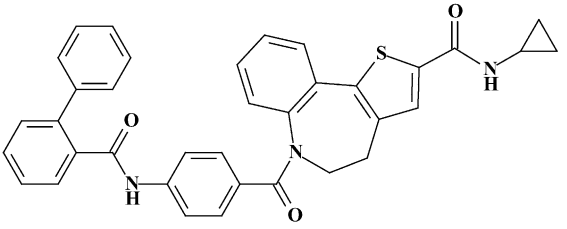


A-60444

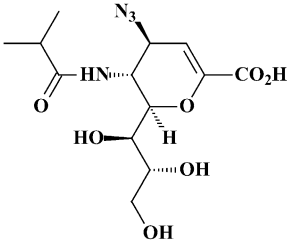
Figure 1. *— Continued.*



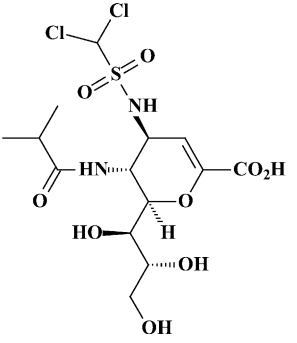
Compound D



YM-53403



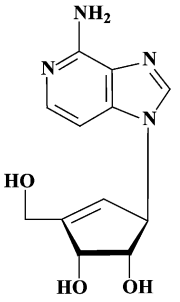
BCX-2798



BCX-2855

Figure 1. *— Continued.*

biosynthesis of GTP, and, although ribavirin may act against distinct viruses by distinct mechanisms (i.e. IMP dehydrogenase inhibition, immunomodulatory effect, RNA capping interference, polymerase in-



3-Deazaneplanocin A

Figure 1. *— Continued.*

hibition, lethal mutagenesis),[57] the predominant mechanism by which ribavirin exerts its antiviral activity *in vitro* against flaviviruses and paramyxoviruses is mediated by inhibition of IMP dehydrogenase.[99]

Recently, a new class of compounds, phosphorodiamidate morpholino oligomers (PMO), conjugated to arginine-rich cell-penetrating peptides (P-PMO) and designed to base pair with the translation start region of Ebolavirus VP35 positive-sense RNA, were reported to inhibit Ebolavirus replication and to protect mice against a lethal Ebolavirus infection.[47] Similarly, siRNAs targeting Ebola virus (Zaire) polymerase (L) gene and delivered using SNALPs (stable nucleic acid–lipid particles) were found to completely protect guinea pigs against death when administered shortly after Ebola virus infection.[53]

IX. REOVIRUSES

Of the reoviruses, rotavirus, which is associated with viral gastrointestinal infections, is by far the most clinically important pathogen. Several attempts have been, and are still being, made to develop an effective vaccine for rotavirus infections. Current treatment for rotavirus diarrhoea is mainly based on the administration of fluids to prevent dehydration. There are no serious attempts to develop an antiviral drug for this disease, although it is noteworthy that the replication of reo- (and rota)viruses is exquisitely sensitive to SAH hydrolase inhibitors such as 3-deazaneplanocin A.[32] Also, small interfering RNA (siRNA) corresponding to the VP4 gene has been shown to efficiently inhibit the synthesis of this protein, and to reduce the yield of viral progeny in virus-infected cells.[35]

X. CONCLUSION

About forty compounds are registered as antiviral drugs, at least half of which are used to treat HIV infections. An even greater number of compounds are under clinical or preclinical development, with again, as many targeting HIV as all the other viruses taken together. This implies that HIV, since its advent, has remained the main stay in antiviral drug development. Antiviral agents can, as guided by the anti-HIV agents as examples, be divided in roughly five categories:

(i) nucleoside analogues,
(ii) nucleotide analogues (or acyclic nucleoside phosphonates),
(iii) non-nucleoside analogues,
(iv) protease inhibitors, and
(v) virus–cell fusion inhibitors.

Molecular targets are for (i) and (ii) the viral DNA polymerase (whether DNA-dependent as in the case of herpesviruses, or RNA-dependent as in the case of HIV or HBV); for (iii) RNA-dependent DNA polymerase (reverse transcriptase), associated with HIV, or RNA-dependent RNA polymerase (RNA replicase) associated with HCV; for (iv) the proteases associated with HIV and HCV; and for (v) the fusion process of HIV (and, potentially, other viruses such as the SARS coronavirus and RSV). Antiviral agents may also exert their antiviral effects through an interaction with cellular targets such as IMP dehydrogenase (ribavirin) and SAH hydrolase (3-deazaneplanocin A). The latter enzymes are essential for viral RNA synthesis (through the supply of GTP) and viral mRNA maturation (through 5′-capping), respectively. Finally, interferons (now generally provided in their pegylated form) may be advocated in the therapy of those viral infections (actually, HBV and HCV; prospectively, Coxsackie B, SARS, . . .) that, as yet, cannot be sufficiently curbed by other therapeutic measures.

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