Highlights in the Development of New Antiviral Agents

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Abstract: The potential of a large variety of new compounds and new strategies for the treatment of virtually all major virus infections has been addressed. This includes, for the treatment of HIV infections, virus adsorption inhibitors (cosalane derivatives, cyanovirin-N), co-receptor antagonists (TAK-779, AMD3100), viral fusion inhibitors (pentafuside T-20, betulinic acid derivatives), viral uncoating inhibitors (azodicarbonamide), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs: emtricitabine, amdoxovir, dOTC, d4TMP prodrugs, tenofovir disoproxil fumarate), non-nucleoside reverse transcriptase inhibitors (NNRTIs: thiocarboxanilide UC-781, capravirine, SJ-3366, DPC 083, TMC 125/R165335), integrase inhibitors (diketo acids), transcription inhibitors (temacrazine, flavopiridol), protease inhibitors (atazanavir, mozenavir, tipranavir); for the treatment of RSV and paramyxovirus infections, viral fusion inhibitors (R170591, VP-14637, NMS03); for the treatment of picornavirus infections, viral uncoating inhibitors (pleconaril); for the treatment of pesti- (hepaci-, flavi-) virus infections, RNA replicase inhibitors (VP-32947); for the treatment of herpesvirus (HSV, VZV, CMV) infections, DNA polymerase inhibitors (A-5021, L- and D-cyclohexenylguanine); for the treatment of VZV infections, bicyclic furopyrimidine analogues; for the treatment of CMV infections, fomivirsen; for the treatment of DNA virus infections at large (papilloma-, polyoma-, herpes-, adeno- and poxvirus infections), cidofovir; for the treatment of influenza, neuraminidase inhibitors (zanamivir, oseltamivir, RWJ-270201); for the treatment of HBV infections, adefovir dipivoxil; for the treatment of HBV and HCV infections, N-glycosylation inhibitors (N-nonyl-deoxynojirimycin); and, finally, IMP dehydrogenase inhibitors and S-adenosylhomocysteine hydrolase inhibitors, for the treatment of various virus infections, including hemorrhagic fever virus infections.

1. INTRODUCTION

Antiviral therapy has definitely come of age [1]. There are now more than 30 compounds that have been formally approved for the therapy of virus infections. The nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine, didanosine, zalcitabine, stavudine, lamivudine and abacavir are used in the treatment of human immunodeficiency virus (HIV) infections, and lamivudine is also licensed for the treatment of hepatitis B virus (HBV) infections. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine and efavirenz are used in the treatment of HIV infections, and so are the HIV protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir. Among the anti-herpesvirus agents, acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine and brivudin are used in the treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections, and ganciclovir, valganciclovir, foscarnet, cidofovir and fomivirsen are indicated in the treatment of cytomegalovirus (CMV) infections. In addition to amantadine and rimantadine, the neuraminidase inhibitors zanamivir and oseltamivir, have now become available for the therapy and prophylaxis of influenza virus infections. Ribavirin is available for the treatment of respiratory syncytial virus (RSV) infections, and is also used, in combination with (pegylated) interferon, for the treatment of hepatitis C virus (HCV) infections.

Here I will describe the different targets that could be envisaged in the design of new antiviral agents, thereby highlighting how this focussed approach has led to the identification of new strategies for the treatment of a wide variety of virus infections. In particular, the following targets will be addressed: virus adsorption (HIV), virus co-receptor interaction (HIV), virus-cell fusion (HIV, RSV, and other paramyxoviruses), viral uncoating (picornaviruses, HIV), viral reverse transcriptase (HIV, HBV), viral RNA replicase (flaviviruses), viral DNA polymerase (herpesviruses, DNA viruses), viral integrase (HIV), viral RNA transcription (HIV), and translation (CMV), viral protease (HIV), viral neuraminidase (influenza), and cellular enzymes (α-glucosidase, IMP dehydrogenase and S-adenosylhomocysteine hydrolase) that are innately associated with the replicative cycle of a number of viruses.

2. VIRUS ADSORPTION INHIBITORS

Numerous polyanionic compounds, i.e. polysulfates [i.e., dextran sulfate, dextrin sulfate, heparin, heparan sulfate, polyvinylalcohol sulfate (PVAS), …], polysulfonates [i.e., suramin, poly(4-styrene)sulfonate, polyvinylsulfonate (PVS), …], polynucleotides [such as zintevir (a 17-mer capable of forming a double guanine quartet)], polyoxometalates, negatively charged albumins, and polycarboxylates [i.e., aurintricarboxylic acid (ATA)] have been reported to block HIV replication through

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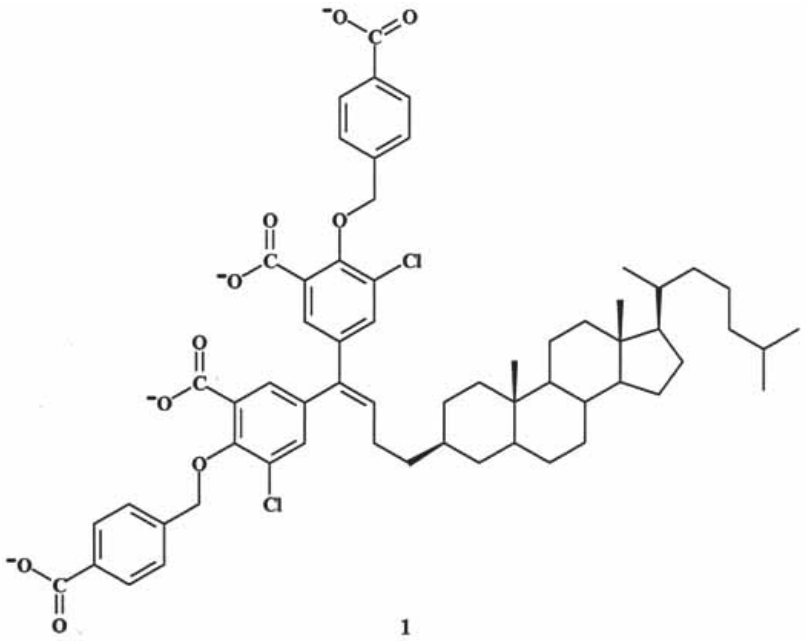

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(H2N)Leu—Gly—Lys—Phe—Ser—Gln—Thr—Cys—Tyr—Asn—Ser—Ala—

e—Gln—Gly—Ser—Val—Leu—Thr—Ser—Thr—Cys—Glu—Arg—Thr—Asn—Gly—Gly—Tyr—Asn—Thr—Ser—

er—Ile—Asp—Leu—Asn—Ser—Val—Ile—Glu—Asn—Val—Asp—Gly—Ser—Leu—Lys—Trp—Gln—Pro—Ser—

n—Phe—Ile—Glu—Thr—Cys—Arg—Asn—Thr—Gln—Leu—Ala—Gly—Ser—Ser—Glu—Leu—Ala—Ala—Glu—

s—Lys—Thr—Arg—Ala—Gln—Gln—Phe—Val—Ser—Thr—Lys—Ile—Asn—Leu—Asp—Asp—His—Ile—Ala—

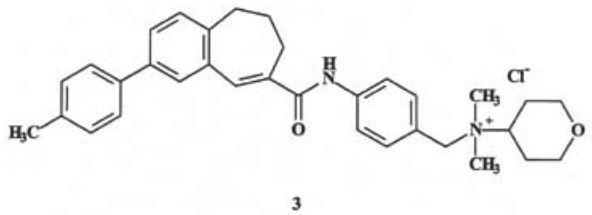
n—Ile—Asp—Gly—Thr—Leu—Lys—Tyr—Glu(COOH)

2

interference with the interaction of the viral envelope glycoprotein (gp120) with the CD4 receptor at the cell surface. To this class of compounds also belong the cosalane derivatives where the polycarboxylate moiety has been attached to a membrane-interactive steroid (i.e., cholestane) (**1**) [2,3]. The major role of the polyanionic substances, including the cosalane derivatives, may reside in their use as topical (i.e., vaginal) microbicides in the prevention of the sexual transmission of HIV infection. Also, cyanovirin-N (**2**), a 11-kDa protein originally isolated from the cyanobacterium *Nostoc ellipsosporum*, prevents the interaction of the viral envelope gp120 with its target cell receptors and thus qualifies as a potential microbicide to prevent the transmission of HIV and AIDS [4,5].

3. VIRAL CO-RECEPTOR ANTAGONISTS

Following their binding to the CD4 receptor, the HIV particles must interact, again through their envelope glycoprotein gp120, with either CXCR4 [the receptor for the CXC chemokine SDF-1 (stromal-cell derivatives factor)] or CCR5 [the receptor for the CC chemokine RANTES (regulated upon activation, normal T-cell expressed and



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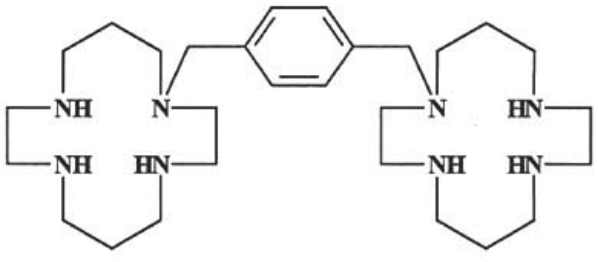

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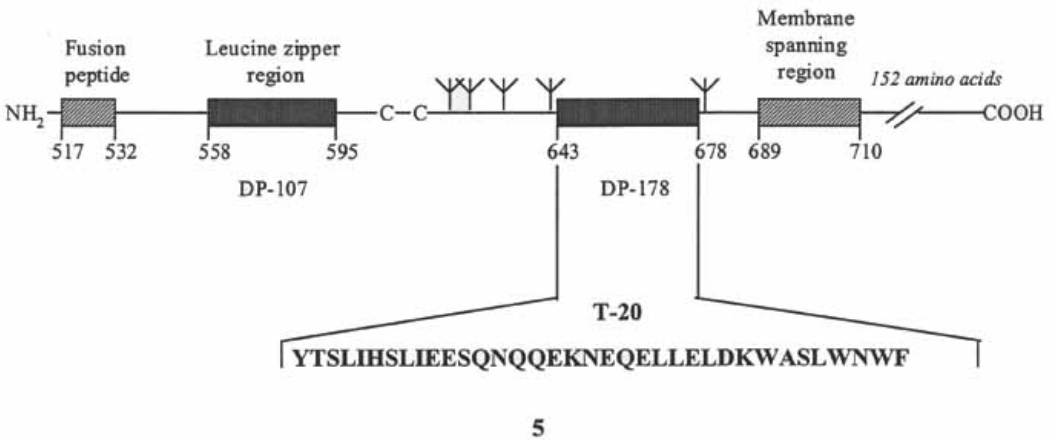

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secreted)] before they can enter the cells: CXCR4 acts as the co-receptor for HIV-1 strain that infect T-cells (T-tropic or X4 strains) and CCR5 functions as the co-receptor for HIV-1 strains that infect macrophages (M-tropic or R5 strains). Viral entry into the cells can be blocked at the CCR5 level, i.e. by TAK-779, a quaternary ammonium derivative (**3**), and at the CXCR4 level, i.e. by the bicyclam AMD3100 (**4**). TAK-779 and AMD3100 have been shown to inhibit, in the nanomolar concentration range, the replication of R5 HIV-1 strains [6] and X4 HIV-1 strains [7], respectively. TAK-779 is a specific CCR5 antagonist, and AMD3100 is a highly selective CXCR4 antagonist, and the crucial binding sites for both compounds at the CCR5 level [8] and CXCR4 level [9] have been identified. So as to simultaneously block X4 and R5 HIV strains, future developmental plans should focus on combinations of CXCR4 and CCR5 antagonists, or, if feasible, compounds that antagonize both receptors.

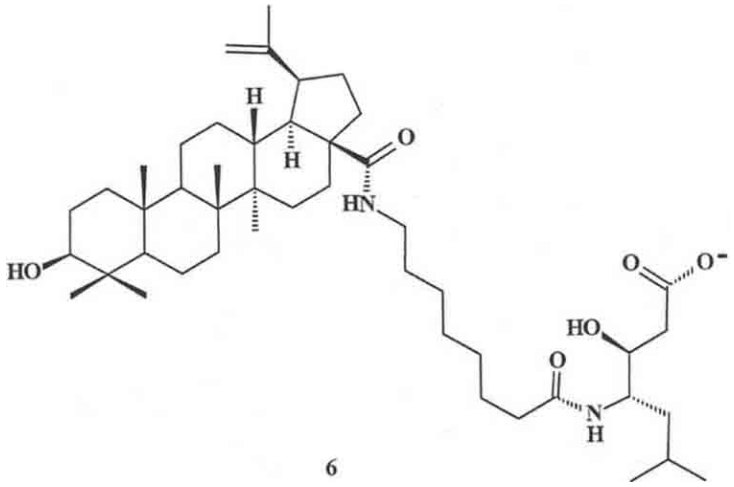
4. VIRAL FUSION INHIBITORS (HIV)

The interaction of the X4 or R5 HIV envelope glycoprotein gp120 with the co-receptors CXCR4 and CCR5, respectively, is followed by a spring-loaded action of the viral glycoprotein gp41 that then anchors through its amino terminus into the target cell membrane and initiates the fusion of the two lipid bilayers (viral envelope and cellular membrane). This fusion process can be blocked by T-20 (pentafuside), previously called DP-178, a synthetic 36-amino acid peptide corresponding to residues 127-162 of the ectodomain of gp41 (or residues 643-678 in the gp120 precursor) (**5**). Proof-of-concept that HIV fusion inhibitors are able to reduce virus replication *in vivo* has been provided by an initial clinical trial, where T-20 was found to achieve a 1.5- to 2.0-fold reduction in plasma viral load at the highest dose used (100 mg, twice daily) [10].

The betulinic acid derivative RPR103611 (**6**) is the only non-peptidic low-molecular-weight compound that has been reported to block HIV-1 infection at the virus-cell fusion level [11], consequently to a specific interaction with the gp41 glycoprotein [12,13]. The mechanism of anti-HIV action of betulinic acid derivatives deserves further investigation, as recent studies have revealed that such derivatives may interact with HIV-1 gp120, rather than gp41 (betulinic acid derivative IC 9564) [14], or even a late step (virion assembly and/or budding) of the HIV replicative cycle (betulinic acid derivative YK-FH312) [15].

5. VIRAL FUSION INHIBITORS (RSV AND OTHER PARAMYXOVIRUSES)

As a "prelude" to the fusion process, the HIV envelope glycoprotein gp41 becomes engaged in the formation of a "trimer-of-hairpins" structure, that is a bundle of six α-



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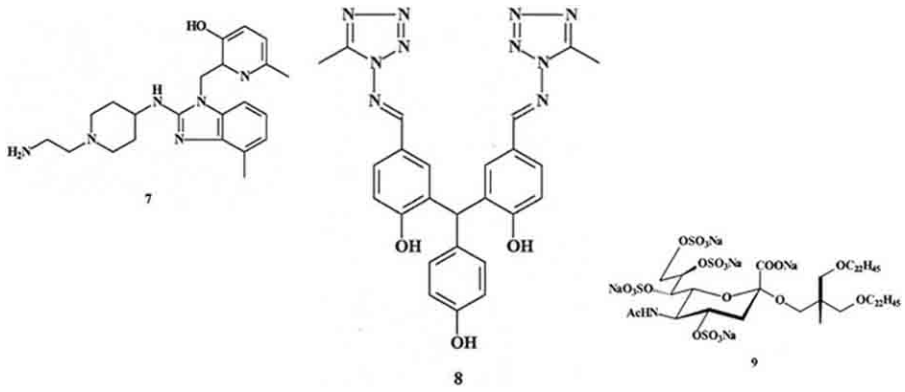
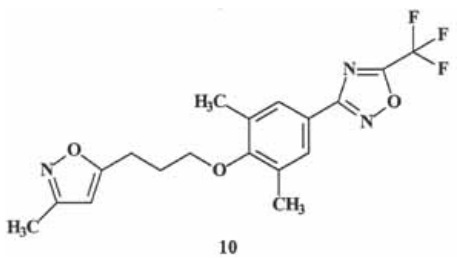


helices (three α-helices formed by the COOH-terminal regions packed in an antiparallel manner with three α-helices formed by the NH_2-terminal regions [16]. Such "trimer-of-hairpins" motifs may also be predicted for other virus families, including paramyxoviridae (parainfluenza, measles, RSV, etc.). Peptides [analogous to DP-178 (**5**)] from conserved regions of paramyxovirus fusion (F) proteins have actually been shown to block viral fusion [17].

Recently, several non-peptidic, low-molecular-weight compounds have been described as RSV inhibitors, interacting with the viral fusion process: R170591 (**7**) [18], VP-14637 (**8**) [19] and NMSO3, a sulfated sialyl lipid (**9**) [20]. The compounds were found to inhibit RSV infection *in vitro* and *in vivo* (cotton rats). They are assumed to interact with the RSV fusion (glyco)protein, but their precise mode of action remains to be elucidated.

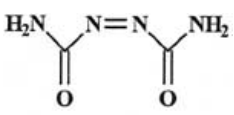
6. VIRAL UNCOATING INHIBITORS (PICORNAVIRUSES)

Picornaviruses encompass entero- and rhinoviruses. Enterovirus infections result in myriad disease syndromes, among which viral meningitis and viral respiratory infections. Rhinoviruses are the leading cause of the common cold. The most advanced drug active against both entero- and rhinoviruses is pleconaril (**10**). This compound binds to a hydrophobic pocket beneath the "canyon floor" of the VP1 capsid protein of picornaviruses, thus rigidifying or "freezing" the viral capsid and preventing its uncoating from the viral RNA genome [21]. Pleconaril is orally bioavailable and achieves plasma concentrations in excess of those required to inhibit 90% of the clinical rhino- and enteroviral isolates *in vitro*; as an additional advantage, it achieves several fold higher drug concentrations within the central nervous system and nasal secretions than in plasma, a highly desirable feature for an antiviral drug targeted towards viruses known to cause central nervous system and upper respiratory tract infections [22]. The clinical efficacy of pleconaril has been assessed in experimentally induced enterovirus (Coxsackie virus A21) respiratory infections in adult volunteers [23] and potentially life-threatening enterovirus infections on a compassionate basis [24].



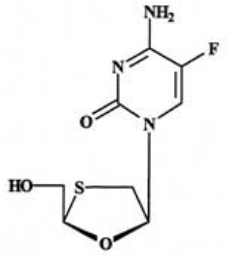
7. VIRAL UNCOATING INHIBITORS (HIV)

The ultimate capsid protein to be dissociated ("uncoated") from the HIV RNA genome, before the latter can be submitted to the "reverse" transcription to proviral DNA, is the nucleocapsid protein (NCp7). NCp7 is firmly attached to the HIV genome, and, as it contains two zinc fingers motifs, targeting these zinc figures with, e.g., zinc-ejecting compounds, such as azodicarbonamide (ADA) (**11**) [25] has been considered as an attractive approach towards anti-HIV therapy. In principle, NCp7-targeted compounds should be able to interfere with both early (i.e. uncoating) and late stages (i.e. assembly) of the retroviral replicative cycle. Of the NCp7-targeted compounds, ADA has been the first to proceed to clinical trials: some preliminary evidence of efficacy was witnessed with add-on ADA in advanced AIDS patients failing current antiretroviral therapy [26]. These studies should be further extended before drawing any conclusions on the potential of ADA, or any other putative NCp7 zinc-finger inhibitors, in the treatment (or prevention) of HIV infections.



8. NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIS)

The substrate (dNTP) binding site of the HIV reverse transcriptase (RT) is the target for a large variety of NRTI



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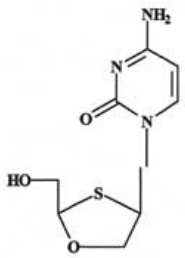

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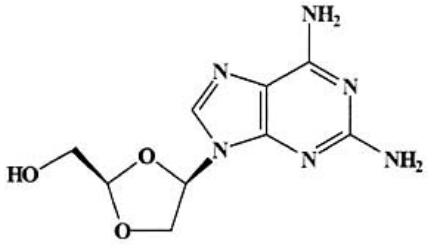

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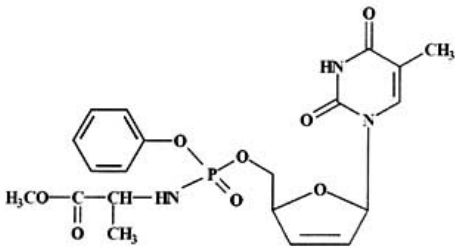

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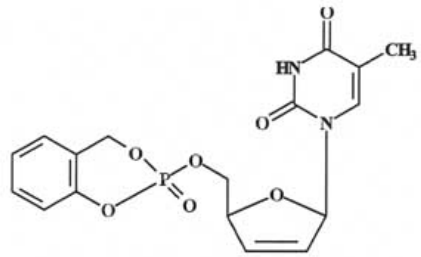

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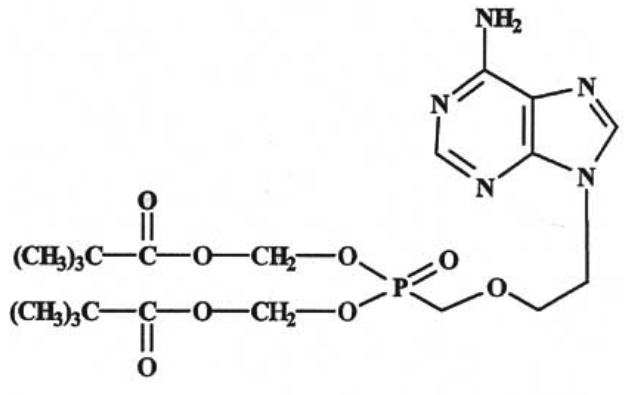
analogues which are currently used in the treatment of HIV infections: i.e., zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC) and abacavir (ABC). In addition to these licensed compounds, several other NRTIs are presently subject of clinical trials: i.e., emtricitabine [(-)2'-deoxy-3'-thia-5-fluorocytidine or (-)FTC (**12**)], (±)2'-deoxy-3'-oxa-4'-thiocytidine(dOTC) (**13**) and amdoxovir [(-)-β-D-2,6-diaminopurine dioxolane or DAPD (**14**)]. As a rule, these newer NRTIs retain activity against AZT- and/or 3TC-resistant HIV-1 strains, as demonstrated for dOTC [27] and DAPD [28]. All NRTIs (whether old or new) have to be converted intracellularly, through three consecutive phosphorylations, to their 5'-triphosphate form before the latter can act as competitive inhibitors/substrate analogues/chain terminators at the RT level. The first phosphorylation step is the rate-limiting step in the intracellular metabolism of the NRTIs, and several nucleotide prodrugs have been designed that directly deliver the 5'-monophosphate form inside the cells, i.e., d4T aryloxyphosphoramidate (**15**) and *cyclo*saligenyl d4TMP (**16**). Both these constructs release the d4T 5'-monophosphate within the cells and thus circumvent or bypass the thymidine kinase needed for the first phosphorylation [29,30].



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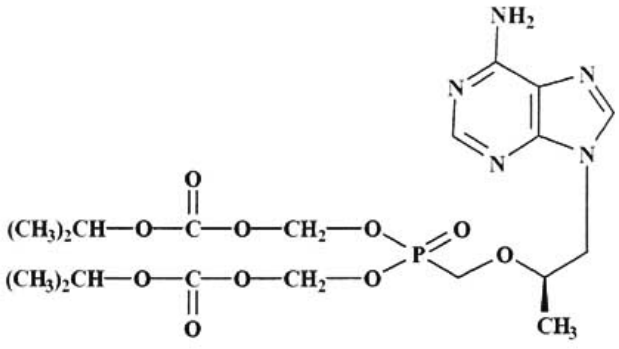
9. ACYCLIC NUCLEOSIDE PHOSPHONATES (ANPS)

The acyclic nucleoside phosphonates 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and (*R*)-9-(2-phosphonylmethoxypropyl)adenine (PMPA) can be considered as nucleotide analogues in which the phosphate group has been built in as an enzymatically stable phosphonate moiety. These compounds thus bypass the first phosphorylation step, and require only two phosphorylations to be converted to their active metabolites, the diphosphate derivatives PMEApp and PMPApp. In this form they act as competitive inhibitors/substrates, with respect to dATP, in the HIV and HBV reverse transcriptase reaction, and when incorporated into the viral DNA chain, they obligatorily terminate further chain elongation [31]. As neither PMEA (adefovir) nor PMPA (tenofovir) are readily bioavailable by the oral route, they have been converted to their oral prodrugs bis(pivaloyloxymethyl)-PMEA (adefovir dipivoxil) (**17**) and bis(isopropyl-oxycarbonyloxymethyl)-PMPA (tenofovir disoproxil) (**18**). The former is in advanced phase III clinical trials for the treatment of HBV infections, whereas the latter, tenofovir disoproxil fumarate (Viread^{TM}), has already completed phase III clinical trials for the treatment of HIV infections. Based on these trials, a new



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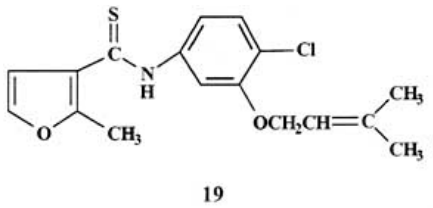

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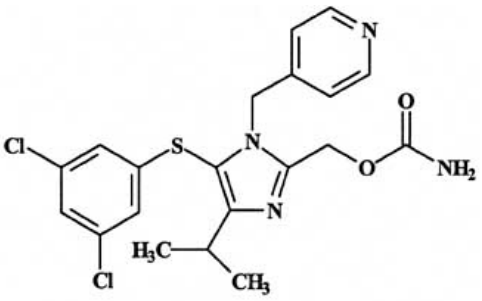

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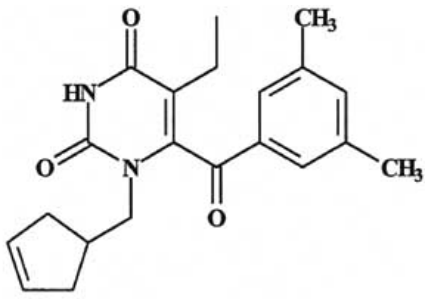

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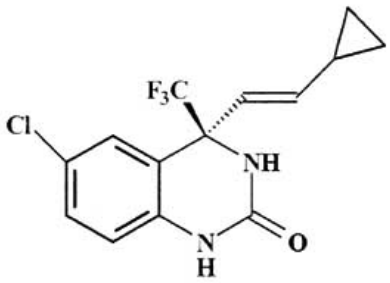

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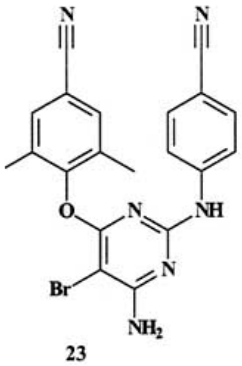

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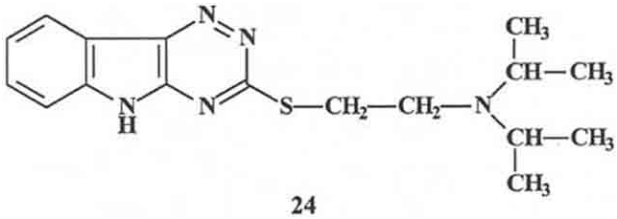
drug application (NDA) and market authorization application (MAA) has been filed for tenofovir disoproxil fumarate with the FDA (U.S.) and EMEA (E.U.), respectively.

10. NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIS)

NNRTIs are defined by the fact that they interact with an allosteric, non-substrate binding site of the HIV-1 reverse transcriptase [32], and, consequently, they are only (or primarily) active against HIV-1, and not any of the other retroviruses. The licensed NNRTIs nevirapine, delavirdine and efavirenz have acquired a definitive "niche" in drug combination regimens for the treatment of HIV infections. As the older NNRTIs are notorious for rapidly eliciting virus-drug resistance (resulting from mutations at amino acid residues surrounding the NNRTI-binding site of HIV-1 RT), attempts have been made to design newer NNRTIs with higher potency and/or resilience to drug resistance mutations. This search has yielded a number of compounds which are now in development as potential drugs for the treatment of HIV-1 infections: e.g. thiocarboxanilide UC-781 (**19**) [33], capravirine (AG1549) (**20**) [34], SJ-3366 (**21**) [35], DPC 083 (**22**) [36] and TMC 125 (R165335) (**23**) [37] .The thiocarboxanilide UC-781 would seem an ideal candidate for application as a vaginal microbicide (virucide), whereas the other new NNRTIs are principally intended for oral administration.

11. VIRAL RNA REPLICASE INHIBITORS

One of the virus infections in the greatest need of antiviral therapy is HCV, and, here, the non-structural protein 5B (NS5B) or RNA-dependent RNA polymerase (RNA replicase) could be considered an attractive molecular target. The experience gathered from the studies with HIV RT inhibitors may be of paramount importance when targeting HCV RNA polymerase, especially, if, as it has been inferred [38], HCV RNA polymerase shows similar kinetics as HIV RT. In fact, NS5B has been validated as a target for antiviral drug discovery, at least as far as NS5B of pestiviruses [i.e., bovine viral diarrhea virus (BVDV)] is concerned. A pestivirus antiviral compound, i.e. VP-32947 (3-[((2-dipropylamino)ethyl)thio]-5H-1,2,4-triazino[5,6-b]indole) (**24**) has been identified that inhibits the replication of BVDV, owing to an inhibition of the viral RNA-dependent RNA polymerase activity [39]. Virus-drug resistance was engendered, and appeared to be associated with a single (F224S) mutation located in a region that might be involved in RNA substrate recognition. If so, VP-32947 may well be reminiscent of an NNRTI, and should, also in view of the similarities among the NS5B of pesti-, hepaci- and flaviviruses, facilitate the search of HCV NS5B inhibitors.



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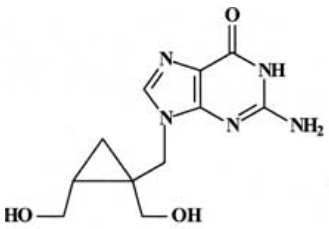
12. VIRAL DNA POLYMERASE INHIBITORS (REQUIRING A VIRUS-INDUCED KINASE FOR ACTIVATION)

All the antiviral agents that are currently available for the treatment of herpesvirus (HSV, VZV, CMV) infections are

nucleoside analogues [i.e., the acyclic guanosine analogues acyclovir, penciclovir, ganciclovir (and their oral prodrug forms valaciclovir, famciclovir and valganciclovir), and the thymidine analogue brivudin]. All these compounds are targeted at the viral DNA polymerase, but, before they can interact with viral DNA synthesis, they need to be phosphorylated intracellularly to the triphosphate form. The first (and, for brivudin, also the second) phosphorylation step is ensured by the HSV- or VZV-encoded thymidine kinase (TK), or, for ganciclovir, by the CMV-encoded protein kinase (PK). These virus-induced kinases thus impart the antiviral specificity of the compounds. At the viral DNA polymerase level, the 5'-triphosphates of the nucleoside analogues then act as competitive inhibitors/alternate substrates with respect to the natural substrates, and, if incorporated, the acyclic guanosine analogues terminate further DNA chain elongation. Within the class of the guanosine analogues, several new congeners, with activity against HSV, VZV and CMV, have been described: i.e., A-5021 [(1'S,2'R)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]guanine] (**25**) [40,41] and the D- and L-enantiomers of cyclohexenylguanine (**26**) [42] .As thymidine derivatives, bicyclic furopyrimidine analogues [bearing a long alkyl or arylalkyl side chain attached to the furane ring (**27**)] have been described that show an exquisitely potent and selective activity against VZV [43,44].



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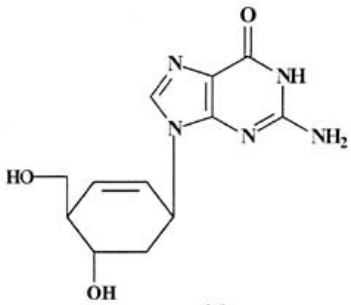

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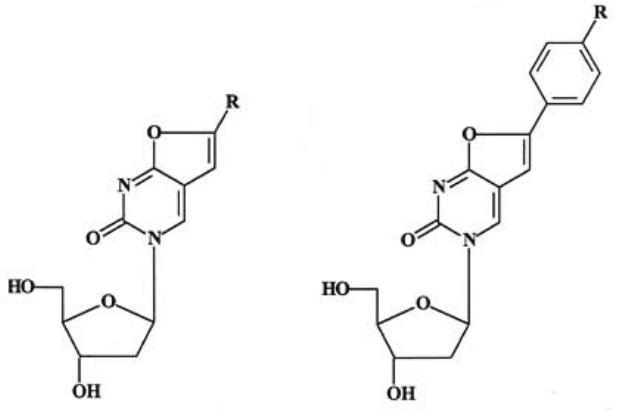

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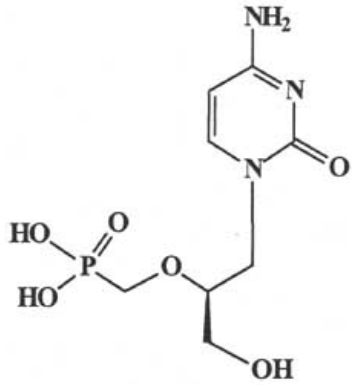
13. VIRAL DNA POLYMERASE INHIBITORS (NOT REQUIRING A VIRUS-INDUCED KINASE FOR ACTIVATION)

In contrast with the aforementioned nucleoside analogues (acyclovir, penciclovir, ganciclovir, etc.), which need three phosphorylations to be converted to their active metabolites, acyclic nucleoside phosphonates, such as (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, cidofovir, Vistide®) (**28**) need only two phosphorylations. The first phosphate is already built in as a phosphonate, which means that this type of compounds no longer depends on the virus-induced thymidine kinase (TK) or protein kinase (PK) for their antiviral activity. Consequently, cidofovir may be expected to show a broadened antiviral activity spectrum, also encompassing those DNA viruses that do not encode for a specific viral TK or PK or have become resistant to the nucleoside analogues because of TK or PK deficiency. These premises have been borne out, and cidofovir has been found effective against a broad range of DNA viruses (papilloma-, polyoma-, adeno-, herpes- and poxviruses) [45]. The antiviral specificity of cidofovir must reside in its mode of interaction with the viral DNA polymerization process. For cidofovir to shut down CMV DNA synthesis, the incorporation of two consecutive cidofovir units is required [46]. Unique features of cidofovir, and of acyclic nucleoside phosphonates in general, are that they possess a particularly long intracellular half-life (lasting for one to several days) and do not easily lead to virus-drug resistance, even after prolonged treatment (for more than one year). Cidofovir has been approved for clinical use in the treatment of CMV retinitis in AIDS patients, but also holds great potential for the treatment of various other herpesvirus infections, as well as human papillomavirus (HPV), polyomavirus, adenovirus and poxvirus infections.



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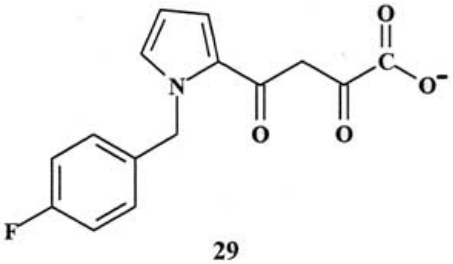
14. HIV INTEGRASE INHIBITORS

Gene expression (i.e. transcription to viral RNA) of the (pro)viral HIV DNA is only possible after the latter has been integrated into the host chromosome, and thus integrase has been considered an attractive target for chemotherapeutic intervention with the HIV replicative cycle. Numerous integrase inhibitors have been described [47,48]. None, however, showed sufficient specificity to be further pursued as an integrase-targeted drug. In some cases, as with zintevir (a G-octet-based 17-mer) [49] and L-chicoric acid [50], selective anti-HIV activity was noted in cell culture, but, then, the antiviral activity could be attributed primarily to inhibition of virus adsorption rather than proviral DNA integration. Thus far, the only compounds that qualify as genuine inhibitors of HIV integrase are the 4-aryl-2,4-dioxobutanoic acid derivatives [51], such as the so-called

diketo acids L-731,988 (**29**) and L-708,906. These compounds were found to inhibit HIV-1 replication in cell culture, on the one hand, and to inhibit the strand transfer function of the integrase, on the other hand; and these two events could be causally linked, as mutations in the HIV-1 integrase conferred resistance to the inhibitory effects of the compounds on both strand transfer and HIV-1 infectivity [52]. *In vivo* efficacy with the diketo acids, or any other HIV integrase inhibitors, remains to be demonstrated.



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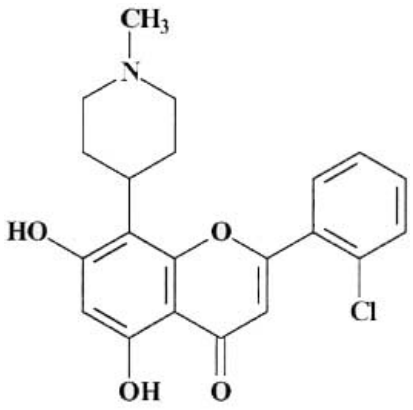
15. HIV transcription inhibitors

At the transcription level, HIV gene expression may be inhibited by compounds that interact with cellular factors that bind to the long terminal repeat (LTR) promoter and that are needed for basal level transcription, such as NF-κB inhibitors [53]. Greater specificity, however, can be expected from those compounds that specifically inhibit the transactivation of the HIV LTR promoter by the viral trans-acting transactivator (Tat) protein. Several compounds have been described as inhibitors of the transcription process, i.e. fluoroquinolines [54] and bistriazoloacridon derivatives such as temacrazine [55]. The latter was found to block HIV-1 RNA transcription starting from the HIV LTR promoter without interfering with the transcription of any cellular genes. Also peptide analogues, i.e. the 9-mer peptoid CGP64222, which is structurally reminiscent of the amino acid 48-56 sequence RKKRRQRRR of Tat, were designed to act as Tat antagonists [56], and, although this 9-mer peptoid is able to interact with the Tat-driven transcription process, its anti-HIV activity in cell culture can be primarily attributed to an interaction with CXCR4, the co-receptor for X4 HIV strains [57]. Viral RNA transcription could also be affected through the targeting of cyclin-dependent kinases (cdks), i.e. cellular proteins required for the replication of many viruses (including HIV). One such cdk, cdk9, forms, together with cyclin T1, the protein kinase P-TEFb, which secures the elongation phase of transcription by RNA polymerase II (through phosphorylation of the carboxyl-terminal domain). Tat forms a ternary complex with P-TEFb and the nascent transcript from the HIV-1 LTR promoter. Flavopiridol (**30**), an inhibitor of the P-TEFb protein kinase, was found to block Tat transactivation and, concomitantly, also inhibited HIV replication [58].



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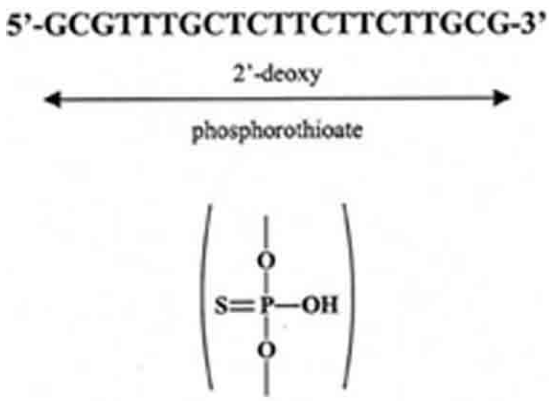
16. VIRAL MRNA TRANSLATION INHIBITORS

Antisense oligonucleotides or ribozymes complementary to specific sequences in the viral mRNA should, in principle, be able to block the translation process for any virus, including HIV. Yet, this approach has, after some initial attempts, not been intensively pursued. In fact, only one antisense construct has been commercialized, that is fomivirsen (ISIS 2922) (**31**), a 21-mer phosphorothioate oligonucleotide that is complementary to the human CMV immediate-early (IE) mRNA [59,60]. Accordingly, fomivirsen was found to inhibit IE gene expression in CMV-infected cells. As a polynucleotide, fomivirsen may also be expected to inhibit virus adsorption. However, the isolation of a CMV mutant with sequence-specific resistance to fomivirsen [61] may be interpreted to mean that at least part of the antiviral activity of fomivirsen is due to a genuine "antisense" mechanism.



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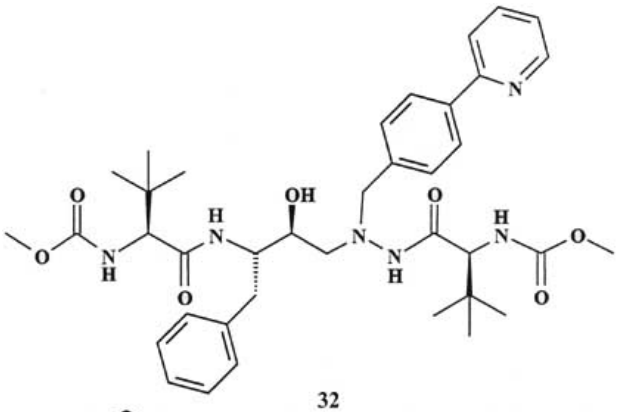
17. HIV PROTEASE INHIBITORS

Viral proteases play critical roles in the life cycle of many different viruses, i.e. HIV, HSV, CMV, HCV, etc. [62]. As to its role in HIV replication, HIV protease secures the cleavage of the gag and gag-pol precursor proteins to the mature structural proteins (p17, p24, p9, p7) and functional proteins (protease, reverse transcriptase/RNAse H, and integrase). HIV protease inhibitors have been tailored after the target peptidic linkages in the gag and gag-pol precursor proteins that have to be cleaved by the protease. All six protease inhibitors that are currently licensed for the treatment of HIV infection, namely saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir, share the same structural determinant, i.e. an hydroxyethylene core (instead of the normal peptidic linkage) that makes them non-scissile, "peptidomimetic", substrate analogues of the HIV protease. The HIV protease inhibitors have proven to be valuable therapeutics in drug combination schedules with



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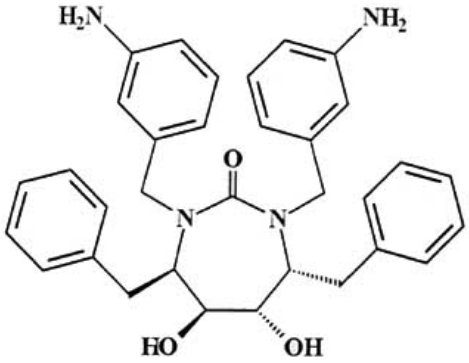

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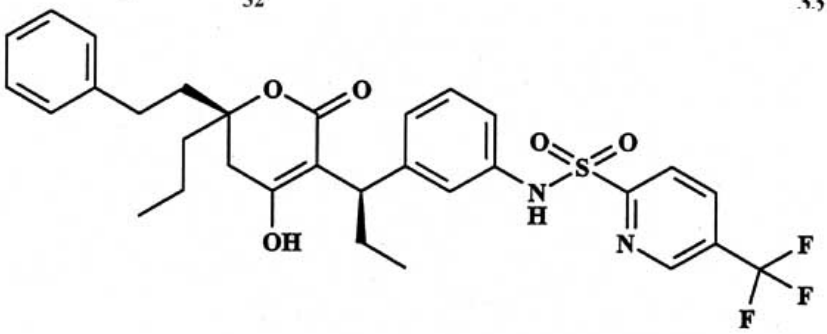

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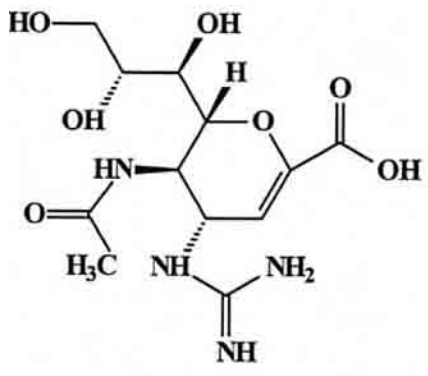
NRTIs and NNRTIs, in the treatment of HIV infections. Yet, they are met by a number of compounding factors, including overlapping resistance patterns and long-term side effects (e.g. metabolic disturbances, such as lipodystrophy). This has prompted the search for new, non-peptidic inhibitors of HIV protease, that would, hopefully, combine excellent anti-HIV potency with little or no cross-resistance with the "older" drugs and better tolerability on both short and long term. To these potential new candidate drugs that may fulfill at least part of the expectations belong atazanavir (BMS-232632) (**32**) [63], mozenavir (DMP-450) (**33**) [64] and tipranavir (**34**) [65].

18. VIRAL NEURAMINIDASE INHIBITORS

Influenza virus (A and B) has adopted a unique replication strategy in using one of its surface glycoproteins, hemagglutinin, to bind to the target cell receptor [containing a terminal sialic acid (N-acetylneuraminic acid or NANA)] and the other surface glycoprotein, neuraminidase, that cleaves off the terminal sialic acid, to egress from the cells, after the viral replicative cycle has been completed. The viral neuraminidase is thus needed for the elution of the newly formed virus particles from the cells, and thus blockade of this elution process, i.e. by neuraminidase inhibitors, may be expected to prevent the spread of the progeny virions to other host cells. Aided by the X-ray crystallographic structure of the influenza virus neuraminidase, specific inhibitors of the enzyme were designed. This led to the identification of 4-guanidino-Neu5Ac2en (zanamivir (GG167) (**35**)) [66], GS4071 (administered as its ethyl ester prodrug (GS4104), oseltamivir (**36**) [67]) and other NANA analogues such as RWJ-270201 (**37**) [68,69], as potent and specific inhibitors of the viral neuraminidase, on the one hand, and the *in vitro* and *in vivo* replication of both influenza A and B virus, on the other hand. Meanwhile, zanamivir (which has to be administered topically, by inhalation) and oseltamivir (which can be administered orally) have already been licensed for the treatment and prevention of influenza virus infections.

19. N-GLYCOSYLATION INHIBITORS

Endoplasmic reticulum (ER) α-glucosidases are responsible for the stepwise removal of terminal glucose residues from the Asn- or N-linked glycan chains of nascent



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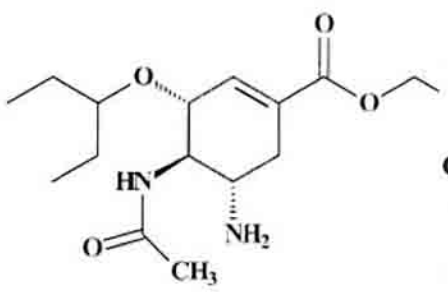

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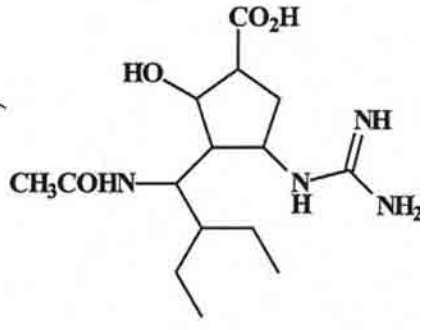

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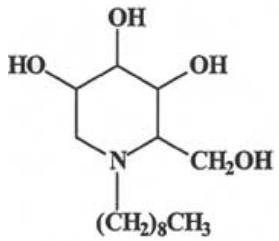
glycoproteins during the N-glycosylation process. This enables the glycoproteins to interact with the ER chaperone proteins (calnexin and calreticulin) (which bind exclusively to monoglucosylated glycoproteins). These interactions are crucial for the correct folding of (some of) the glycoproteins and for the morphogenesis and trafficking of the enveloped viruses, such as HBV and HCV, which originate through budding from the ER into intracellular vesicles. Consequently, inhibitors of the ER α-glucosidases, such as N-nonyl-deoxynojirimycin (NN-DNJ) (**38**), have been shown to prevent the formation and secretion of infectious particles of ER-budding viruses, i.e. woodchuck hepatitis virus (WHV) (as a surrogate for HBV) [70] and BVDV (as a surrogate for HCV) [71]. *In vivo*, NN-DNJ caused a significant, dose-dependent decrease of the WHV secretion, without affecting the glycosylation of most serum glycoproteins, and as viral secretion seems to be selectively sensitive to glucosidase inhibition relative to the secretion of cellular proteins, glucosidase inhibitors may be entertained as potential broad-spectrum anti-hepatitis virus agents, active against both HBV and HCV.



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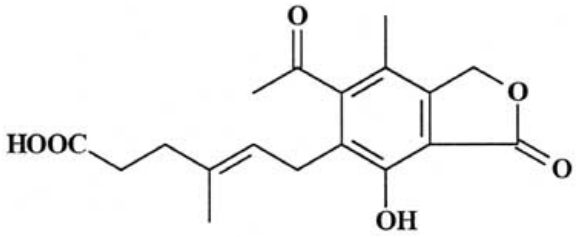
20. IMP DEHYDROGENASE INHIBITORS

IMP dehydrogenase is a key enzyme in the *de novo* biosynthesis of purine mononucleotides: it is responsible for the NAD-dependent oxidation of IMP to XMP, which is then further converted to GMP, GDP and GTP, and from GDP, *via* dGDP, also to dGTP. IMP dehydrogenase inhibitors may be expected to affect both RNA and DNA synthesis, and more so in virus-infected cells where there is an increased need for such syntheses. There are two classes of IMP dehydrogenase inhibitors, i.e. competitive or uncompetitive with respect to the normal substrate, IMP. To the first class belongs ribavirin, which has been officially approved for clinical use, as an aerosol for the treatment of RSV infections, and in combination with (pegylated) interferon-α for the treatment of HCV infections. To the second class belongs mycophenolic acid (**39**), an immunosuppressing agent which has been approved, as its morpholinoethyl ester prodrug, for the prevention of acute allograft rejection following kidney transplantation. Mycophenolic acid and congeners (i.e. VX-497) have broad-spectrum activity against a wide variety of DNA and RNA viruses (picorna-, toga-, flavi-, bunya-, arena-, reo-, rhabdo-, and, particularly ortho- and paramyxoviruses) [72]. Mycophenolic acid has marked activity against yellow fever virus, and, in addition, markedly potentiates the inhibitory effects of acyclic guanosine analogues (acyclovir, penciclovir, ganciclovir) against HSV, VZV and CMV infections, which could be of great clinical utility in organ transplant recipients suffering from these infections [73]. Also, mycophenolic acid potentiates the activity of guanine-derived dideoxynucleoside analogues, such as abacavir, against HIV [74], and this may be further exploited as a new combination strategy in the treatment of HIV infections.



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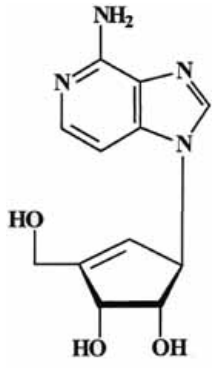
21. S-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS

S-adenosylhomocysteine (SAH) hydrolase is a key enzyme in methylation reactions depending on S-adenosylmethionine (SAM) as the methyl donor, including those methylations that are required for the maturation of viral mRNAs. In particular, (-)RNA viruses (bunya-, arena-, rhabdo-, filo-, ortho- and paramyxoviruses) are critically dependent on these methylations. SAH is both a product and inhibitor of the SAM-dependent methyltransferase reactions. SAH is rapidly hydrolyzed by SAH hydrolase into homocysteine and adenosine. In the presence of SAH hydrolase inhibitors, however, SAH can accumulate and thus exert its inhibitory effect on the methylation reactions. Various adenosine analogues, i.e. carbocyclic adenosine, carbocyclic 3-deazaadenosine, neplanocin A, 3-deazaneplanocin A (**40**), and their 5'-nor derivatives, have been described as potent inhibitors of SAH hydrolase [75]. They possess a characteristic antiviral activity spectrum, encompassing the (-)RNA viruses mentioned above but also (±)RNA viruses (reo) and poxviruses. SAH hydrolase inhibitors offer a real potential for the treatment of hemorrhagic fever virus infections, such as Ebola hemorrhagic fever; 3-deazaneplanocin A was recently shown to protect mice from an otherwise lethal Ebola virus infection [76].



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