

# Antiviral Therapy for Human Immunodeficiency Virus Infections

ERIK DE CLERCQ\*

*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

<b>INTRODUCTION</b> .....	<b>200</b>
<b>ANTI-HIV AGENTS</b> .....	<b>201</b>
<b>Virus Adsorption Inhibitors</b> .....	<b>201</b>
<b>Virus-Cell Fusion Inhibitors</b> .....	<b>207</b>
<b>Virus Uncoating Inhibitors</b> .....	<b>207</b>
<b>Reverse Transcription Inhibitors</b> .....	<b>209</b>
Substrate analogs.....	209
Nonsubstrate analogs.....	210
Miscellaneous RT inhibitors.....	215
<b>Integration Inhibitors</b> .....	<b>216</b>
<b>DNA Replication Inhibitors</b> .....	<b>216</b>
<b>Transcription Inhibitors</b> .....	<b>216</b>
<b>Translation Inhibitors</b> .....	<b>217</b>
<b>Maturation Inhibitors</b> .....	<b>218</b>
Protease inhibitors.....	218
Myristoylation inhibitors.....	220
Glycosylation inhibitors.....	220
<b>Budding (Assembly/Release) Inhibitors</b> .....	<b>222</b>
<b>COMBINATION THERAPY</b> .....	<b>223</b>
<b>VIRUS-DRUG RESISTANCE</b> .....	<b>224</b>
<b>CONCLUSION</b> .....	<b>225</b>
<b>ACKNOWLEDGMENTS</b> .....	<b>226</b>
<b>REFERENCES</b> .....	<b>226</b>

## INTRODUCTION

Numerous compounds have been reported to inhibit the replication of human immunodeficiency virus (HIV) in vitro (118, 410), yet only four agents have at this time been formally licensed (in the United States) for clinical use in the treatment of AIDS. These are zidovudine (3'-azido-2',3'-dideoxythymidine or azidothymidine [AZT]; Retrovir) (269), didanosine (2',3'-dideoxyinosine [ddI]; Videx) (156), zalcitabine (2',3'-dideoxycytidine [ddC]; Hivid) (479), and stavudine (2',3'-dideoxy-2',3'-dideoxythymidine [D4T]; Zerit). The basic strategies and molecular targets for anti-HIV therapy have been repeatedly reviewed starting from 1985, thus shortly after HIV had been identified as the causative agent of AIDS (116-121, 127, 324, 327). More recent reviews have addressed the challenges and prospects for the therapy of HIV infection (236, 490).

The replicative cycle of HIV comprises a number of steps that could be considered adequate targets for chemotherapeutic intervention (Fig. 1). In fact, HIV follows a replicative pathway that is similar to that of the classical cytolitic RNA viruses, except for reverse transcription (step 4) and integration (step 5), which lead to the formation and integration of the proviral DNA into the cellular DNA genome. Most of the substances that have been identified as anti-HIV agents can be assigned to one of the 10 classes of HIV inhibitors according to

the stage at which they interfere with the HIV replicative cycle (Table 1).

However, not all substances to which anti-HIV activity has been attributed easily fit within the proposed scheme (Fig. 1; Table 1). For example, some recombinant (chimeric) proteins in which a toxin, *Pseudomonas aeruginosa* toxin (7, 16, 17, 65) or diphtheria toxin (18), has been linked to the HIV envelope glycoprotein (gp120)-binding domain of human CD4 have been described: by virtue of their affinity for gp120, these hybrid toxins selectively bind to and kill HIV-infected cells. Although both acutely and chronically HIV-infected cells can be selectively killed by this gp120-targeted cytotoxicity approach, it does not prevent the emergence of HIV-infected cells that are resistant to the chimeric toxins (18). Also, gene therapy approaches have been advocated to introduce the diphtheria toxin gene directly to HIV-infected cells (198), which should ultimately result in the eradication of the cells when the diphtheria toxin gene is expressed.

Another approach that could not be readily accommodated by the proposed scheme (Fig. 1; Table 1) is that based on the targeting of antiviral agents (i.e., pokeweed antiviral protein) to CD4<sup>+</sup> cells (whether infected or not) by conjugation of these antiviral agents with monoclonal antibodies reactive with normal antigens on CD4<sup>+</sup> cells. Such conjugates have been shown to inhibit HIV type 1 (HIV-1) replication in CD4<sup>+</sup> cells and were surmised to inhibit the replication of other viruses as well (152, 497).

Also, various other compounds that have been reported to inhibit HIV replication cannot be unequivocally allocated to one of the 10 classes of HIV inhibitors (Table 1; Fig. 1), primarily because their target of action has not been elucidated

\* Mailing address: Rega Institute for Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. Phone: 32-16-33.73.41. Fax: 32-16-33.73.40.

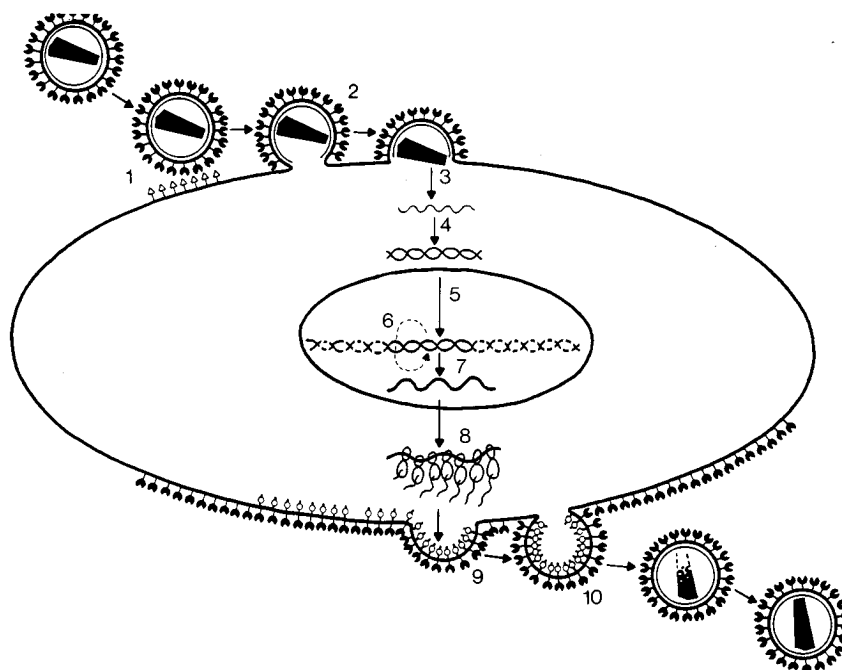


FIG. 1. Essential steps in the HIV replicative cycle: 1, adsorption; 2, fusion; 3, uncoating; 4, reverse transcription; 5, integration; 6, DNA replication; 7, transcription; 8, translation; 9, maturation; 10, budding (assembly/release).

or does not fall within the proposed scheme. To the more recent group of HIV inhibitors, for which the mechanism of anti-HIV action needs to be elucidated, belong diphenylhydantoin (97), ascorbic acid (194), pradimycin (444), oxophenarsine (188), fluoroquinolones (354), prostaglandins (10), glutathione, glutathione ester, *N*-acetylcysteine (241), (-)-gossypol (282) and various analogs of gossypol (284), and the HIV-1 inhibitors produced by myxobacteria (237) or induced by *Pinus parviflora* extracts (440). Other HIV inhibitors such as the C<sub>60</sub> fullerene derivatives seem to interact at multiple steps of the viral life cycle, i.e., direct virus inactivation as well as inhibition of the HIV reverse transcriptase (RT) and HIV protease (166, 411, 423). Until the modes (targets) of action of these compounds are better delineated, it would seem difficult to assess their position or potential for the treatment of HIV infections.

## ANTI-HIV AGENTS

### Virus Adsorption Inhibitors

Since the CD4 molecule on helper T4 lymphocytes and monocytes/macrophages is the principal receptor for the HIV-1 envelope glycoprotein gp120, various forms of recombinant soluble CD4 (rsCD4), including truncated CD4 molecules (i.e., CD4 [segment 74-95] or CD4 [segment 81-92] peptides [384, 418] and benzylated or phenylalanine-substituted derivatives thereof [275]) as well as CD4-immunoglobulin conjugates (i.e., CD4 immunoadhesins [86, 268]) and CD4-albumin constructs (491), have been created with the aim of blocking HIV-1 binding (adsorption) to the cells. The chimeric forms (CD4 immunoadhesins and CD4-albumin constructs) were obviously made to increase the plasma half-life of the otherwise short-lived CD4. The CD4 immunoadhesin (CD4-immunoglobulin G) did not offer much protection against simian immunodeficiency virus infection in macaques (268) but proved capable of preventing HIV-1 infection in chimpanzees (471), and this offers hope for the use of CD4-immunoglobulin

in HIV-infected pregnant women for the prevention of HIV infection of the fetus, since CD4-immunoglobulin G, like the parent immunoglobulin G molecule, efficiently crosses the placenta. Yet, there are several problems linked to the use of CD4-based therapeutics, in particular, the fact that much higher concentrations of CD4 are needed to inhibit primary HIV-1 isolates than laboratory strains of HIV-1 (109), for reasons that still have to be clarified (15). Also, cell-associated virus may be less easily inhibited by CD4 derivatives than cell-free virus.

As CD4 is not only the receptor for HIV but also the receptor for class II major histocompatibility complex antigens, soluble forms of CD4 may also interfere with immune processes involving the class II major histocompatibility complex proteins, and in addition, the CD4 derivatives may have delivery, stability, and expense problems. The smaller the peptides, the smaller these problems may turn out to be, and in this perspective the *N*-carboxymethoxycarbonyl-prolyl-phenylalanyl benzyl esters (CPFs) were conceived (160). These compounds interact directly with the viral glycoprotein gp120, block binding of the HIV to the CD4 receptor, do not interfere with the binding of CD4 to class II major histocompatibility complex proteins, and prevent the spread of HIV from a small number of afflicted cells to a larger population of uninfected cells (160). The questions of how the CPFs perform *in vivo* and whether they indeed block dissemination of HIV-1 *in vivo* have so far remained unanswered. Given their poor aqueous solubility, these compounds might also have bioavailability problems.

In addition to the CPFs, several other, miscellaneous compounds have been postulated to inhibit HIV infection through an interaction with the viral glycoprotein gp120, thus blocking the binding of gp120 to the CD4 receptor: pyridoxal 5'-phosphate (187a), *Prunella vulgaris* extract (488), tannins (474), caffeoylquinic acid derivatives (294), flavans (i.e., daphnodorins [495a]), and flavanoids (i.e., (-)-epicatechin 3-*O*-gallate) (295). In contrast with the sulfated polysaccharides (i.e., dex-

TABLE 1. Review of HIV inhibitors according to stage of intervention with the HIV replicative cycle

Stage of HIV intervention	HIV inhibitor
Adsorption	rsCD4 constructs (CD4 fragments, CD4 immunoadhesins, and CD4-albumin constructs) CPFs ( <i>N</i> -carboxymethoxycarbonyl-prolyl-phenylalanyl benzyl esters), tannins, and flavanoids [(–)epicatechin-3- <i>O</i> -gallate] Polysulfates (heparin, dextran sulfate, dextrin sulfate, curdlan sulfate, pentosan polysulfate, mannan sulfate, sulfoevernan, fucoidan, polyvinylalcohol sulfate, polyacetal polysulfate, <i>O</i> -acylated heparin, cyclodextrin sulfate, and modified cyclodextrin sulfates) Polysulfonates [suramin, Evans blue, bis(naphthalene disulfonate) derivatives, polyvinyl sulfonate, polystyrene sulfonate] Polycarboxylates (ATA), polyhydroxycarboxylates (phenyl-derived polyhydroxycarboxylates), and polyfluoroalkylcarboxylates (MAA-HFPO5) Polyoxometalates {H <sub>4</sub> SiW <sub>12</sub> O <sub>40</sub> (JM1493), K <sub>7</sub> [PTi <sub>2</sub> W <sub>10</sub> O <sub>40</sub> ] · 6H <sub>2</sub> O [PM-19], K <sub>13</sub> [Ce(SiW <sub>11</sub> O <sub>39</sub> ) <sub>2</sub> ] · 26H <sub>2</sub> O [JM1590], and [Me <sub>3</sub> NH] <sub>8</sub> [Si <sub>2</sub> Nb <sub>6</sub> W <sub>18</sub> O <sub>77</sub> ] (JM2820)}
Fusion	Plant lectins (from <i>Listera ovata</i> , <i>Hippeastrum</i> hybrid, <i>Cymbidium</i> hybrid, <i>Epipactis helleborine</i> , and <i>Urtica dioica</i> ) Peptide T22 [(Tyr-5,12,Lys-7)polyphemusin II] Succinylated and aconitylated HSA Betulinic acid RPR 103611
Uncoating	Bicyclams (JM2763 and JM3100)
Reverse transcription	Substrate analogs 2',3'-Dideoxynucleoside analogs (zidovudine [AZT], didanosine [ddI], zalcitabine [ddC], stavudine [D4T], lamivudine [3TC], FTC, and FddCIUrd) Acyclic nucleoside phosphonates (PMEA, FPMPA, PMPA, and PMPDAP) Nonsubstrate analogs (NNRTIs: TIBO [R82150, R82913, and R86183], HEPT [E-EPU, E-EBU-dM, and I-EBU], nevirapine [BI-RG-587], pyridinone [L-696,229 and L-697,661], BHAP [U-88204 and U90152], TSAO, α-APA, and PETT) Miscellaneous RT inhibitors, including antisense oligonucleotides
Integration	Antisense constructs
DNA replication	Antisense constructs
Transcription	Antisense ODNs Tat antagonists (benzodiazepines [Ro 5-3335 and Ro 24-7429] and 3-keto/enol-4,5-epoxy steroids) LTR-directed gene expression inhibitors (topotecan) and PKC inhibitors (indolocarbazoles)
Translation	Antisense ODNs (phosphorothioates, phosphorodithioates, and methylphosphonates) Ribozymes (hammerhead and hairpin ribozymes) that can be delivered exogenously or endogenously via retroviral vectors) Trichosanthin (?)
Maturation	Protease inhibitors: transition-state peptidomimetics (Ro 31-8959, U-81749, A-77003, and KNI-227), and nonpeptide cyclic ureas (XM323) Myristoylation inhibitors (12-azidododecanoic acid) Glycosylation inhibitors (NBuDNJ and its prodrug [ <i>N</i> -butyldeoxynojirimycin-6-phosphate])
Budding (assembly/release)	IFN (also interferes with other stages) Hypericin (?) Cyclosporine analogs (SDZ NIM 811) (also interfere with transport of viral DNA into the nucleus)

tran sulfate), whose action is reversible, the flavanoids irreversibly inactivate virus infectivity (295). Also, some of the flavanoids have been shown to inhibit the RT of certain retroviruses (including HIV), but this effect would not contribute to their anti-HIV action observed in cell culture. Other compounds that have been postulated to interfere with several steps of the HIV replicative cycle, i.e., cosalane (disalicyl methane linked to cholestane [106a]) and GTOs (oligonucleotides composed entirely of guanosine and thymidine [356a]), may owe their anti-HIV activity primarily to inhibition of gp120-CD4 binding.

Various sulfated polysaccharides (e.g., heparin, dextran sulfate, dextrin sulfate, cyclodextrin sulfate, curdlan sulfate, pentosan polysulfate, mannan sulfate, sulfoevernan, and fucoidan) and derivatives thereof (e.g., *O*-acylated heparin, polyacetal

polysulfate, polyvinylalcohol sulfate, and modified cyclodextrin sulfates) (Fig. 2) have been found to inhibit HIV replication in vitro at<sup>45</sup> concentrations that are up to 10,000-fold lower than the cytotoxic concentration (124). These compounds are targeted at the interaction between the viral envelope glycoprotein gp120 and the CD4 receptor, and as a consequence, they inhibit not only virus adsorption to the cells but also virus-induced syncytium (giant cell) formation (29). The inhibitory effects of dextran sulfate and its congeners on viral binding, viral replication, and syncytium formation appear to be mediated by a specific interaction with the V3 region of gp120 (64, 82). In addition, sulfated polysaccharides may also directly interfere with the binding of HIV particles to the heparan sulfate proteoglycan at the cell surface, whether or not this process occurs independently of, or cooperatively with, the

**A**

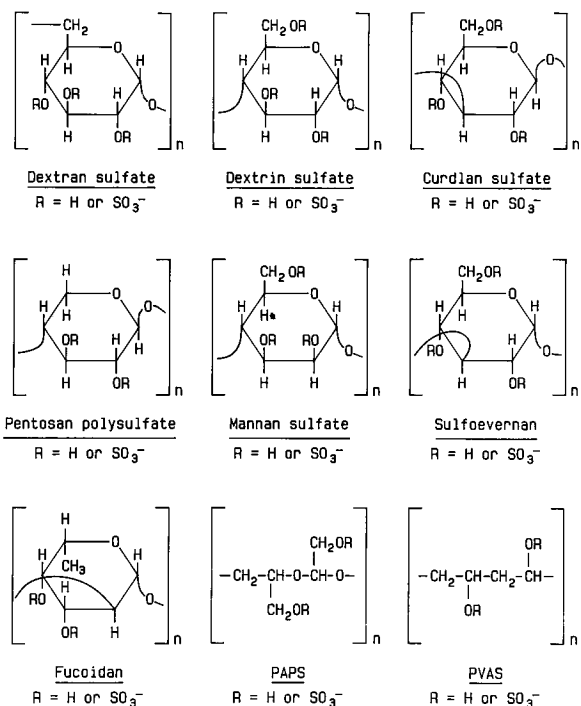
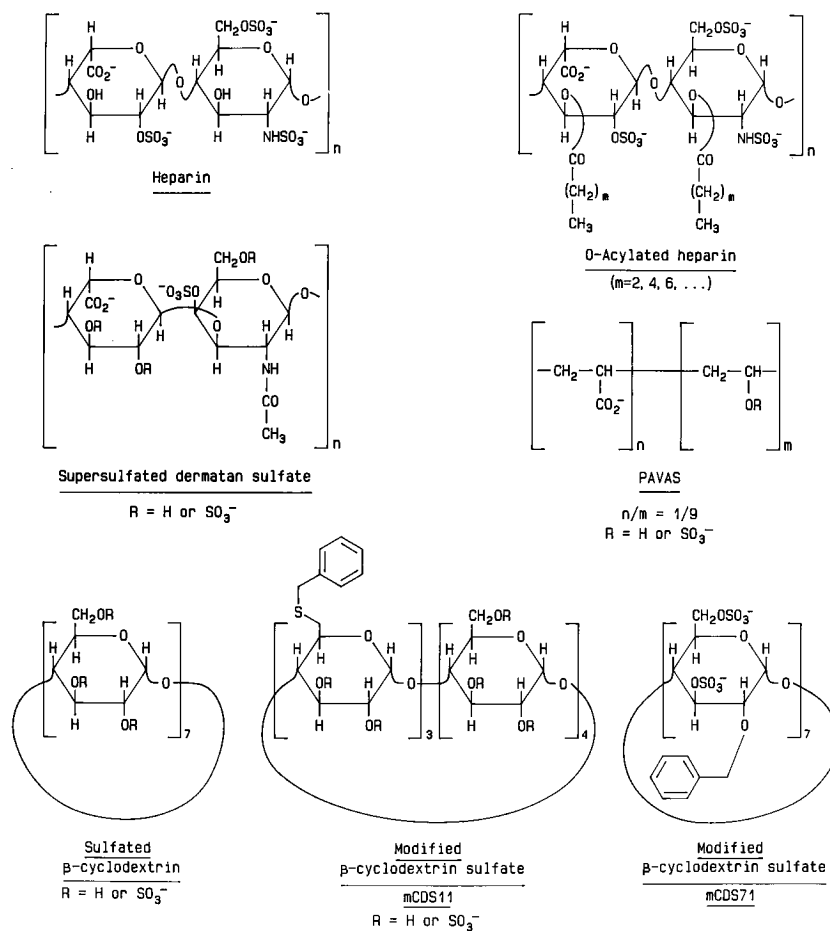


FIG. 2. Structures of polysulfates. (A) Dextran sulfate [sulfated (1 → 6)-α-D-glucan], dextrin sulfate [sulfated (1 → 4)-α-D-glucan], curdlan sulfate [sulfated (1 → 3)-β-D-glucan], pentosan polysulfate [sulfated (1 → 4)-β-D-xylan], mannan sulfate [sulfated (1 → 4)-α-D-mannan], sulfoevernan [sulfated (1 → 3) [80%], (1 → 4) [20%]-α-D-glucan], fucoidan [composed of sulfated (1 → 2)-linked L-fucose units], PAPS (polyacetal polysulfate prepared from dextran), and PVAS (polyvinyl alcohol sulfate). (B) Heparin [composed of L-iduronic acid or D-glucuronic acid (1 → 4) linked to D-glucosamine], O-acylated (butyrylated or hexanoylated) heparin, supersulfated dermatan sulfate [chondroitin sulfate B; consists of L-iduronic acid (1 → 3) linked to D-(N-acetyl)galactosamine], PAVAS [poly(acrylic acid vinyl alcohol sulfate) copolymer], and sulfated β-cyclodextrin [cyclic dextrin consisting of seven (1 → 4)-linked α-D-glucans] and derivatives thereof (mCDS11 and mCDS71 [containing 6-benzylthio-6-deoxy or 2-O-benzyl substituents, respectively]).

**B**



viral envelope-CD4 receptor interaction (364). Yet, sulfated polysaccharides would be unable to block the viral gp120 interaction with the CD4 of monocytes (292a).

Among the more promising congeners of dextran sulfate rank polyacetal polysulfate (484) and polyvinylalcohol sulfate (27), which show potent activity against HIV-1, HIV-2, and several other enveloped viruses, including simian immunodeficiency virus, herpes simplex virus (HSV), cytomegalovirus (CMV), influenza A virus, and respiratory syncytial virus, as well as toga-, flavi-, arena-, bunya-, and rhabdoviruses (8, 124, 215, 414). Thus, the spectrum of activity of the polysulfates extends to various viruses other than HIV that may occur as opportunistic pathogens in immunosuppressed (i.e., AIDS) patients.

Of additional importance is the fact that the polysulfates can be obtained from natural sources (i.e., marine invertebrates) (66). They can be prepared and made available in large quantities at reasonable cost. They can act synergistically with other anti-HIV drugs (i.e., AZT, ddI, and ddC) (415). They are not known to lead to the development of virus-drug resistance, and they should be effective against HIV mutants that are resistant to AZT or other RT inhibitors (461).

However, polysulfates (such as dextran sulfate) suffer from a number of drawbacks which seem to argue against their potential usefulness in vivo. They are poorly absorbed after oral administration, as noted in humans (2, 288), rats (200), and mice (256). However, high oral bioavailability can be obtained by the appropriate chemical modifications, as shown for the modified  $\beta$ -cyclodextrin sulfates (mCDS11 and mCDS71) (338, 339, 359). Dextran sulfate, upon intravenous administration, produces thrombocytopenia (164). Sulfated polymers are also notorious for their anticoagulant activity, but as has been demonstrated with periodate-treated heparin (19) and O-acylated heparin (63), this problem can be overcome by appropriate chemical modifications.

The sulfated polymers owe their anti-HIV activity to the presence of the sulfate groups, which in turn are responsible for the inhibition of virus-cell binding. In this sense, any compound could be turned into an anti-HIV agent targeted at virus-cell binding provided it contains the necessary hydroxyl groups for attachment of the sulfate groups, and thus various compounds, i.e., glycyrrhizin, lentinan, amphotericin B, and gangliosides (191, 204, 347, 358, 453), were found to gain anti-HIV activity following sulfation.

Given their widely varying molecular weights and degrees of sulfation, it is very difficult to obtain standardized preparations of dextran sulfate or other sulfated polymers. This lack of homogeneity, together with the inherent variability of the molecular target (V3 loop of gp120) with which the sulfated polymers interact, may account for the differences in susceptibility of different HIV strains to different polysulfates (79, 416). This differential virus-drug susceptibility obviously raises questions as to the in vivo efficacy that may be expected for the polysulfates in each particular HIV infection.

There is little, if any, evidence for the in vivo efficacy of sulfated polysaccharides against HIV infection or any other viral infection. Dextran sulfate did not prove efficacious against feline leukemia virus infection in cats (299) or duck hepatitis B virus (HBV) infection in ducklings (356). On the other hand, sulfoglycocalyxin was reported to completely suppress Rauscher leukemia virus infection in mice if administered at a dose of 20 mg/kg/day for 8 days, starting 1 day after infection (477). Equally impressive have been the protective effects of dextran sulfate and, recently, pentosan polysulfate (139) in mice infected with the unconventional scrapie agent.

Pentosan polysulfate has been further pursued for its phar-

macokinetic properties in HIV-infected individuals (372). It has also been investigated, but found inactive, against HIV-associated Kaposi's sarcoma (375). Since Kaposi's sarcoma is characterized by microvascular proliferation (angiogenesis) in the initial stage of lesion development, it would seem justified to study sulfated polysaccharides because of their angiostatic potential against Kaposi's sarcoma. Perhaps pentosan polysulfate was not the best choice, and other sulfated polysaccharides such as the sulfated polysaccharide-peptidoglycan produced by *Arthrobacter* sp. (343) might be more efficacious against Kaposi's sarcoma.

In the wake of any solid evidence for the in vivo efficacy of the polysulfates against HIV or other viral infection, one should consider their potential application in the (systemic) prophylaxis of HIV infection following an accidental needle stick injury or stab wound, i.e., conditions in which AZT has proved inefficacious, and/or topical prophylaxis of HSV or HIV infection contracted through sexual intercourse.

The principles guiding the anti-HIV activity of polysulfates are also applicable to the polysulfonates. Several polysulfonates of varying molecular weights and degrees of sulfonation have been described as potent anti-HIV agents (Fig. 3): e.g., naphthalene sulfonates (330, 330a, 332-334) {i.e., 4,4'-[1,6-hexanediylbis(carbonylamino)]bis(5-hydroxy-2,7-naphthalenedisulfonic acid) (335)}, stilbene sulfonates (87), Evans blue and various other sulfonated dyes (47, 96, 263, 363, 473), polystyrene sulfonate, polyanethole sulfonate, and polyvinyl sulfonate (331). These compounds would bind primarily to the viral envelope gp120 glycoprotein (28) and thus interfere with the interaction between the viral gp120 glycoprotein and the cellular CD4 receptor and block virus adsorption and virus-induced syncytium formation. Like the polysulfates, the polysulfonates inhibited not only the replication of HIV but also that of other enveloped viruses, i.e., CMV (24).

In fact, the prototype of the polysulfonates, suramin (117), was the first compound to be recognized as an anti-HIV agent (325) and also the first to be used in the clinic for the treatment of AIDS (74). It was originally assumed that suramin, as well as Evans blue (47), inhibits the replication of HIV through an inhibitory effect on the viral RT. Hence, initial structure-function relationship studies were based on the inhibitory effect of suramin and its congeners on the viral RT (230). It has now become evident, however, that the polysulfonates also interfere with the viral adsorption process (412). Inhibition of virus-cell binding may well be their principal mode of anti-HIV action, since as a rule, inhibition of the RT does not correlate with inhibition of HIV replication in the virus-cell assay, probably due to the lack of cellular entry of the polysulfonates (330a).

Suramin may interfere with a number of processes, i.e., protein kinase C (PKC)-mediated processes (296), involved in virus infectivity. Furthermore, suramin and other polysulfonates (i.e., sulfonated distamycin A derivatives) (95) are known to block basic fibroblastic growth factor and other factors involved in tumor angiogenesis and should therefore be pursued for their antitumor potential, i.e., against Kaposi's sarcoma. Also, suramin is notorious for its stickiness to plasma proteins, i.e., albumin (70); thus, albumin reverses the ability of suramin to block the CD4-gp120 interaction, thereby attenuating its anti-HIV activity (489). Although the high affinity of suramin for plasma proteins, a propensity it undoubtedly shares with other polysulfonates, is likely to affect the in vivo efficacy of these compounds, suramin has proved to be effective in suppressing retrovirus (Rauscher leukemia virus) replication in mice (398). Now that so many more polysulfonates have been shown to be antivirally active, not only against HIV but also

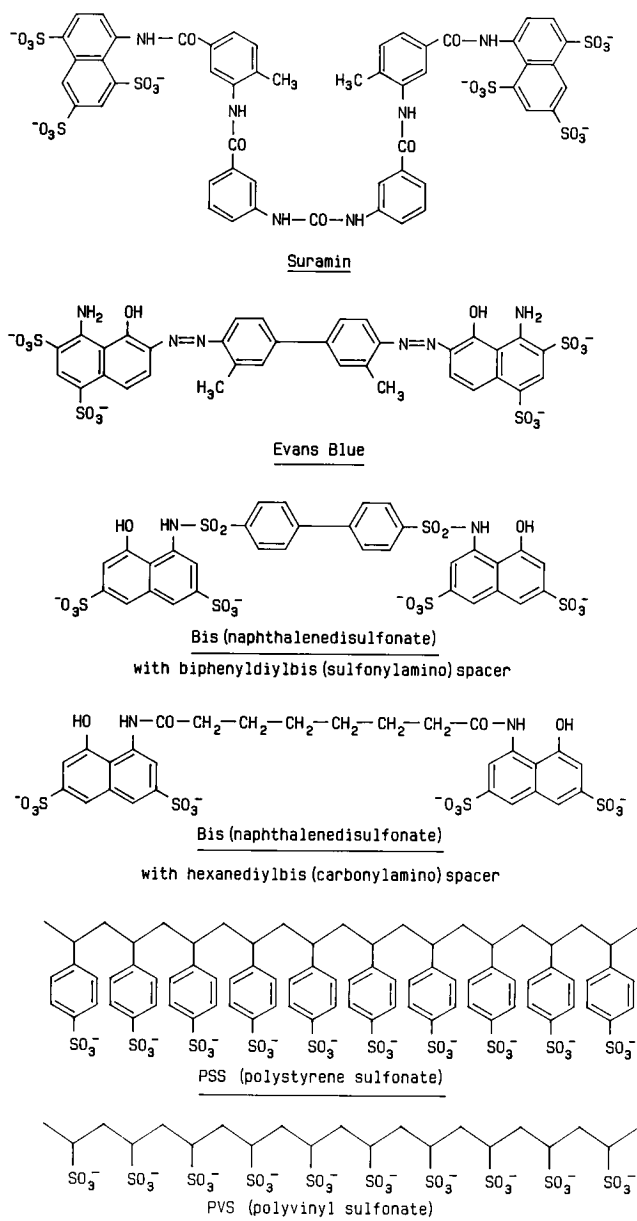


FIG. 3. Structures of polysulfonates: suramin, Evans blue, bis(naphthalenedisulfonate) derivatives, polystyrene sulfonate, and polyvinyl sulfonate.

against other viruses (e.g., CMV), it would seem imperative to explore their *in vivo* antiviral activity in the appropriate animal models.

Akin to the polysulfonates (i.e., Evans blue), the polycarboxylates (i.e., aurintricarboxylic acid [ATA]) (Fig. 4) were originally assumed to inhibit HIV replication through inhibition of the viral RT (47). Later it was ascertained that ATA inhibits HIV replication primarily through a specific interaction with the CD4 receptor (413), thus preventing the binding of the viral gp120 with its receptor (413, 472). In addition to the cellular CD4 receptor, the viral gp120 glycoprotein (V3 loop) may also serve as a target for the interaction of ATA (351, 413). Different fractions of ATA, with varying molecular weights, have been prepared, and a direct correlation was found between antiviral potency and molecular weight; thus,

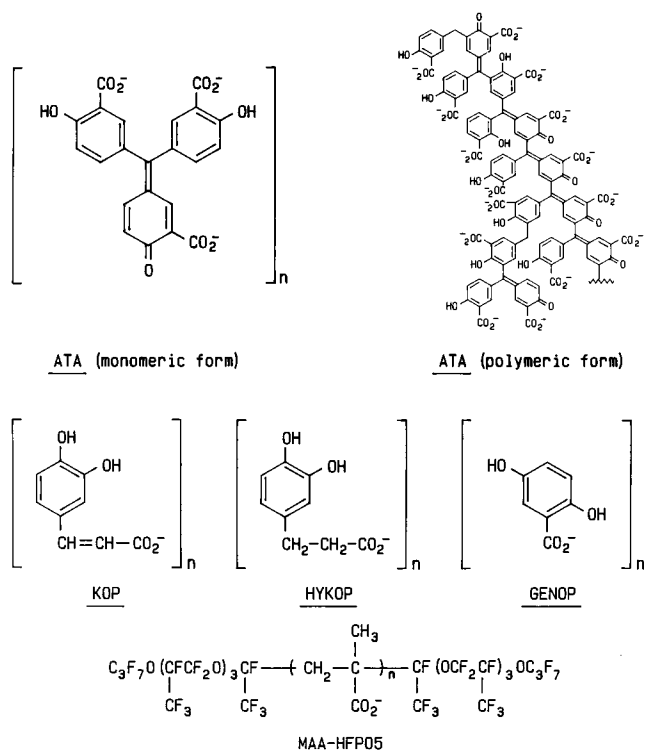


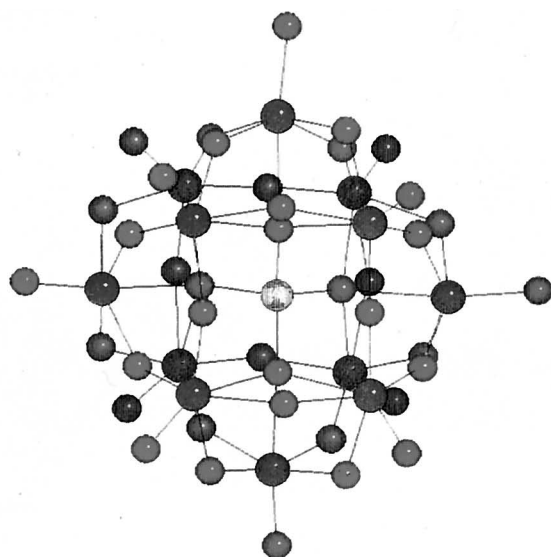
FIG. 4. Structures of polycarboxylates: aurintricarboxylic acid (ATA), phenol-derived polyhydroxycarboxylates [KOP (from caffeic acid), HYKOP (from hydrocaffeic acid), and GENOP (from gentisic acid)], and polyfluoroalkylcarboxylates [bis(perfluoro-1,4,7,10-tetramethyl-2,5,8,11-tetraoxatetradecyl) methacrylic acid oligomer (MAA-HFPO5)]. Polymeric form for ATA, as proposed by Cushman et al. (108).

the higher the molecular weight, the higher the capacity of the ATA fractions to block HIV binding to the cells, HIV replication, and HIV-induced syncytium formation (107, 108).

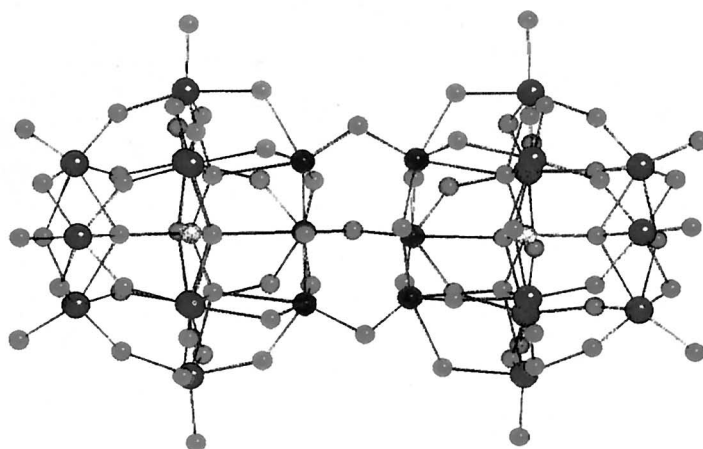
Also, polyhydroxycarboxylates derived from phenolic (PDP) compounds have been found to block HIV binding to the cells, HIV replication, and HIV-induced syncytium formation (417). The anti-HIV activity of the polyhydroxycarboxylates can be ascribed to inhibition of the gp120-CD4 interaction, and this inhibitory effect would depend essentially on the presence of the carboxylate groups (417). A similar mode of action may be postulated for the polyfluoroalkylcarboxylates (i.e., MAA-HFPO5), which have been recently shown to inhibit HIV-1 replication, HIV-1 binding to the cells, and HIV-1-induced syncytium formation (23).

As noted above for the polysulfonates, the poly(hydroxy)carboxylates (i.e., ATA and PDP) were also found to inhibit the replication of herpesviruses (i.e., HSV and CMV) (353), which again could be ascribed to inhibition of the viral adsorption process (353). As for the polysulfonates, the poly(hydroxy)carboxylates need to be further explored for their *in vivo* efficacy in the appropriate animal virus infection models.

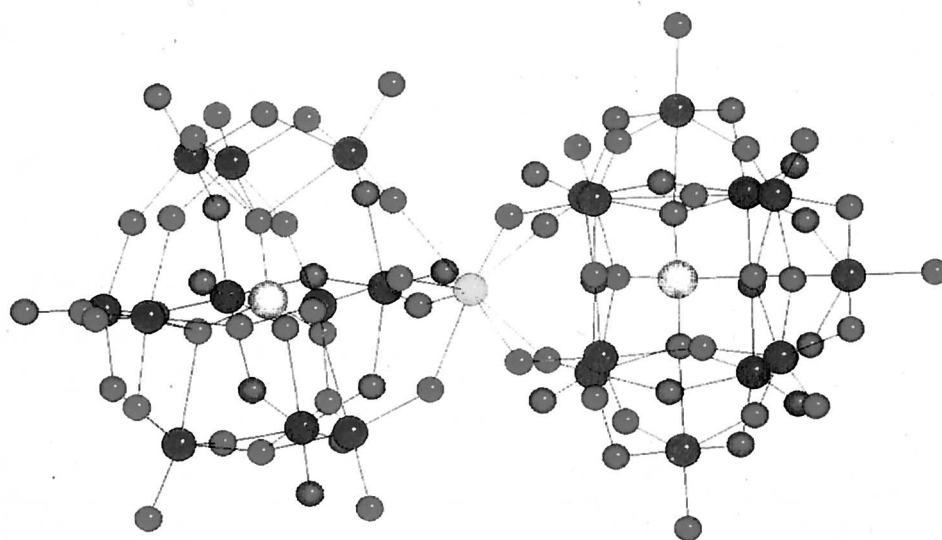
Beginning with HPA-23  $[\text{NH}_4]_{17}\text{Na}[\text{NaSb}_9\text{W}_{21}\text{O}_{86}] \cdot 14\text{H}_2\text{O}$  as the prototype (142), numerous polyoxometalates have been synthesized and found to be effective as anti-HIV agents (210, 223, 439, 475, 487). Representative examples (Fig. 5) of these inorganic complexes are  $\text{H}_4\text{SiW}_{12}\text{O}_{40}$  (JM1493) (210),  $\text{K}_7[\text{PTi}_2\text{W}_{10}\text{O}_{40}] \cdot 6\text{H}_2\text{O}$  (PM-19) (439),  $[\text{NH}_4]_2\text{H}_2[\text{EU}_4(\text{MoO}_4)(\text{H}_2\text{O})_{16}(\text{Mo}_7\text{O}_{24})_4] \cdot 13\text{H}_2\text{O}$  (PM-104) (223),  $\text{K}_{13}[\text{Ce}(\text{SiW}_{11}\text{O}_{39})_2] \cdot 26\text{H}_2\text{O}$  (JM1590) (487),  $\text{K}_6[\text{BGa}(\text{H}_2\text{O})\text{W}_{11}\text{O}_{39}] \cdot 15\text{H}_2\text{O}$  (JM2766) (487), and  $[\text{Me}_3\text{NH}]_8$



JM1493



JM2820



JM1590

FIG. 5. Structures of polyoxometalates:  $\text{H}_4\text{SiW}_{12}\text{O}_{40}$  (JM1493),  $[\text{Me}_3\text{NH}]\cdot 8[\text{Si}_2\text{Nb}_6\text{W}_{18}\text{O}_{77}]$  (JM2820), and  $\text{K}_{13}[\text{Ce}(\text{SiW}_{11}\text{O}_{39})_2]\cdot 26\text{H}_2\text{O}$  (JM1590). JM1493 represents a “keggin” structure; JM2820, a “double keggin” structure; and JM1590, a “keggin sandwich” structure.

[Si<sub>2</sub>Nb<sub>6</sub>W<sub>18</sub>O<sub>77</sub>] (JM2820) (487). Like all of the other poly-anionic substances, polyoxometalates inhibit HIV replication, HIV binding to the cells, and HIV-induced syncytium formation.

Although the polyoxometalates also inhibit the viral RT, their mechanism of anti-HIV action can be attributed primarily to inhibition of virus-cell binding. This mode of action was suggested by "time of addition" experiments, in which the polyoxometalates were added at different times after virus infection (487). Inhibition of virus-cell binding apparently results from the interaction of the polyoxometalates with the viral glycoprotein gp120.

In keeping with the other polyanionic substances, polyoxometalates are also inhibitory to various enveloped viruses (other than HIV), including herpesviruses (i.e., HSV and CMV) and ortho- and paramyxoviruses (influenza A and respiratory syncytial virus) (167, 221, 487). This broad-spectrum antiviral activity adds to the therapeutic potential of the polyoxometalates and also justifies their further follow-up in the appropriate animal virus infection models. In fact, the polyoxotungstate PM-19 has proved effective against HSV-2 infection in mice when given intraperitoneally over a dosage range of 0.1 to 50 mg/kg/day under conditions in which acyclovir was ineffective at doses of up to 100 mg/kg/day (222).

### Virus-Cell Fusion Inhibitors

To qualify as a specific virus-cell fusion inhibitor, a given compound, while not inhibitory to virus-cell binding, should inhibit syncytium formation in the direct syncytium formation assay. The latter test is based on the formation of giant cells following cocultivation of uninfected CD4<sup>+</sup> cells with HIV-infected cells expressing the viral glycoproteins gp120 and gp41. This giant cell (or syncytium) formation requires the interaction of the CD4 receptor with the viral glycoproteins. Direct syncytium formation should be distinguished from indirect syncytium formation, in which giant cells are induced by virus that has gone through its replicative cycle. The indirect syncytium formation assay cannot be used for identifying compounds that specifically interfere with virus-cell fusion, since inhibition of indirect syncytium formation may reflect interference with any step of the virus replicative pathway.

The mannose-specific plant lectins (i.e., from *Listera ovata*, *Hippeastrum* hybrid, *Cymbidium* hybrid, and *Epipactis helleborine*) and *N*-acetylglucosamine-specific plant lectin (i.e., from *Urtica dioica*) qualify as specific inhibitors of the virus-cell fusion process: they do not inhibit virus attachment to the cells, yet they block syncytium formation between HIV-infected cells and uninfected cells (52, 58). Those plant lectins that inhibit syncytium formation also inhibit HIV replication, and it is likely that they intervene with the virus replicative cycle at the fusion step. This may also be the case for mannose-specific lectins from *Gerardia savaglia* (340) (although the latter lectin was mentioned, but not shown, to block virus binding to the cells) and other sources (*Machaerium biovulatum* and *Machaerium lunatus*) (9).

Mannose- and *N*-acetylglucosamine-specific plant lectins may be assumed to interact with specific glycosylation sites within the viral envelope glycoproteins gp120 and/or gp41, particularly those sites that are rich in mannose (or *N*-acetylglucosamine). These plant lectins were also found to inhibit a number of viruses other than HIV, i.e., CMV, respiratory syncytial virus, and influenza A virus (52). As these antiviral effects were achieved at concentrations well below the cytotoxicity threshold, the most promising plant lectins should be further

pursued for their therapeutic potential in the treatment of retro-, herpes-, and myxovirus infections *in vivo*.

The peptide T22 ([Tyr-5,12,Lys-7]polyphemusin II), a derivative of polyphemusin that is highly abundant in hemocyte debris of the horseshoe crab *Limulus polyphemus*, also qualifies as an HIV-cell fusion inhibitor: it is only weakly inhibitory to virus-cell binding, yet it is strongly inhibitory to syncytium formation, and from time of addition experiments it appears to interact with a stage of the virus replicative cycle that may well correspond with virus-cell fusion (346). It would seem mandatory to examine whether the antiviral activity spectrum of T22 extends to viruses other than HIV (i.e., HSV, CMV, or respiratory syncytial virus) and/or whether it is as efficacious *in vivo*, as its *in vitro* potency tends to suggest.

Another class of molecules that is apparently targeted at the fusion process is the succinylated lectins (i.e., succinylated concanavalin A [300]) and succinylated albumins (whether or not these albumins are also glycosylated [228]). The anti-HIV activity of the succinylated albumins increases with their negative charge; they inhibit syncytium formation at concentrations that correspond to (or are slightly higher than) the concentrations required to inhibit HIV replication, while virus-cell binding is inhibited only partially at much (100-fold) higher concentrations (228). In addition to the succinylated human serum albumins (HSA), aconitylated HSA (Fig. 6) have also been found to inhibit HIV replication (229). Aconitylated albumins inhibit HIV-1 replication at lower concentrations than succinylated albumins, probably because in addition to their inhibitory effect on virus-cell fusion, aconitylated albumins also inhibit virus-cell binding by shielding off viral gp120. Both succinylated and aconitylated HSA are less active against HIV-2 than HIV-1, and in contrast to the sulfated polysaccharides (dextran sulfate), they are inactive against viruses other than HIV. Also in contrast to dextran sulfate, succinylated and aconitylated HSA lack anticoagulant activity. Succinylated and aconitylated albumins offer the potential to block HIV infectivity in blood, plasma, and plasma products and should be further examined for this purpose.

A novel class of triterpene (i.e., betulinic acid) derivatives has been recently identified as a potent and selective HIV-1 inhibitor (303). These betulinic acid derivatives (Fig. 7) are inactive against HIV-2 and apparently targeted at a postbinding, virus-cell fusion step. As some HIV-1 strains (i.e., NDK) are not susceptible to betulinic acid RPR 103611, the compound may be surmised to interact with a very specific molecular site. The precise mode of action of RPR 103611, as well as its potential therapeutic usefulness, remains a subject for further study.

### Virus Uncoating Inhibitors

Of all the retrovirus inhibitors that have been described to date, the bicyclams, consisting of 2 cyclam (1,4,8,11-tetraazacyclotetradecane) units tethered by various bridges (Fig. 8), are the only ones that have been postulated to interfere with the uncoating process. This assumption has been based on the fact that the prototype (JM2763) of this class of compounds inhibits the replicative cycle of HIV at a stage that follows the virus adsorption step but precedes the reverse transcription step, and as the compound had apparently no effect on syncytium formation (in a direct syncytium formation assay), its mode of action could be attributed to an inhibition of the viral uncoating event (129). This hypothesis was corroborated by "uncoating" experiments in which sensitivity to RNase A was monitored for the viral RNA that was recovered from HIV-infected cells that had been exposed to JM2763: the compound



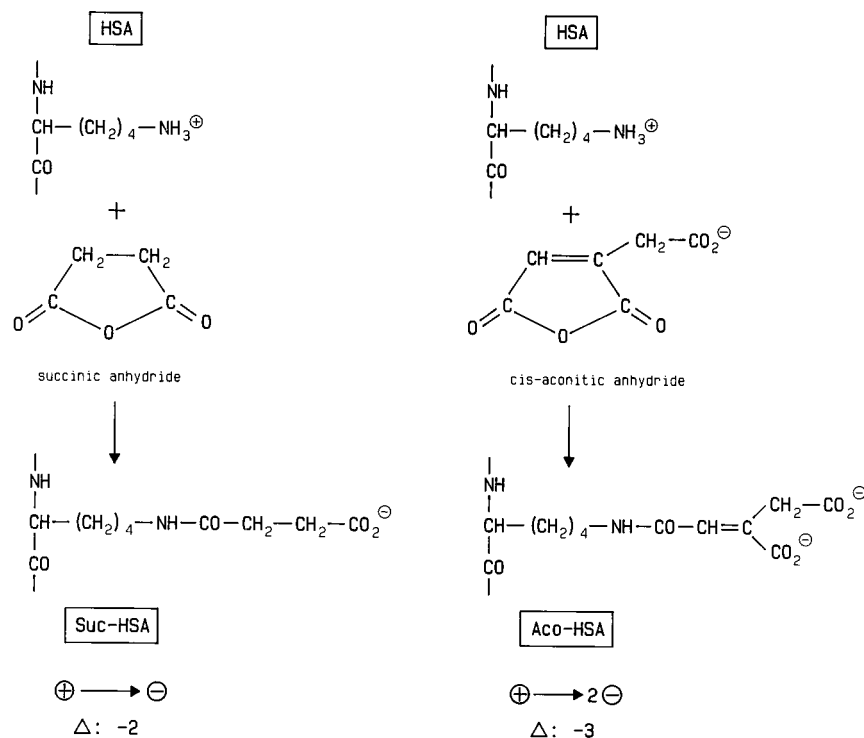


FIG. 6. Succinylated (Suc) and aconitylated (Aco) HSA, following treatment of HSA with succinic anhydride or *cis*-aconitic anhydride. Per lysine residue, suc-HSA and aco-HSA acquire one or two negative charges, respectively, which means a gain ( $\Delta$ ) of two or three negative charges overall.

effected a concentration-dependent inhibition of the degradation of viral RNA by RNase A, as could be anticipated if the uncoating (i.e., dissociation) of the viral RNA from the surrounding viral proteins had been blocked (123).

Bicyclams represent an entirely new class of HIV inhibitors and new approach toward the treatment of HIV infections. Some of the recently synthesized bicyclams (e.g., JM3100), in which the cyclam moieties are tethered via an aromatic phenylenebis(methylene) bridge (Fig. 8), inhibit the replication of HIV-1 and HIV-2 at concentrations which are more than 100,000-fold lower than the cytotoxic concentration (130). In primary T4 lymphocytes or monocytes, JM3100 inhibits HIV-1 replication at concentrations lower than 1 nM. From time of addition experiments, JM3100 appeared to interfere with viral uncoating, and this was further corroborated by uncoating experiments in which the RNase A sensitivity of the viral RNA was monitored (130). JM3100 was also found to interfere directly with virus-induced syncytium formation formation, albeit at a higher concentration ( $\sim 1 \mu\text{M}$ ) than that required for inhibition of viral replication.

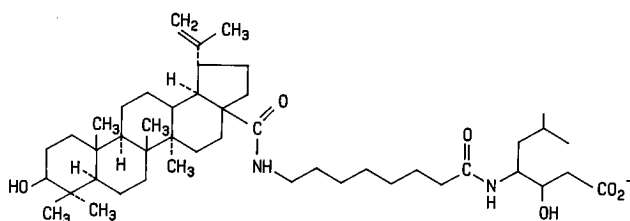


FIG. 7. Betulinic acid, RPR 103611: *N'*-[*N*-[3 $\beta$ -hydroxy-1up-20(29-ene-28-oyl]-8-aminoctanoyl]-L-statine.

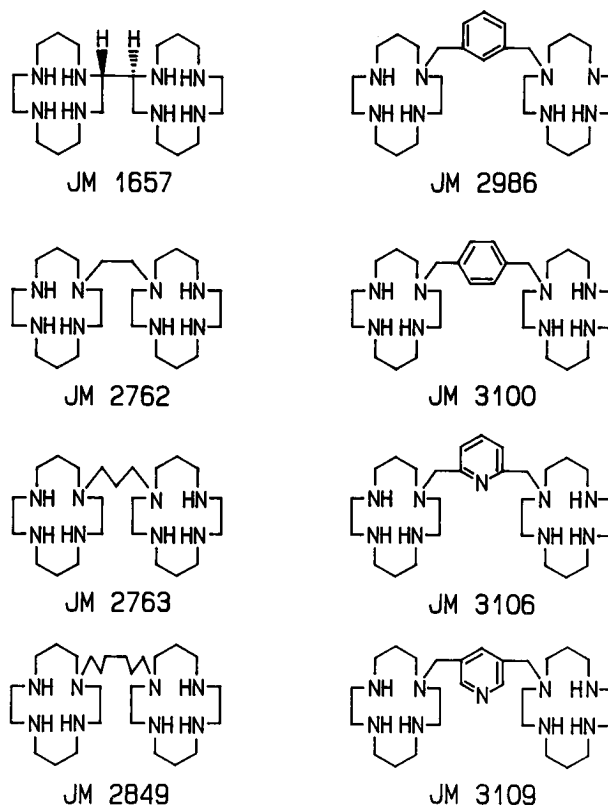


FIG. 8. Bicyclams, consisting of two cyclam (1,4,8-11-tetraazacyclotetradecane) moieties, tethered via an aliphatic bridge (i.e., propylene, as in JM 2763) or an aromatic bridge [i.e., phenylenebis(methylene), as in JM 3100].

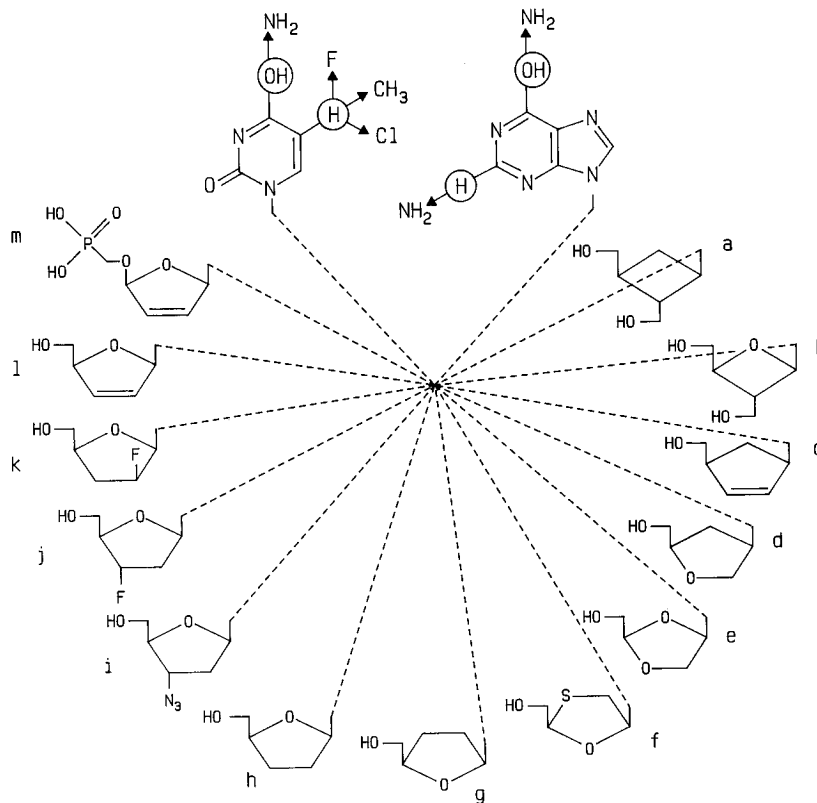


FIG. 9. 2',3'-Dideoxynucleoside analogs (clockwise): a, carboxylic oxetanocin analogs; b, oxetanocin analogs; c, carbocyclic 2',3'-didehydro-2',3'-dideoxynucleosides; d, 2',3'-dideoxynucleoside isomers; e, 1,3-dioxolane nucleosides; f, 1-oxo-3-thiolane nucleosides (3TC and FTC); g, 2',3'-dideoxy-L-nucleosides; h, 2',3'-dideoxynucleosides (ddI and ddC); i, 3'-azido-2',3'-dideoxynucleosides (AZT); j, 3'-fluoro-2',3'-dideoxynucleosides (FddCIUrd); k, 2'-fluoro("up")-2',3'-dideoxynucleosides; l, 2',3'-didehydro-2',3'-dideoxynucleosides (D4C and D4T); and m, phosphonate isosteres of 2',3'-didehydro-2',3'-dideoxynucleoside 5'-monophosphates.

### Reverse Transcription Inhibitors

**Substrate analogs.** All four anti-HIV drugs that have been formally approved for the treatment of HIV infection, namely, AZT, ddI, ddC, and D4T, belong to the class of the 2',3'-dideoxynucleoside analogs (Fig. 9). Their anti-HIV activity was disclosed (323, 326) shortly after suramin had been described as an anti-HIV agent (235). Following the saturated 2',3'-dideoxynucleosides (323), their 2',3'-unsaturated derivatives (i.e., 2',3'-didehydro-2',3'-dideoxycytidine or 2',3'-dideoxycytidinene [also referred to as D4C] and 2',3'-didehydro-2',3'-dideoxythymidine or 2',3'-dideoxythymidinene [also referred to as D4T]) (26, 53, 190, 281, 283) and various other 2',3'-dideoxynucleoside analogs were reported to inhibit HIV replication, with selectivity indexes that in some instances (i.e., 5-chloro-3'-fluoro-2',3'-dideoxyuridine [FddCIUrd]) (60, 128, 463) approached the selectivity index of AZT (118, 122, 349). While its selectivity index is comparable to that of AZT, FddCIUrd is much less toxic for the host cells than are AZT and various other 2',3'-dideoxynucleoside analogs (60, 128, 463). This compound (BW 935U83) has been selected for further development (109a).

All 2',3'-dideoxynucleoside analogs may be assumed to act in a similar fashion; that is, following intracellular phosphorylation to the 5'-triphosphate form, they serve as chain terminators of the RT reaction (as has been clearly demonstrated with AZT) (169, 218, 434). As attested to by the inactivity of 2',3'-dideoxyuridine (ddU) against HIV replication (despite the potent inhibitory effect of its 5'-triphosphate form on the

viral RT), the anti-HIV activity (or inactivity) of 2',3'-dideoxynucleosides may be more critically dependent on the initial intracellular phosphorylation than on their eventual interaction with their target enzyme (192, 193).

The bottleneck in the intracellular metabolism of the 2',3'-dideoxynucleosides is the first phosphorylation step by nucleoside kinases. Many dideoxynucleosides (such as ddU) have a low affinity for nucleoside kinases (such as thymidine kinase), and moreover, the nucleoside kinase activity of some cells (such as monocytes/macrophages) at rest may be insufficient to satisfactorily phosphorylate even those dideoxynucleoside analogs (i.e., AZT) that have high affinity for the enzyme. In attempts to overcome this problem, special prodrugs, i.e., aryl methoxyglycyl derivatives (308) and bis[*S*-(2-hydroxyethylsulfidyl)-2-thioethyl] esters (379), have been designed that deliver the monophosphate forms intracellularly and thus bypass the first phosphorylation step.

Among the most promising 2',3'-dideoxynucleoside analogs that have recently been described are 3TC, the (–)-β-enantiomer of 2',3'-dideoxy-3'-thiacytidine (BCH-189), and the (–)-β-enantiomer of 2',3'-deoxy-5-fluoro-3'-thiacytidine [(–)FTC] (83, 405, 406, 409, 429). In both cases the (–)-β-enantiomer was found to be less toxic and/or more potent than the (+)-β-enantiomer. The absolute configuration of (–)FTC has been determined by X-ray crystallography, and the results confirmed that the L-isomer [or (–)-β-enantiomer] is indeed the most active enantiomer (465). Akin to all other 2',3'-dideoxynucleoside analogs, 3TC and (–)FTC function, following their intra-

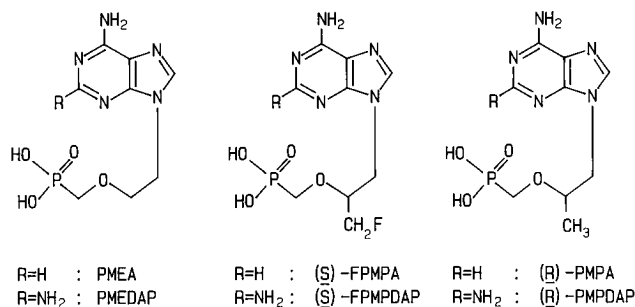


FIG. 10. Acyclic nucleoside phosphonates: 9-(2-phosphonylmethoxyethyl)-adenine (PMEA) and -2,6-diaminopurine (PMEDAP), (S)-9-(3-fluoro-2-phosphonylmethoxypropyl)-adenine (FPMPA) and -2,6-diaminopurine (FMPDAP), and (R)-9-(2-phosphonylmethoxypropyl)-adenine (PMPA) and -2,6-diaminopurine (PMPDAP).

cellular phosphorylation to the 5'-triphosphate, as DNA chain terminators in the HIV RT reaction. In fact, the 5'-triphosphates of the (-) and (+) enantiomers of FTC interact equally well with the HIV-1 RT (481). Since HBV replicates through an RNA template-driven RT process, it should come as no surprise that 2',3'-dideoxynucleosides, namely 3TC and (-)FTC, by virtue of their DNA chain-terminating capacity, not only inhibit HIV RT but also inhibit HBV RT (141, 168).

In addition to the (-)- $\beta$ -enantiomers 3TC and (-)FTC, which both have the L-configuration, other L-nucleosides, i.e., 2',3'-dideoxy- $\beta$ -L-cytidine ( $\beta$ -L-ddC) and 2',3'-dideoxy- $\beta$ -L-5-fluorocytidine ( $\beta$ -L-FddC), have been recently shown to inhibit HIV-1 and HBV replication in vitro (183a, 280). The L-nucleosides  $\beta$ -L-ddC and  $\beta$ -L-FddC must act according to the same mechanism as 3TC and (-)FTC, since HIV-1 strains resistant to 3TC and (-)FTC are cross-resistant to  $\beta$ -L-ddC and  $\beta$ -L-FddC (183a), and like the 5'-triphosphates of 3TC and (-)FTC, the 5'-triphosphates of  $\beta$ -L-ddC and  $\beta$ -L-FddC have been found to inhibit HIV-1 RT (155a).

4'-Azidothymidine, another potent HIV inhibitor (292b), runs counter to many of the structural trends: although it inhibits HIV replication via a mechanism similar to that of the 2',3'-dideoxynucleoside analogs, it retains a hydroxyl group at the 3'-position, and this 3'-hydroxyl group is mandatory, since if the 3'-hydroxyl group of 4'-azidothymidine is removed, all anti-HIV activity is lost.

The acyclic nucleoside phosphonates, i.e., 9-(2-phosphonylmethoxyethyl)adenine (PMEA), (S)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine (FPMPA), (R)-9-(2-phosphonylmethoxypropyl)adenine (PMPA), and their 2,6-diaminopurine derivatives [9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) and (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine (PMPDAP)] (Fig. 10), represent another class of selective HIV inhibitors that interact, as chain terminators, with the viral RT reaction (35, 38, 39, 370). They do so after they have been converted intracellularly to their diphosphate form, i.e., PMEApp, PMEDAPpp, FPMPApp, PMPApp, or PMPDAPpp. PMEA and its congeners have proven to be effective in vitro in a wide variety of retrovirus-cell systems, including HIV in monocytes/macrophages and human peripheral blood lymphocytes (57), feline immunodeficiency virus in feline peripheral blood lymphocytes (201), and maedi or visna virus in sheep choroid plexus cells (451). PMEA and PMEDAP have also proved selectively inhibitory to the replication of both human and duck HBV infections (492, 493), the latter in both duck hepatocytes and Pekin ducks (208).

PMEA and its congeners are more effective in vivo than may

be predicted from their in vitro potency. PMEA has been found efficacious in several animal models for retrovirus infection, including Friend leukemia virus, Rauscher leukemia virus, Moloney sarcoma virus and LP-BM5 (murine AIDS) virus infection in mice (49, 59, 171), feline leukemia virus and feline immunodeficiency virus infection in cats (150, 214), and simian immunodeficiency virus infection in monkeys (51, 456).

When PMEA was compared with AZT for in vivo effectiveness against retrovirus infections (49, 59), PMEA proved clearly superior in terms of potency and/or selectivity. A unique feature common to all acyclic nucleoside phosphonates is their prolonged antiviral action, lasting for up to 1 week or even longer after a single-dose administration. This long-lasting antiviral action may be related to the long half-life of the active metabolites (i.e., PMEApp and PMEDAPpp) within the cells and would allow infrequent dosing in the prophylaxis and therapy of retrovirus infections (48, 342).

An additional advantage of some of the acyclic nucleoside phosphonates (i.e., PMEA and PMEDAP) and the closely related 9-[2-(phosphonomethoxy)alkoxy]purines (145) and 9-[2-phosphonomethylthio]alkoxy]purines (196) is that their activity spectrum is not limited to retroviruses but also extends to herpesvirus. Thus, PMEA and PMEDAP may have a dual usefulness in AIDS patients: for the treatment of both the underlying HIV infection and the intercurrent HSV infections. Furthermore, PMEA and other acyclic nucleoside phosphonates have been found to enhance natural killer activity and stimulate interferon (IFN) production, at least in mice (81).

Drawbacks of the acyclic nucleoside phosphonates are their slow cellular uptake (by an endocytosis-like process) and their poor oral bioavailability. Thus, recent efforts have been focused on the development of prodrugs (esters) that would be better taken up by the cells (in vitro) and the gastrointestinal tract (in vivo). This approach has yielded the bis(pivaloyloxymethyl) or bis(pom) derivative of PMEA (432), which shows a >100-fold-increased cellular uptake and 5-fold better oral bioavailability than the parent compound (106, 431).

**Nonsubstrate analogs.** While the acyclic nucleoside phosphonates (i.e., PMEA) have only recently become the subject of clinical trials, much clinical expertise has been accumulating for the 2',3'-dideoxynucleoside analogs AZT, ddI, ddC, and D4T. In general, these compounds lead to an improvement of virological, immunological, and clinical parameters, namely, a decrease in p24 antigen levels (and/or virus load), an increase in CD4 cell counts, and an increase in body weight (and/or delay in the progression of the disease). Also, the long-term use of AZT in AIDS patients is accompanied by a significant increase in survival rate. However, the clinical usefulness of the dideoxynucleoside analogs AZT, ddI, ddC, and D4T is limited by their toxic side effects. These toxic side effects differ from one compound to another: anemia or neutropenia for AZT, peripheral neuropathy for ddC and D4T, and acute pancreatitis (as well as peripheral neuropathy) for ddI. These toxic side effects may be related to the interference of the 2',3'-dideoxynucleoside metabolites (i.e., 5'-mono-, di-, and triphosphates) with 2'-deoxynucleoside metabolism and, in particular, interference of the 2',3'-dideoxynucleoside 5'-triphosphates with the cellular DNA polymerization processes. Therefore, nonsubstrate analogs that do not interact with the substrate binding site of DNA polymerases, whether RNA dependent or DNA dependent, may be expected not to cause any of the toxic side effects that compromise the clinical usefulness of the 2',3'-dideoxynucleoside analogs (122, 125).

The first compounds ever shown to specifically inhibit HIV-1, but not HIV-2, replication were 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) (31, 328) and tetrahy-

droimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) (113, 369). The unprecedented specificity of the TIBO derivatives (R82150 and R82913) was attributed to a specific interaction with the HIV-1 RT (113, 369). For the HEPT derivatives it became evident that they also interact specifically with HIV-1 RT after a number of derivatives, i.e., E-EPU, E-EBU, and E-EBU-dM, had been synthesized that were more active than HEPT itself (20, 21). Subsequently to the discovery of HEPT and TIBO, several other compounds, i.e., nevirapine (BI-RG-587) (262, 317), pyridinone derivatives (L-696,229 and L-697,661) (179, 180), and bis(heteroaryl)piperazine (BHAP) (U-88204 and U-90152) (392, 393), were described as HIV-1-specific RT inhibitors.

Whereas HEPT and TIBO were discovered as the result of a systematic evaluation for anti-HIV activity in cell culture (and later found to achieve their anti-HIV-1 activity through an interaction with the HIV-1 RT), the other compounds (nevirapine, pyridinone, and BHAP) emerged from a screening program for HIV-1 RT inhibitors. The anti-HIV-1 activity of the latter compounds was then confirmed in cell culture. Like the HEPT and TIBO derivatives, the 2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-3'-spiro-5''-(4'-amino-1'',2''-oxathiole-2'',2''-dioxide)pyrimidine (TSAO) derivatives (TSAO-T and TSAO-m<sup>3</sup>T) (55, 56) and  $\alpha$ -anilino phenylacetamides ( $\alpha$ -APA R89439) (368) were discovered through the evaluation of their anti-HIV activities in cell culture and then proved to act through inhibition of HIV-1 RT. HEPT, TIBO, nevirapine, pyridinone, BHAP, TSAO, and  $\alpha$ -APA can be regarded as HIV-1-specific RT inhibitors. These compounds have also been referred to as "non-nucleoside RT inhibitors" (NNRTIs).

Which compounds could be considered NNRTIs that specifically inhibit HIV-1 RT? To qualify, the compound should, due to a specific interaction with HIV-1 RT, inhibit HIV-1, but not HIV-2, replication in cell culture at a concentration that is significantly lower than the concentration required to affect normal cell viability. On the basis of these premises, several classes of compounds (Fig. 11) could be considered NNRTIs that are specifically targeted at HIV-1 RT: TIBO derivatives (111, 113, 367, 369, 478), HEPT derivatives (20, 21, 30, 32, 495), nevirapine (54, 262, 317), pyridinones (179, 180), bis(heteroaryl)piperazines (147, 392, 393), TSAO derivatives (54–56),  $\alpha$ -APA (368), PETT derivatives (448), oxathiin carboxanilide (Uniroyal) (for which in the original studies no inhibitory effect on HIV-1 RT could be witnessed [34]), quinoxaline S-2720 (251), dihydrothiazoloisoinolones (i.e., BM+51.0836) (293, 318, 404), imidazodipyridodiazepine UK-129,485 (449), 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide (L-737,126) (480), and a series of 4-(arylethynyl)-6-chloro-4-cyclopropyl-3,4-dihydroquinazolin-2(1H)-ones (457a). These compounds were found to inhibit HIV-1 cytopathicity at a concentration that was at least 1,000-fold and in some instances (E-EBU-dM [21] and  $\alpha$ -APA R89439 [368]) even 100,000-fold, below the cytotoxicity threshold. Also, most of these compounds proved inhibitory to HIV-1 replication at concentrations of 1 to 10 nM, i.e., concentrations that would be much lower than those attainable in the organism following therapeutic use of the compounds. Exceptional activity against the HIV-1 RT (50% inhibitory concentration, 0.65 nM) was noted for a member of the imidazo[1,5-b]pyridazine series (286) carrying an additional imidazole at position 2 and 1-phenyl-1-heptanone at position 7.

The following compounds have also been claimed to be specific inhibitors of HIV-1 replication: thiazolo[3,4-a]benzimidazole NSC 625487 (76, 90, 91), pyrrolo-[1,2-d]-(1,4)-benzodiazepin-6-one (132), 2-nitrophenyl phenyl sulfone (309), naphthalenone TGG-II-23A (4), 3,4-dihydro-2-alkoxy-6-ben-

zyl-4-oxypyrimidine derivatives (14), and benzothiadiazine (NSC 287474) derivatives (75a). However, these compounds showed only moderate selectivity and/or weak potency. Calanolide A, a dipyrano coumarin derivative from the tropical rainforest tree *Calophyllum lanigerum* (245) and the related inophyllums, isolated from the Malaysian tree *Calophyllum inophyllum* (365), are examples of natural products that act as HIV-1 RT-specific inhibitors (245, 365). The interaction of calanolide A with HIV-1 RT may be distinct from that of the other NNRTIs; in particular, a segment located between amino acids 225 and 427 in HIV-1 RT may be important for specifying susceptibility to the drug (211).

How do the NNRTIs interact with the HIV-1 RT? NNRTIs show marked differences in their inhibitory potency. Their RT inhibitory potency is greatly influenced by the choice of the template/primer; it is much greater with poly(C)·oligo(dG) than with poly(A)·oligo(dT) as the template/primer (20, 21, 54, 113, 179, 317, 369, 455). In fact, TSAO-T is inhibitor to HIV-1 RT only with poly(C)·oligo(dG), and not with poly(A)·oligo(dT), poly(U)·oligo(dA), or poly(I)·oligo(dC), as template/primer (54). With rRNA as the template, TIBO R82913 inhibits HIV-1 RT at a 50% inhibitory concentration of 0.006  $\mu$ M, which is more than 1,000-fold lower than that obtained for R82913 with poly(A)·oligo(dT) as the template/primer (478).

The HIV-1 RT controls three consecutive functions: RNA transcription to DNA, degradation of the RNA template by RNase H, and duplication of the remaining DNA strand. The TIBO derivatives (e.g., R82150) and their congeners (i.e., nevirapine) preferentially inhibit the first step, i.e., RNA-dependent DNA polymerization (113, 317, 455). Inhibition of HIV-1 RT by the NNRTIs is noncompetitive with respect to both the substrate (dGTP) and the template/primer, as demonstrated, in particular, for TIBO (113, 165), HEPT (20, 21), nevirapine (258, 317), pyridinone (179, 180), BHAP (6), TSAO (54), and  $\alpha$ -APA (368). This contrasts with the behavior of the 2',3'-dideoxynucleoside 5'-triphosphates, which competitively inhibit the incorporation of the natural substrates (deoxynucleoside triphosphates) into the growing DNA chain. The noncompetitive type of inhibition of HIV-1 RT by TIBO and the other NNRTIs suggests that these compounds may interact with a nonsubstrate binding site of the HIV-1 RT. Through photoaffinity labeling, the binding site for nevirapine was shown to span the region 174 to 199, the tyrosine residues at positions 181 and 188 being crucial in the binding of nevirapine to HIV-1 RT (98, 486).

While TIBO and its congeners can be considered allosteric inhibitors of the HIV-1 RT (112, 115), their target site may be functionally and/or spatially related to the substrate binding site (114). While generally noncompetitive, the TIBO congeners under some conditions act as competitive inhibitors of HIV-1 RT: i.e., TIBO R82150 with respect to dATP, if poly(U)·oligo(A) is used as the template/primer (54); HEPT with respect to dGTP if poly(C)·oligo(dG) is used as the template/primer (114); and E-EPU and E-EBU-dM with respect to dTTP, if poly(A)·oligo(dT) is used as the template/primer (20, 21). That the HIV-1 RT binding site of the NNRTIs may be functionally and/or spatially related to the substrate binding site is also suggested by the fact that NNRTIs (BHAP and U-88204) and 2',3'-dideoxynucleoside 5'-triphosphates (ddGTP) can bind simultaneously to HIV-1 RT but the presence of one ligand decreases the affinity of RT for the second (146).

Unequivocal proof that Tyr-181 and Tyr-188 are involved in the susceptibility (and binding) of HIV-1 RT to NNRTIs such as nevirapine and TIBO came from chimeric RT constructs in

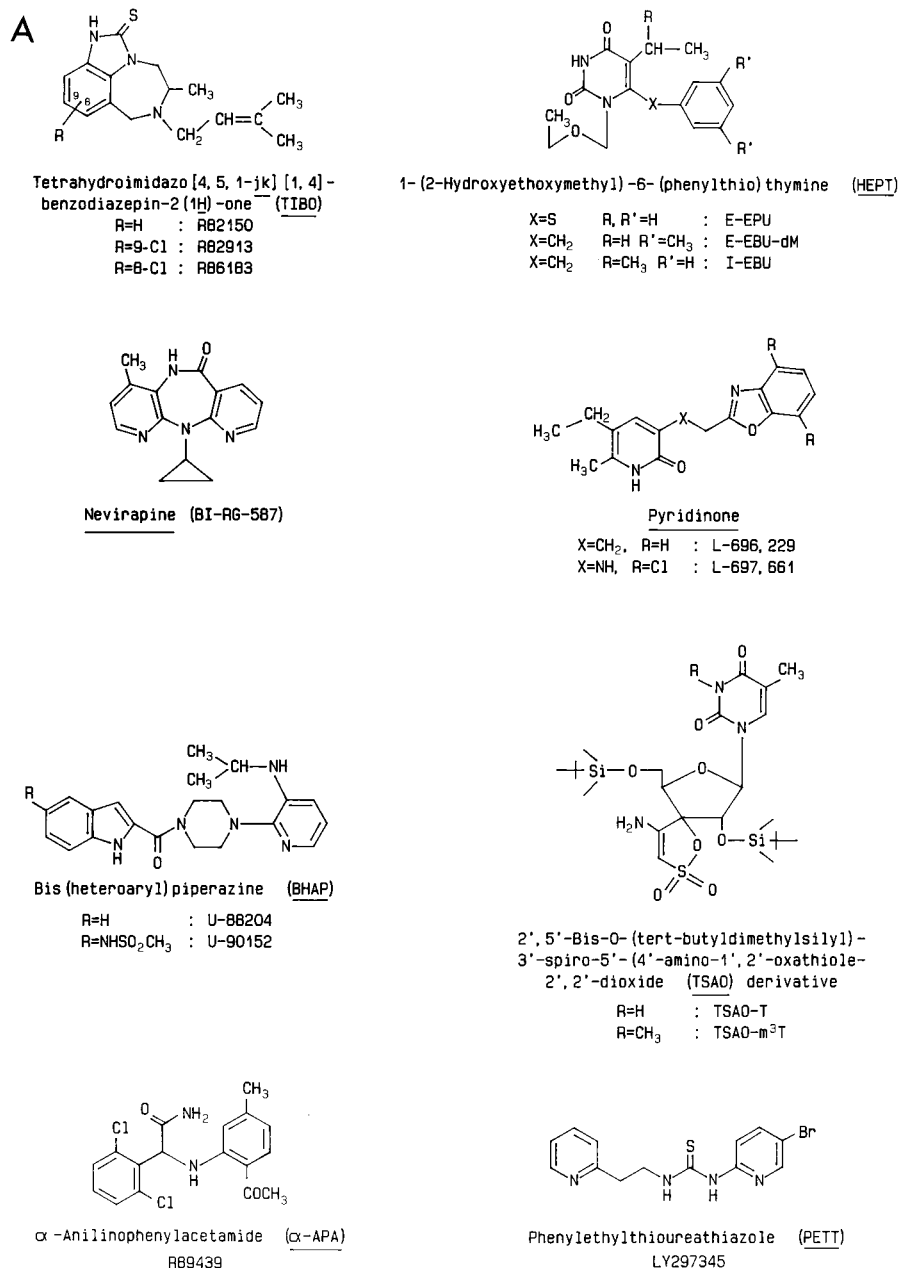


FIG. 11. HIV-1-specific RT inhibitors, which have also been referred to as NNRTIs. (A) TIBO (R82150, R82913, and R86183), HEPT (E-EPU, E-EBU-dM, and I-EBU), nevirapine (BI-RG-587), pyridinone (L-696,229 and L-697,661), BHAP (U-88204 and U-90152), TSAO (TSAO-T and TSAO-m<sup>3</sup>T),  $\alpha$ -APA (R89439), and PETT (LY297345). (B) Oxathiin carboxanilide, quinoxaline S-2720, thiazolobenzimidazole NSC 625487, pyrrolo[1,2-d]benzodiazepinone, thiazolo[2,3-a]isoidolone, imidazodipyrrodoiazepine UK-129,485, phenylsulfonylindolecarboxamide L-737,126, and 2-nitrophenyl phenyl sulfone (NPPS). (C) Oxazolinylnaphthalenone TGG-II-23A, DABO, calanolide A, inophyllums B and P, and imidazo[1,5-b]pyridazines.

which the tyrosine residues at position 181 or 188 were replaced by the HIV-2 RT counterparts isoleucine and leucine (420): the Y181I and Y188L RT constructs proved resistant to nevirapine, TIBO R82913, TIBO R82150, and E-EPU, while retaining full susceptibility to the 2',3'-dideoxynucleoside triphosphates (135, 420). The substitution Y181C, which arises as the most frequent mutation upon passage of HIV-1 in cell culture in the presence of the NNRTIs, did not cause a more than 10-fold decrease in susceptibility to TIBO R82150 (135). While Y181 and Y188 are essential for the susceptibility (and binding) of HIV-1 RT to NNRTIs (such as HEPT, TIBO, and

nevirapine), they alone do not suffice, since HIV-2 RT constructs containing I181Y and L188Y are virtually resistant to nevirapine (420). This suggests that in addition to Y181 and Y188, other amino acid residues must be involved in the susceptibility (and binding) of nevirapine and other NNRTIs to HIV-1 RT.

Which amino acids are involved in the interaction of HIV-1 RT with the NNRTIs? Through the use of HIV-1 or HIV-2 chimeric RT constructs, it was ascertained that RT susceptibility to NNRTIs largely, though not exclusively, depends on the RT region defined by amino acid residues 176 to 190, with

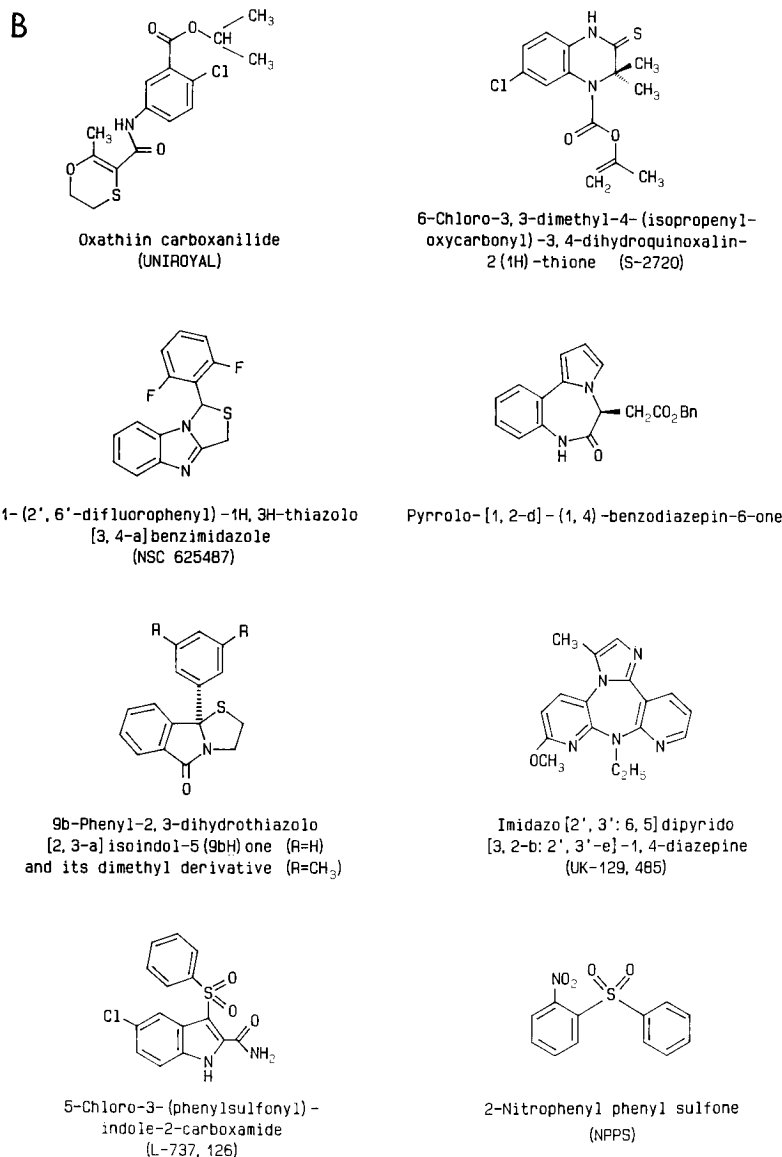


FIG. 11—Continued.

specific contributions by residues 181 and 188, and that other regions, in particular the region defined by residues 101 to 106, and a segment located between amino acids 225 and 427 may also be important for specifying drug susceptibility (99, 211). Characterization of drug-resistant virus mutants that arise in vitro, upon passage of HIV-1 in the presence of the NNRTIs, revealed that the amino acid residues 100, 103, 106, 138, 179, 181, 188, 190, and 236 (at either the p66 or the p51 subunit) of HIV-1 RT are crucial in the susceptibility of the virus to the NNRTIs. Amino acid substitutions at these positions invariably lead to resistance of the enzyme and the virus to one or more of the NNRTIs (43, 44, 61, 80, 148, 251, 314, 464, 466, 499, 500). The amino acid substitution Y181C, or Tyr → Cys at position 181, is responsible for resistance to virtually all of the NNRTIs (i.e., TIBO, HEPT, nevirapine, pyridinone, BHAP,  $\alpha$ -APA, quinoxaline S-2720, and dihydrothiazoloisoindolone BM+51.0836) (40–42, 80, 135, 251, 293, 313, 355, 368, 388, 400, 480, 500). The role of amino acid residues at positions 100,

103, 106, 138, 181, 188, and 236 in the susceptibility and resistance patterns of HIV-1 RT to TIBO, HEPT, nevirapine, pyridinone, TSAO, and BHAP has been confirmed by site-directed mutagenesis. Also, drug-resistant virus strains emerging upon passage of HIV-1 in the presence of NNRTIs in cell culture may be predictive of the mutations that could arise in the clinic in patients treated with the NNRTIs.

The structure of the HIV-1 RT, complexed with either nevirapine (255) or double-stranded DNA (13, 227), has been determined at 3.5- and 3.0-Å (0.35- and 0.30-nm resolution, respectively). In analogy with the DNA polymerase Klenow fragment, the polymerase subdomains of the HIV-1 RT p66 subunit have been named fingers, palm, and thumb and a fourth subdomain, which is missing in the Klenow fragment, has been designated the connection subdomain, for it links the RNase H subdomain with the polymerase domain. The mutations conferring resistance to the NNRTIs appear to be located on general segments ( $\beta$ 5b- $\beta$ 6 connecting loop [100 Leu → Ile,

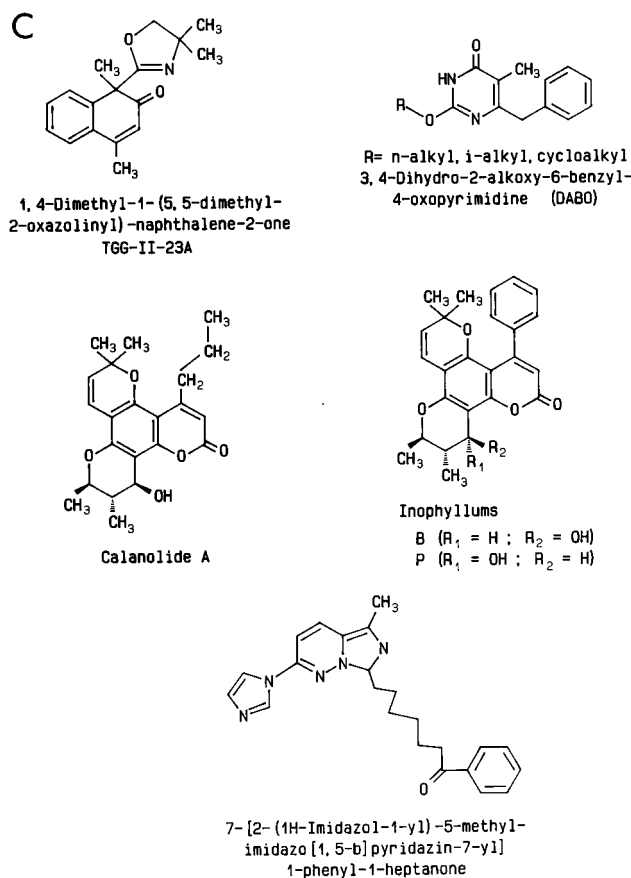


FIG. 11—Continued.

103 Lys → Asn]; β6 sheet [106 Val → Ala, 108 Val → Ile]; β-β8 connecting loop [138 Glu → Lys]; β9 sheet [181 Tyr → Cys]; β10 sheet [188 Tyr → His, Cys]; and β13-β14 reverse turn [236 Pro → Leu]) surrounding a flexible, highly hydrophobic pocket, where the NNRTIs may bind. The β5b-β6 connecting loop (with positions 100 and 103) would encircle the backside of the pocket; the tyrosine residues 181 and 188 would be located in the floor of the pocket, whereas Pro 236 would be located in the roof of the pocket (348).

The TSAO mutation 138 Glu → Lys occurs in the β7-β8 connecting loop of the fingers subdomain. In the p66 subunit, the β7-β8 hairpin is far removed from the NNRTI-binding pocket, but in p51 the β7-β8 hairpin is adjacent to the NNRTI-binding pocket of p66, and therefore the mutation 138 Glu → Lys may be expected to confer drug (i.e., TSAO) resistance when occurring in the p51, rather than the p66, subunit (73a, 236a). As dictated by the proximity of Leu-100, Lys-103, Val-106, Val-108, Val-179, Tyr-181, Tyr-188, and Gly-190 with the catalytic triad Asp-110, Asp-185, Asp-186 (which is directly responsible for substrate binding), the NNRTI pocket must be situated in the immediate vicinity of the polymerase active site, and thus any conformational changes of the NNRTI pocket resulting from its interaction with the NNRTIs may alter the conformation of the deoxynucleoside triphosphate binding site as well (446a).

To the extent that the different mutations involved in HIV-1 RT resistance to the NNRTIs affect their binding to the NNRTI pocket and/or the conformation of this pocket, cross-resistance may be expected among the different NNRTIs. This

has indeed been observed with the Y181C mutation, but for most of the other mutations, resistance is generally limited to one, two, or three classes of the NNRTIs. If, for example, Glu-138 is mutated to Lys, only resistance to TSAO, and not to any of the other NNRTIs, is seen (61). This may be attributed to the fact that the TSAO compounds, unlike all other NNRTIs, interact probably via the 4'-amino group of the 3'-spiro substituent, with the carboxylic acid group of Glu-138 (44). Other amino acid substitutions, i.e., 100 Leu → Ile and 103 Lys → Asn, lead to resistance to TIBO but not HEPT (40, 305). The 106 Val → Ala substitution confers resistance to nevirapine but not pyridinone; it also confers resistance to TIBO but much less so than to nevirapine (80). Also, α-APA is active against the TIBO-resistant 100 Leu → Ile mutant, while it is inactive against the TIBO-resistant 181 Tyr → Cys mutant (368). Substitution of Cys, Ser, or His for Tyr at position 181 results in a decreased susceptibility to TIBO, nevirapine, and pyridinone, but while substitution of Cys for Tyr at position 188 also reduces the susceptibility to TIBO, nevirapine, and pyridinone, the 188 Tyr → His substitution does not appear to affect the sensitivity of the HIV-1 RT to nevirapine (400). The fact that resistance of HIV-1 RT mutants to some NNRTIs does not necessarily lead to cross-resistance to others clearly indicates that while all of these HIV-1-specific RT inhibitors may share a common binding site ("pocket"), significant differences must exist with regard to the exact amino acid residues (within this common pocket) and/or the affinity by which they bind to their target. This thus means that while the binding sites of the different NNRTIs at the HIV-1 RT level may overlap, they are not necessarily identical (73).

Several NNRTIs have been the subjects of phase I and phase II clinical studies. When TIBO R82913 was administered daily by intravenous infusion for 2 to 50 weeks at daily doses of up to 300 mg, the drug appeared to be well tolerated, with no serious hematological, biochemical, or clinical side effects; as the patient population of this pilot study (22 patients) was small and heterogeneous, efficacy could not be assessed (373). Most of the HIV-1 isolates obtained from these patients were as susceptible to R82913 as wild-type virus; only two HIV-1 variants showing a 20- or >100-fold reduced susceptibility could be isolated; the latter appeared to contain the Y188L mutation in its RT, and this mutation was lost upon passaging the virus in vitro in cord blood lymphocytes (464). Another phase I dose-finding study with oral TIBO R82913 indicated that the oral bioavailability of this particular derivative was low (7 to 10%) and that improvement of oral bioavailability would be needed before implementation of long-term efficacy and tolerability studies (136).

Initial single-dose studies with nevirapine (given by mouth as tablets of 2.5 up to 400 mg) in humans indicated that the drug was rapidly absorbed and well tolerated and would achieve, if given daily at 12.5 mg, trough concentrations in the plasma that would be sufficient to totally inhibit replication of wild-type HIV-1 in cell culture (89).

Pyridinone L-697,661 has been subjected to a short-term clinical evaluation (6 or 12 weeks) with one of the following dosage regimens: oral L-697,661 at 25 mg twice a day, 100 mg three times a day, or 500 mg twice a day. The compound was well tolerated and exhibited a significant dose-related activity against HIV-1 (as monitored by plasma viremia or p24 antigen dlevels) (110, 399). However, this antiviral response subsided after 6 to 12 weeks, when drug-resistant virus variants appeared. The latter contained the characteristic pyridinone resistance mutations at positions 103 (Lys → Asn) and 181 (Tyr → Cys) of the HIV-1 RT. The authors (399) concluded that the rapid emergence of drug-resistant virus may limit the ef-

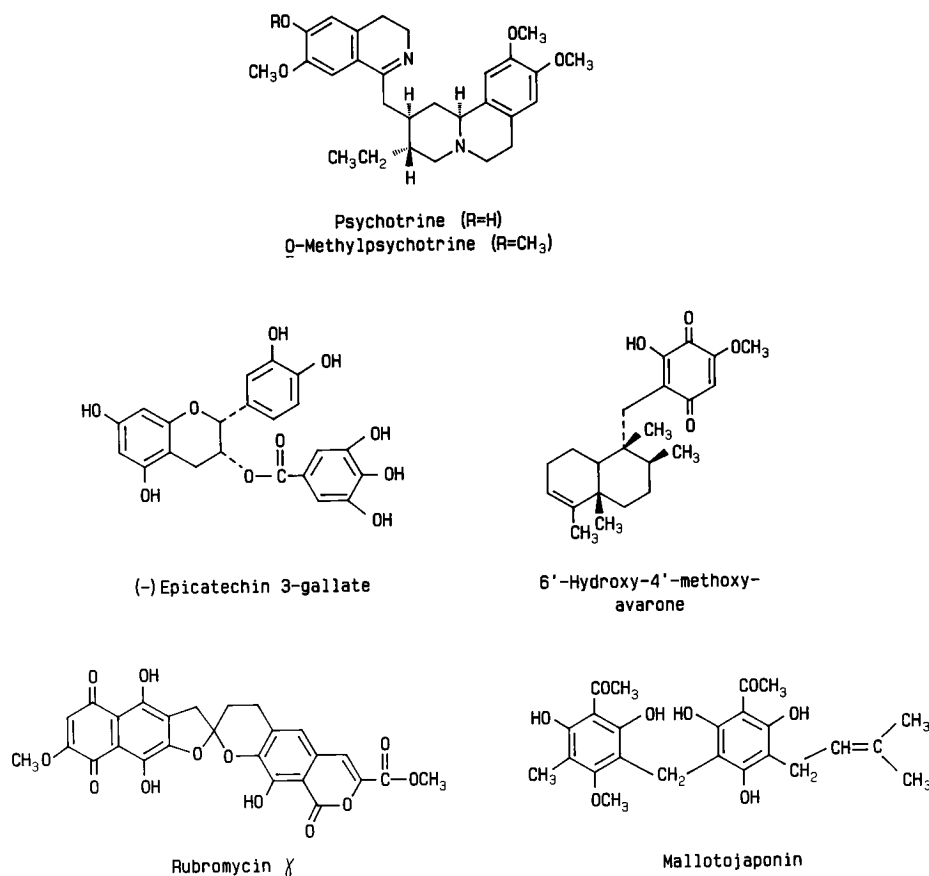


FIG. 12. Miscellaneous RT inhibitors: psychotrine, epicatechin, avarone, rubromycin, and mallotojaponin (a phloroglucinol derivative).

fectiveness of NNRTIs if used as monotherapy for HIV-1 infection but that these agents may still be useful in combination regimens. They further advised that because the emergence of resistant isolates occurred in the setting of established infection, when the genetic complexity of the virus is extensive and subpopulations of resistant virus are more likely, the use of NNRTIs for very early infection or postexposure prophylaxis may be especially advantageous (399).

**Miscellaneous RT inhibitors.** In addition to the substrate analogs (AZT, ddI, ddC, etc., which need to be phosphorylated intracellularly to their 5'-triphosphate form before they can interact with the viral RT) and the nonsubstrate analogs (TIBO, HEPT, nevirapine, etc., which are as such able to interact with the HIV-1 RT), various other substances of widely varying origins have been shown to inhibit HIV RT activity: for example (Fig. 12), rifamycins (62), rubromycins (182), avarone and avarol (289), psychotrine and *O*-methylpsychotrine (442), (-)-epicatechin-3-gallate (345), phloroglucinol derivatives (i.e., mallotojaponin) (344), pyrophosphate analogs (i.e., *N*-hydroxyphosphonoforamidate [140]), and 2',5'-oligoadenylates (341). In fact, rubromycin  $\gamma$ , avarol, and avarone were also evaluated for their inhibitory effects on HIV-1 replication in cell culture, in which they displayed little, if any, antiviral selectivity (182, 402). For (-)-epicatechin-3-gallate conflicting data have appeared: while in one study (345) the compound was found inactive at subtoxic concentrations, in another study (295) it proved inhibitory to HIV-1 infectivity at a concentration that was at least 100-fold below the cytotoxicity threshold. The anti-HIV-1 activity of (-)-epicatechin-3-gallate,

and that of other flavanoids, was attributed to its interaction with the viral envelope glycoprotein gp120 (295) rather than the RT. Another compound that has been recently found to inhibit HIV-1 replication, albeit at rather high concentrations (IC<sub>50</sub>, 14.8  $\mu$ M), is the dithiole derivative oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione] (378). Oltipraz behaves kinetically as an irreversible inhibitor of HIV-1 RT in the template-primer binding domain (378), but it is unclear to what extent, if any, the inhibitory effect of oltipraz on HIV-1 RT could account for its inhibitory effect on virus replication.

The HIV RT-associated RNase H activity has also been considered a possible target for HIV inhibitors. A number of totally unrelated products, i.e., sulfated polysaccharides (329), illimaquinone (a metabolite isolated from the Red Sea sponge *Smenospongia*) (291), a cephalosporin degradation product (189), and the 5'-monophosphate of AZT (441), have been reported to inhibit RNase H from HIV or other retroviruses. Illimaquinone would interact with HIV RT in close proximity to cysteine residue 280 and thus affect the RNase H function of the HIV RT (290). The fact that AZT 5'-monophosphate is inhibitory to RNase H, albeit at a higher concentration than is AZT 5'-triphosphate to RT, may be relevant to the mode of anti-HIV action of AZT, since AZT 5'-monophosphate is known to accumulate inside the cells to levels that are in excess of those required for the inhibition of RNase H activity (36, 169, 212). AZT 5'-monophosphate is also inhibitory to the 3'-exonuclease(s) that would otherwise cleave off AZT 5'-monophosphate from the DNA 3'-terminal ends (197).

The reverse transcription process can also be inhibited by



antisense oligonucleotides, i.e., oligonucleotides that are complementary to a template sequence adjacent to (downstream from) the primer binding site: the RT molecule travelling on the RNA template would thus be blocked by the hybrid formed by the RNA and the antisense oligonucleotide (67, 69). Phosphorodithioate-linked deoxynucleotides that bind to the primer-template binding site of HIV RT provide another type of potential therapeutic agent (298): S2dKY<sub>14</sub>, a dithioate deoxynucleotide (CTGTTCGGGCGCCA) complementary to the 5' end of the viral RNA primer binding sites was found to inhibit HIV-1 RT at a  $K_i$  of 0.5 nM.

Antisense oligodeoxynucleotides (ODNs) can also block the reverse transcription process by an RNase H-dependent mechanism, i.e., when the ODN is bound to a template sequence remote from the primer binding site and allows the RNA template to be cleaved by the RT-associated RNase H (67). Antisense phosphorothioate ODNs (S-oligos) would exert a biphasic effect on RNase H activity: at low concentration, S-oligos could enhance the cleavage of the RNA portion of the S-oligo-RNA duplex, whereas at high concentrations, S-oligos could inhibit RNase H and protect the complementary RNA from degradation (173).

Finally, the HIV RT could be blocked by RNA pseudoknots, selected by the SELEX procedure (systematic evolution of ligands by exponential enrichment), that act as high-affinity ligands of the enzyme and thus suppress enzymatic activity (459).

### Integration Inhibitors

After it has been formed in the cytoplasm by the viral RT, the duplex viral DNA is transported into the nucleus, where it is integrated into the host DNA genome through the aid of the viral integrase (IN). In fact, the IN protein is the only HIV protein necessary for integration of the viral DNA. It is also responsible for generating the recessed 3'-termini of the viral DNA before it is inserted (as proviral DNA) into the host DNA (78, 467). A relatively simple assay has been developed that should allow a high-throughput evaluation of candidate IN inhibitors (78). Potential candidate IN inhibitors may include antisense ODNs that lead to triple helix formation with the duplex viral DNA sequences that are recognized by the viral IN protein. Instead of inhibiting the function of the IN protein, one might also envisage preventing its formation, i.e., through the aid of ribozymes that cleave the RNA containing the IN gene (424). Such ribozymes have been shown to block expression of HIV-1 integrase in *Escherichia coli*. Whether aimed at blocking the action or the expression of the viral integrase, antisense ODNs and/or ribozymes will be therapeutically useful only if integration of the viral DNA into the host genome is indeed required for efficient HIV replication. This has not been ascertained for all cell types that can serve as host for the HIV infection.

### DNA Replication Inhibitors

Once integrated, the proviral DNA is replicated concomitantly with, and inseparably from, the cellular DNA genome. To operate at the level of the integrated proviral DNA, any construct, whether antisense or not, should be able to specifically recognize proviral DNA sequences. They should firmly bind to these target sequences and inactivate, or even better, delete, them from the cellular genome. Approaches to "cure" the cellular genome from any untoward genes have become an area of intense research and speculation. One of the most imaginative approaches is based on antisense constructs that (i) specifically bind to the target duplex DNA sequences, thus

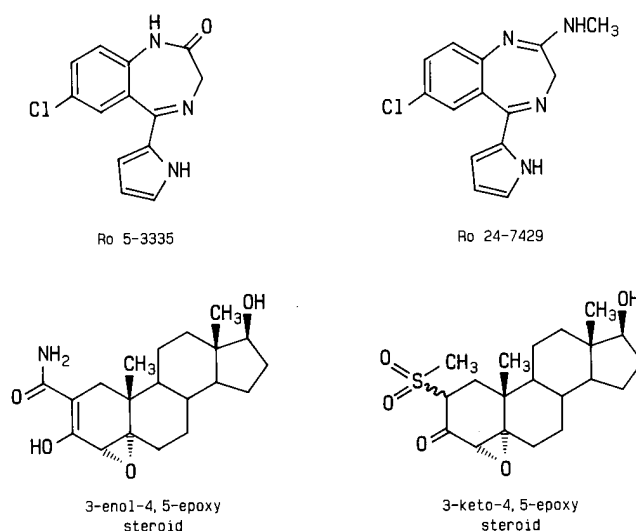


FIG. 13. Tat antagonists: Ro 5-3335 [7-chloro-5-(2-pyrrolyl)-3*H*-1,4-benzodiazepin-2(*H*)-one], Ro 24-7429 [7-chloro-*N*-methyl-5-(1*H*-pyrrol-2-yl)-3*H*-1,4-benzodiazepin-2-amine], and 3-keto/enol-4,5-epoxy steroids.

forming a local triple helix, which (ii) could be stabilized by a triple helix-specific ligand [i.e., benzo(*e*)pyridoindole (316)] and (iii) then cleaved by a specific DNA-cleaving functionality. Such "magic bullets" aimed at genetically curing the cells from any undesirable intruder still need to be worked out. Obviously, their implications reach much further than a cure for AIDS.

### Transcription Inhibitors

Antisense ODNs designed to form DNA triple helices with specific proviral DNA target sequences may be expected to inhibit transcription of viral mRNA in intact cells carrying the HIV proviral DNA genome (311). In principle, the antisense ODNs could be targeted at any region of the proviral DNA genome (i.e., *trans*-activation-responsive [TAR] region, Rev-responsive element [RRE], etc.), and they may prevent transcription by triplex formation with the proviral duplex DNA or arrest translation by duplex formation with the viral RNA, as will be discussed in the next section.

Another approach toward blocking HIV gene expression is based on the inhibition of *trans*-activation by the *trans*-activator protein, Tat. The Tat protein interacts with the TAR region (380), located immediately downstream of the transcription initiation site of the proviral DNA. A number of cellular factors seem to cooperate with Tat in the overall transactivation process (242). Some of these factors (i.e. NF- $\kappa$ B, SP-1, TFIID, LBP-1, and LBP-2) (297) may bind directly to the proviral DNA near the transcriptional initiation site, whereas other cellular factors (i.e., MSS1) (419) might directly modulate Tat-mediated transactivation. Better insight into the different factors and steps involved in the transactivation process should help in developing Tat inhibitors.

The best known Tat antagonists (Fig. 13) are Ro 5-3335 [7-chloro-5-(2-pyrrolyl)-3*H*-1,4-benzodiazepin-2(*H*)-one] (217, 483) and its congener Ro 24-7429, in which the —NH—CO— functionality has been replaced by the —N=C(NHCH<sub>3</sub>)— functionality (216). More recently, some keto/enol epoxy steroids (Fig. 13) have also been reported to act as HIV-1 Tat inhibitors (321). The Tat antagonists Ro 5-3335 and Ro 24-7429 are inhibitory (at a concentration of about 1  $\mu$ M) to both

HIV-1 and HIV-2, which contrasts with the TIBO-type RT inhibitors which are solely inhibitory to HIV-1. Again, in contrast with the RT inhibitors, the Tat antagonists are active against both acute and chronic HIV infection. They also act synergistically with the dideoxynucleoside analogs, show activity against AZT-resistant HIV strains, and do themselves not lead to the development of resistance, even after prolonged (2-year) exposure in cell culture (216). The latter is not surprising, since as suggested for the marked cell type-dependent differences in anti-HIV activity noted for Ro 5-3335 (483), this class of compounds may be assumed to interact with one of the cellular factors involved in the transactivation process rather than the Tat protein per se (483). A possible target protein for transactivation inhibitors is the cellular serine or threonine kinase that seems to mediate Tat function (209). It remains to be established whether TAT antagonists, such as Ro 5-3335 and Ro 24-7429, or any other, that are targeted at cellular proteins rather than the Tat protein itself may be effective in vivo, in the clinical setting, in suppressing HIV replication without untoward effects on the host.

Transcription of the HIV-1 provirus is governed by the viral long terminal repeat (LTR), and the activity of the HIV-1 LTR is determined by a number of both positive and negative transcriptional regulators. In particular, phorbol 12-myristate 13-acetate and tumor necrosis factor are potent activators, whereas three other compounds (topotecan,  $\beta$ -lapachone, and curcumin) have been reported to block activation and/or suppress the activity of the HIV-1 LTR (279). The latter compounds may thus prevent induction of viral expression in latently infected cells. Assuming that PKC is involved in activation of the latent HIV-1 infection, PKC inhibitors, such as the recently described indolocarbazoles (366, 382), may also be postulated to act, albeit indirectly, as HIV-1 LTR transcription inhibitors.

### Translation Inhibitors

Antisense oligonucleotides may inhibit HIV replication at a number of stages: virus adsorption, reverse transcription (RNA  $\rightarrow$  DNA) proviral DNA replication, transcription (DNA  $\rightarrow$  RNA), and finally, translation. As a rule, the antisense ODNs are expected to form a stable duplex with complementary sequences of the viral mRNA and thus arrest viral mRNA translation (301, 401, 496). This has been shown particularly with an antisense phosphorothioate ODN against the regulatory HIV gene Rev (302), as well as the antisense phosphorothioate ODN GEM91, a 25-mer complementary to the HIV-1 *gag* mRNA initiation site (Fig. 14). GEM 91 may block the translation of *gag* mRNA and also disrupt the secondary structure of RNA (3). Antisense oligonucleotides may also be targeted at the RRE of the viral mRNA and, through disruption of Rev-RRE complexes, assist in blocking expression of the viral glycoproteins (158a).

The phosphodiester-, phosphorothioate- and phosphorodithioate-based ODNs, once they have been hybridized to the mRNA, may allow the cellular RNase H to cleave the RNA, and hence multiple copies of each target mRNA could be eliminated via the RNase H cleavage mechanism. However, only the phosphodiester-, phosphorothioate-, and phosphorodithioate-based ODNs are competent for RNase H-activated cleavage of RNA, while methylphosphonate ODNs, phosphoramidate ODNs, and many other backbone-modified ODNs (Fig. 14) are not (322). These other ODNs must, if active in inhibiting mRNA translation, do so through simple steric blocking, thus preventing the RNA from interacting with

the cellular components required for translation of the mRNA into protein.

The backbone-modified ODNs (Fig. 14) have been designed in attempts to overcome the hurdles that generally compromise the therapeutic efficacy of ODNs: poor cellular permeability, premature degradation by nucleases, and insufficient affinity for their target RNA sequences (322). Modification of the phosphodiester backbone has indeed been shown to impart stability and may also allow for enhanced affinity and increased cellular permeation, but none of the currently available ODNs meet all the requirements for a therapeutically useful molecule. Thus, further ingenuity will be needed to construct antisense molecules (i.e., uniformly modified 2'-deoxy-2'-fluorophosphorothioate oligonucleotides [246], self-stabilized at their 3'-ends by hairpin loop structures [446]) that have both high affinity for their RNA target and stability toward nucleases (246) and, moreover, remain sufficiently stable in vivo (446).

Antisense ODNs could be added exogenously: for example, antisense ODN phosphorothioates targeting different sequences of the viral genome have been applied in a rotating manner so as to reduce the viral burden and to minimize the risk of escape mutants (285). Because of the difficulties encountered in delivering the antisense oligonucleotides intracellularly (390), different approaches using viral vectors (460) (i.e., murine leukemia virus [287] or adeno-associated virus [88]) have been elaborated to introduce antisense oligonucleotides into the cells. Constitutive expression of the antisense RNA may then lead to inhibition of HIV gene expression in the cell that has already been infected by HIV as well as confer "intracellular immunity" of noninfected cells against subsequent HIV infection. The constitutively expressed antisense RNA may block HIV replication by several mechanisms: by blocking the reverse transcription of genomic RNA to proviral DNA or by arresting translation of the targeted mRNA (460).

An interesting approach (394) toward the therapy of HIV infections is based on the use of ribozymes (403), namely, RNA molecules that following hybridization with their target RNA sequences, also cleave a specific phosphodiester bond in this target RNA (Fig. 15). Most of the ribozymes that have been constructed are of the "hammerhead" (374) or hairpin (494) type. They can be targeted at different sites of the viral RNA, including the RNA fragments that encode the regulatory proteins (i.e., Rex and Tax in the case of bovine leukemia virus) (85). The stability of the ribozymes toward nucleases can be increased without a serious decrease in catalytic efficiency (206, 207). Ribozymes can be delivered exogenously to the cells (457), and this delivery can be enhanced by electroporation, liposome encapsulation (395), or conjugation to polycations. As mentioned above for the antisense oligonucleotides, ribozymes can also be delivered intracellularly via retroviral vectors (287, 476), and this should then allow constitutive expression of the ribozymes and thus protect ("immunize") the cells against HIV infection (144, 494). The efficacy of ribozymes for the rapid and specific cleavage of RNA might be enhanced by endogenous proteins or addition of p7 nucleocapsid (Nc) protein (457), and such proteins may be introduced along with the ribozyme by means of a gene therapy approach. Also, constructs in which the ribozyme is covalently linked to antisense oligonucleotides or to the (3'-end of the) tRNA primer may be envisaged. The latter may be able to cleave the viral RNA as soon as it has been attached to the primer binding site.

Many problems remain to be addressed before the true potential of ribozymes can be fully assessed. These questions concern their delivery forms (exogenous or endogenous), their

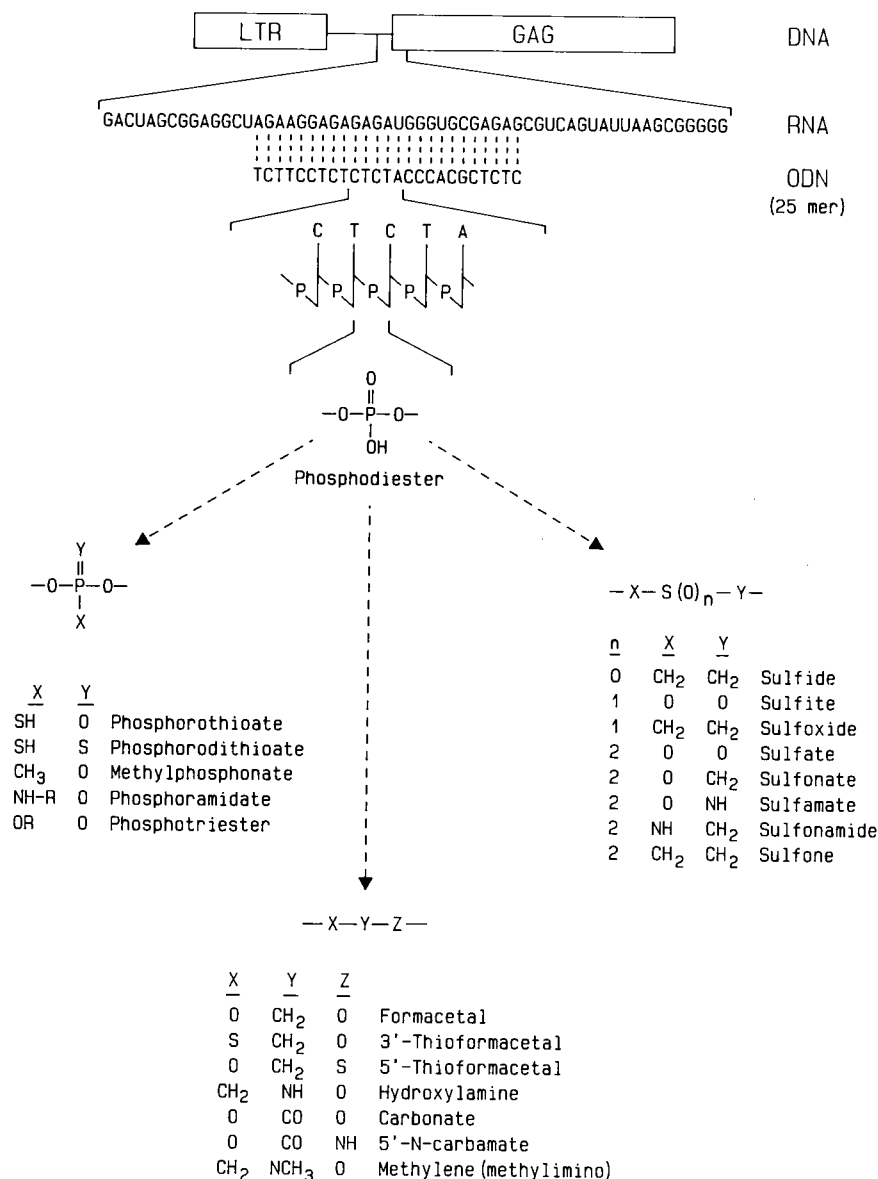


FIG. 14. Antisense ODNs: for example, GEM 91, a 25-mer ODN phosphorothioate, complementary to the *gag* mRNA of HIV-1 at the initiation codon (AUG) site. In attempts to increase cellular permeation of ODNs, protect them against degradation by cellular nucleases, and/or enhance their affinity for binding to their target mRNA sequences, the natural phosphodiester linkage can be replaced by various other linkages (i.e., phosphorothioate, phosphorodithioate, etc.).

specificity (for viral compared with cellular RNAs), their stability (intracellular turnover), their catalytic activity in physiological conditions, and their accessibility, inside the cells, to the viral RNAs and to the target sequences of the viral RNA. Tethering the ribozyme to the HIV packaging signal may enhance the ribozyme's efficiency by colocalizing it with the HIV mRNA transcripts inside the cells (436). Other issues remaining to be addressed concern the propensity of ribozymes for mispairing and the development of resistance by mutations at their target site.

A separate class of translation inhibitors, also known as SCRIPs (for single-chain ribosome-inactivating proteins) because they cleave the eukaryotic ribosomal 28S RNA, is represented by trichosanthin, also referred to as GLQ223, a 26-kDa protein isolated from *Trichosanthes kirilowii* (307). The compound was found to inhibit HIV replication in acutely and

chronically infected lymphocytes and macrophages. GLQ223 has been pursued for its clinical potential (174), despite its overt toxicity to the host cells (i.e., for MT-4 cells at a concentration of 0.25  $\mu\text{g/ml}$ ) (159, 381). Another protein from *T. kirilowii*, termed TAP 29, a 29-kDa protein, would be less toxic yet still active in inhibiting HIV replication (277). Although TAP 29, like trichosanthin and other SCRIPs, is assumed to owe its anti-HIV activity to its "SCRIP" effect, namely, cleavage of ribosomal 28S RNA and thus abrogation of polypeptide chain elongation, a causal link between SCRIP and anti-HIV activity has not been established.

#### Maturation Inhibitors

**Protease inhibitors.** An aspartyl protease encoded by the viral *pol* gene is responsible for the cleavage of the *gag* and

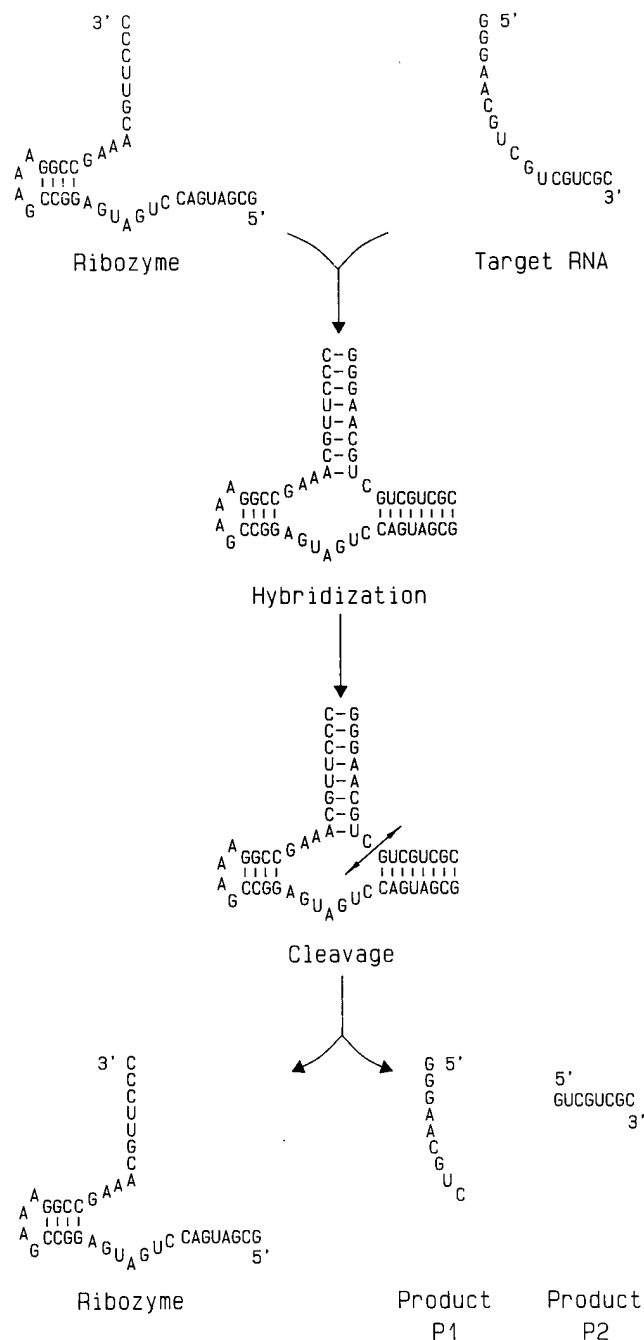


FIG. 15. Ribozyme (hammerhead ribozyme HH16 [403]) hybridizes to a specific RNA sequence (containing GUCN) and then cuts it at the specific cleavage point C ↑ N to give two products, P1 (5' product, ending with a 2',3'-cyclic phosphate) and P2 (3' product, starting with a 5'-hydroxyl group).

*gag-pol* precursor proteins (Pr55 and Pr160, respectively) into the mature *gag* and *pol* proteins. The search for HIV protease inhibitors was launched after it was ascertained that the HIV protease is required for viral infectivity (254). This search was facilitated by the vast knowledge of other aspartyl protease (i.e., renin) inhibitors, the cloning and purification of the HIV protease, the elucidation of its three-dimensional structure (first at 3-Å [0.3-nm] resolution [350] and later at 2.8-Å [0.28-nm] resolution [154]), and the development of rapid enzyme

assays for screening potential inhibitors. In the past few years there has been a virtual explosion of new X-ray crystal structures from numerous laboratories aimed at the characterization, on an atomic level, of the structures of the HIV protease-inhibitor complexes (485).

The identification of the HIV protease cleavage sites (Tyr † Pro, Phe † Pro, Leu † Ala, Met † Met, Phe † Tyr, Phe † Leu, and Leu † Phe) proved useful in designing the appropriate inhibitors: protease inhibitors with high specificity (Tyr † Pro or Phe † Pro), protease inhibitors of the renin inhibitor type (Leu † Ala), and symmetrical inhibitors (Met † Met). In the design of these inhibitors, the "transition state peptidomimetic" principle was followed, thus replacing the hydrolyzable peptide linkage by a nonhydrolyzable transition state isostere, i.e., statine, hydroxyethylene, reduced amide, hydroxyethylamine, (hydroxyethyl)urea, or dihydroxyethylene (Fig. 16). Thus emerged a variety of HIV protease inhibitors (Fig. 17): hydroxyethylamine derivatives (i.e., Ro 31-8959 [391]), hydroxyethylene derivatives (i.e., U-81749 [310], UK-88947 [33], and L-687,908 [462]), (hydroethyl)urea derivatives (i.e., SC-52151 [175]), norstatine derivatives (i.e., KNI-227 [238]), the C<sub>2</sub> symmetric dihydroxyethylene derivatives A-74704 (154, 250), A-77003 (249), and L-700,414 (68) and other dihydroxyethylene derivatives (450), and various other protease inhibitors (133, 239, 266, 292, 386, 454).

Various new HIV protease inhibitors containing the dihydroxyethylene transition state isostere have been synthesized, and starting from Ro 31-8959 as the model compound, various novel and high-affinity ligands have been introduced at the P<sub>2</sub> (3-tetrahydrofuran and pyran urethanes [177], cyclic sulfolanones [176], and tetrahydrofuranylglucines [183]) and P<sub>3</sub> (pyrazine amides [183]) positions of the molecule. Novel constrained "reduced amide"-type inhibitors of HIV protease have been constructed in which three amino acid residues of the polypeptide chain were locked into a  $\gamma$ -turn conformation and designated  $\gamma$ -turn mimetics (352).

As an alternative to the peptide-based approach, penicillin-derived compounds have been pursued as HIV-1 protease inhibitors: (i) penicillin-C<sub>2</sub>-symmetric dimers held together by an ethylenediamine linker (220) and (ii) monomeric penicillins linked to peptide isosteres (i.e., statine) (213). On the bases of the knowledge of the X-ray crystal structure of the HIV protease dimer and the role of a structural water molecule in linking the protease inhibitor to the HIV protease dimer, an entirely new class of HIV protease inhibitors, that of the non-peptide cyclic ureas, has been developed (265, 361). XM323, the prototype of this series of HIV protease inhibitors, inhibits the enzyme at a  $K_i$  of 0.27 nM and inhibits HIV-1 replication in vitro at a 50% inhibitory concentration of 0.036  $\mu$ M (50% cytotoxic concentration, 61.5  $\mu$ M); in contrast to most of the peptide-based HIV protease inhibitors, XM323 also has good oral bioavailability (27% in rats and 37% in dogs) (265).

The HIV protease inhibitors Ro 31-8959 (391) and A-77003 (259) have been the subjects of extensive preclinical evaluation. These compounds offer an interesting profile as candidate anti-HIV drugs: i.e., Ro 31-8959 is active against HIV-1 in cell culture at a concentration of 1 to 2 nM and inhibitory to the HIV-1 protease at a  $K_i$  of 0.1 nM. It is not inhibitory to renin, pepsin, cathepsin, elastase, prolidase, or collagenase. It is active in both acutely and chronically HIV-infected cells (170). It is active against AZT-resistant HIV-1 strains and acts synergistically with 2',3'-dideoxynucleosides (ddC) and Tat antagonists (Ro 24-7429). Although virus resistance to the HIV protease inhibitors may develop resistance to Ro 31-8959 would seem to arise less readily than that found for the RT inhibitors (105).

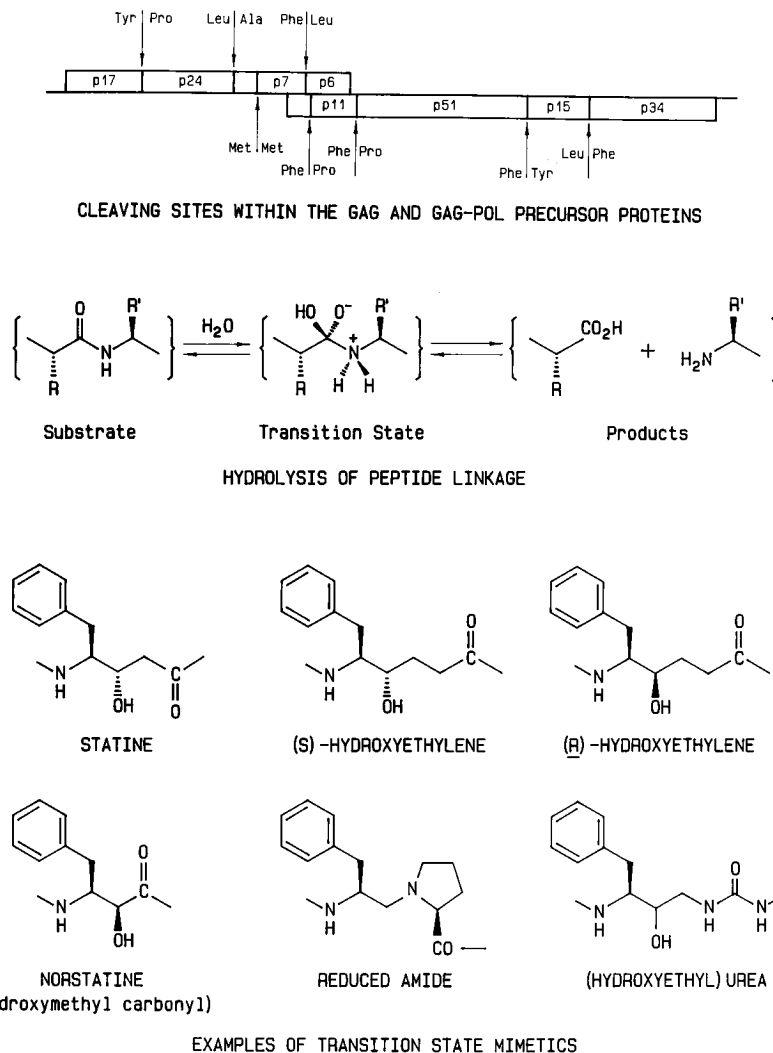


FIG. 16. Concept of HIV-1 protease inhibitors as peptidomimetics of the transition state formed during hydrolysis of the peptide linkage, with special reference to the peptide bonds that are cleaved during the maturation of the viral *gag* and *gag-pol* precursor proteins.

Peptide-based drugs generally have a short half-life (due to degradation by proteolytic enzymes) and poor oral bioavailability. As demonstrated with the renin inhibitor A-72517 (253), oral bioavailability can be significantly enhanced by the appropriate chemical modifications, and thus A-77003, which has poor oral bioavailability (<1%), has been further modified to yield A-80987, which is still equally as active as an HIV-1 protease inhibitor ( $K_i$ , 0.2 nM) but has better oral bioavailability ( $\approx 25\%$  in the rat). The HIV protease inhibitor Ro 31-8959 would achieve plasma levels upon oral administration that for several hours are far in excess of those required to inhibit HIV replication.

Also, most HIV protease inhibitors are notoriously hydrophobic and thus poorly soluble in aqueous medium. These compounds also appear to be rapidly cleared by the liver. In attempts to remedy these problems, phosphate prodrugs in which the phosphate group was introduced via the hydroxyl functionality of serine or threonine have been designed: they are highly water soluble and maintain significantly higher blood levels *in vivo* (93).

It remains to be established whether HIV-1 protease inhibitors are able to arrest progression of AIDS in patients. Clin-

ical trials with the prominent HIV-1 protease inhibitors (i.e., Ro 31-8959) are under way. In the meantime, it has been demonstrated that inhibitors of retroviral proteases, in particular, KH164, a statine-based protease inhibitor, impedes progression of Friend murine leukemia virus-induced disease in mice (264).

**Myristoylation inhibitors.** The *gag* precursor protein (Pr55) and *gag-pol* precursor protein (Pr160), as well as the *nef* protein, need to be myristoylated; that is, they require attachment of myristic acid via an amide bond to their N-terminal Gly; otherwise, no mature infectious virus particles can be formed (184). This myristoylation is carried out by a cellular enzyme, protein *N*-myristoyltransferase. Several myristic acid derivatives, i.e., *N*-myristoyl glycinal diethylacetal (447), 13-oxatetradecanoic acid (75), and 12-azidododecanoic acid (134), have been found to inhibit HIV-1 production in both acutely and chronically infected cells. However, these myristoylation inhibitors are active only at a relatively high concentration (10 to 50  $\mu\text{M}$ ), which may not be therapeutically meaningful.

**Glycosylation inhibitors.** The HIV envelope glycoproteins gp120 and gp41 undergo extensive glycosylation, and as these glycoproteins are involved in virus-cell binding and virus-cell

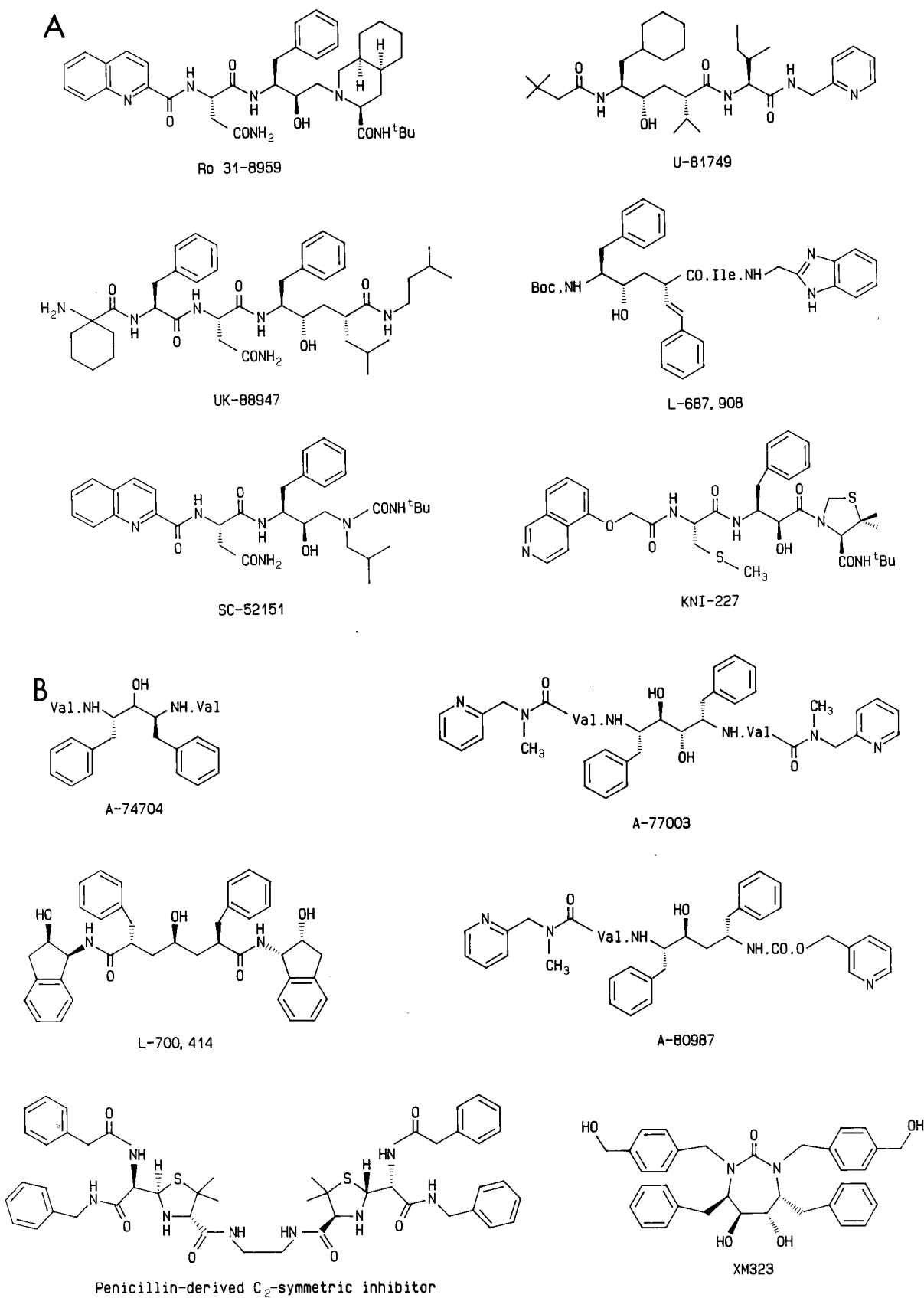


FIG. 17. HIV-1 protease inhibitors. (A) Ro 31-8959, U-81749, UK-88947, L-687,908, SC-52151, and KNI-227. (B) A-74704, A-77003, L-700,414, A-80987, penicillin-derived  $C_2$ -symmetric inhibitors, and nonpeptide cyclic ureas (XM323). Boc, *tert*-butoxycarbonyl; <sup>t</sup>Bu, *tert*-butyl.

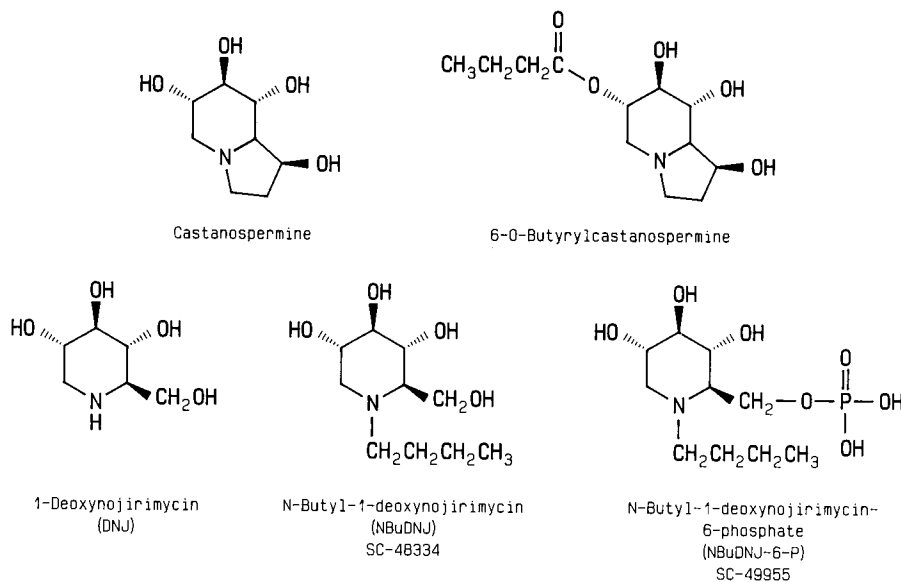


FIG. 18. Glycosylation inhibitors: castanospermine, 6-*O*-butyrylcastanospermine, 1-deoxynojirimycin (DNJ), *N*-butyl-1-deoxynojirimycin (NBuDNJ), and *N*-butyl-1-deoxynojirimycin-6-phosphate (NBuDNJ-6-P). The last should be considered a prodrug of NBuDNJ.

fusion, the glycosylation process has been pursued as a target for chemotherapeutic intervention. Thus, a number of aminosugar derivatives (Fig. 18) (castanospermine [470], 1-deoxynojirimycin [185], *N*-butyl-1-deoxynojirimycin [NBuDNJ; 131, 243], 1-deoxymannojojirimycin [337], and 6-*O*-butyrylcastanospermine [396]) have been reported to inhibit HIV infectivity, albeit at relatively high concentrations (0.1 to 10 mM). All of these glycosylation inhibitors, except 1-deoxymannojojirimycin, which is a mannosidase inhibitor (482), are inhibitory to  $\alpha$ -glucosidase I, the enzyme which is responsible for the cleavage of the terminal  $\alpha$ -glucose unit and thus initiates the trimming of the N-linked oligosaccharides.

The attenuated infectivity of HIV particles released from chronically infected cells that have been exposed to the glycosylation inhibitors is paralleled by reduced binding of these virions to the cells and, consequently, syncytium formation (362). The anti-HIV activity of 1-deoxynojirimycin and its congeners may obviously be attributed to the altered glycosylation of the HIV envelope glycoproteins ensuing from their inhibitory effect on  $\alpha$ -glucosidase I, but how then may this aberrant glycosylation give rise to an attenuation of HIV infectivity? Among the several possibilities that could be envisaged are (i) abnormal folding of the nascent glycoprotein gp120 (158), (ii) diminished processing of the gp160 precursor glycoprotein to gp120 and gp41 (362, 383, 421), and possibly, (iii) impaired processing of the gp120 to gp70 and gp50 (which would be catalyzed by a trypsin-like protease, once gp120 has been docked to the CD4 receptor) (225).

Castanospermine, when given orally at doses as high as 100 or 400 mg/kg/day, was found to inhibit murine Rauscher leukemia virus-induced splenomegaly by 37 and 78%, respectively; however, when compared with AZT in the same murine system, castanospermine was less active and more toxic (397). In patients, gastrointestinal side effects (diarrhea, flatulence, and abdominal pain) have been noted with NBuDNJ (SC-48334) given orally (1,000 mg every 8 h) (161). These problems would be caused by the inhibitory effect of NBuDNJ on the intestinal  $\alpha$ -glucosidases (such as maltase and sucrase) and might be overcome by prodrugs (i.e., NBuDNJ 6-phosphate

[SC-49955]), which do not inhibit gut  $\alpha$ -glucosidases (226). Admittedly, these prodrugs must as such be able to cross the intestinal barrier before they are hydrolyzed so as to release the active compound (NBuDNJ).

#### Budding (Assembly/Release) Inhibitors

IFN- $\alpha$  has been shown to directly prevent the release of HIV virions from chronically infected cells (376); this is in accord with earlier studies on IFN in murine retroviral systems. IFN may affect the budding of new HIV particles through an alteration of the fluidity of the plasma membrane or it may render the viral proteins unable to interact, assemble, and bud from the cell (428). In addition to its action targeted at the viral budding process, IFN has been found to interfere with various other stages of the HIV-1 replication cycle: (i) at an early step preceding or coinciding with the integration of proviral DNA (320, 422); (ii) at the transcriptional level, an effect that is overruled by the Tat protein (377); and (iii) at the posttranscriptional level, through induction of a cellular factor that antagonizes Rev function (101). All of these effects enable IFN to restrict HIV replication in both acutely and chronically infected cells and this suggests that IFN should be effective *in vivo* in AIDS patients, if the outcome would depend solely on the antiviral effects of IFN.

Although originally hailed as "therapeutic agents with dramatic antiretroviral activity" (319), the aromatic polycyclic diones (naphthodianthrones) hypericin (Fig. 19) and pseudohypericin have so far not fulfilled their promise. These natural products from *St. Johnswort* (*Hypericum*) would have the capacity to block viral assembly (or release) as well as directly inactivate properly assembled (released) virions (276). Hypericin is a photodynamic agent (149), causing hypericemia in cattle ingesting large amounts of *Hypericum* sp. on pastures. It inhibits PKC activity (438) and epidermal growth factor receptor tyrosine kinase activity (137). Its antiviral activity is not restricted to retroviruses but extends to various other viruses (7, 219, 445). Light is essential for all of the antiviral effects of hypericin (278). Upon illumination by visible light, it inacti-

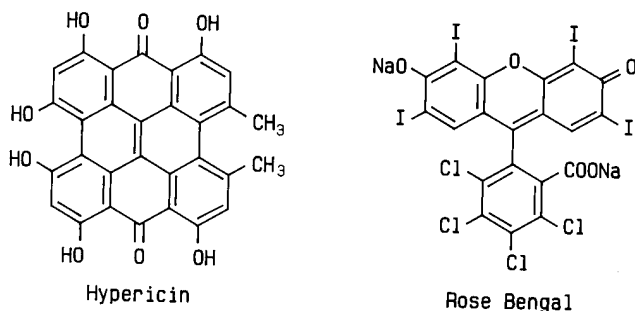


FIG. 19. Hypericin and rose bengal: virucidal agents that, upon illumination by visible light, are able to inactivate HIV and other enveloped viruses.

vates enveloped (but not unenveloped) viruses (278, 435) and thus acts as a virucidal agent. Rose bengal (Fig. 19) acts similarly to hypericin (278): both compounds are known to generate singlet oxygen (upon illumination) that may be responsible for their virus-inactivating effect. While hypericin and rose bengal might prove to be suitable agents for photodynamic inactivation of enveloped viruses in blood or blood products, it is hard to conceive how these compounds could be useful in the systemic treatment of HIV-infected patients.

Other agents that have been found to directly interact with the viral envelope, and thus block HIV-1 infectivity, are the aurothiolates, aurothioglucose and aurothiomalate (357). These compounds interact directly with the cysteine residue at position 532 of the envelope glycoprotein gp120, which is then no longer capable of interacting with the viral glycoprotein 41 and is thus released from the budding virus particles (357).

Recently, a nonimmunosuppressive cyclosporine analog (SDZ NIM 811) was shown to inhibit HIV-1 replication (393a). It was postulated that the compound may interfere with both the assembly process and an early step of viral replication, e.g., transport of the viral DNA into the nucleus. Cyclophilins would be involved in both processes through their capacity to bind to the HIV-1 *gag* protein and SDZ NIM 811 would interfere with the cyclophilin-*gag* protein interaction. This working hypothesis remains to be proven, however (393a).

### COMBINATION THERAPY

It has become increasingly clear that as for the chemotherapy of a variety of bacterial and malignant diseases, the ultimate strategy for the treatment of AIDS will be based on the combination of two, three, or even more, anti-HIV drugs. Different anti-HIV drugs, whether targeted at different viral proteins (enzymes) or at different molecular sites within the same enzyme, may thus be combined. Combination therapy is often understood in the sense of simultaneous use of different drugs. Although alternating the use of two, three, or more drugs may be an equally, if not more, valuable approach than simultaneous use of the drugs for the treatment of HIV infections, alternating drug regimens have proved less effective in inhibiting HIV-1 infection *in vitro* than giving all drugs of an alternating regimen simultaneously (306). Three "virtues" are generally expected from the (simultaneous or alternating) combination of different anti-HIV drugs: (i) diminished toxicity, because of a reduction in the dosage of the individual compounds; (ii) reduced risk of virus-drug resistance development, if resulting from different mutations in the viral genome; and (iii) synergistic antiviral activity, if anti-HIV action is targeted at different viral proteins or different sites within the same protein.

These premises have been borne out, at least under some conditions. Thus, when the individual compounds do not have overlapping toxicity profiles, as with AZT and ddC, combination therapy may be well tolerated and not result in toxicity (315), and in addition, it may show increased efficacy (430). Also, treatment can be readily switched from one drug (i.e., AZT) to another (i.e., ddI or ddC); patients with HIV infection who no longer respond to AZT treatment may still respond, by a delayed progression of the disease, to ddI (240) or ddC (1). Different anti-HIV drugs, such as AZT, ddI, and nevirapine (or pyridinone), that lead to virus-drug resistance resulting from different point mutations if used individually may prevent virus breakthrough when combined (94a). Synergistic anti-HIV activity has been demonstrated with a large number of combinations, including phosphonoformate (foscarnet [PFA]) with IFN- $\alpha$  (202), AZT with IFN- $\alpha$  (203), IFN- $\alpha$  with ddC (468), AZT with rsCD4 (232), AZT with castanospermine (235), AZT with PMEAs (425), PFA with AZT (155, 261), AZT with quartromicins (443), IFN- $\alpha$  with coumermycin (437), PFA with FddThd (FLT) (260), AZT with FLT (195), ddI with FLT (103), AZT with nevirapine (387), AZT with TIBO R82913 or TIBO R86183 (77), AZT with BHAP U-90152 (92), IFN- $\alpha$  with HEPT (224), AZT with HEPT derivatives (i.e., E-EPU) (22), AZT (ddC or nevirapine) with the Tat antagonist Ro 24-7429 (100), and the HIV protease inhibitor Ro 31-8959 with either AZT (104) or the Tat antagonist Ro 24-7429 (100). Also, AZT acts synergistically with ddI (12, 143), and AZT combined with ddI or IFN- $\alpha$  has been found to act synergistically against AZT-resistant HIV-1 mutants (234); likewise, AZT combined with BHAP (U-87201E) acts synergistically against AZT-resistant clinical isolates of HIV-1 (84). In addition to two-drug combinations, a number of three-drug combinations (AZT, rsCD4, and IFN- $\alpha$  [233] and AZT, PFA, and ddThd [257]) and even four-drug combinations (306) have proved to confer increased anti-HIV activity. As a rule, it can be stated that multidrug regimens are more effective in inhibiting HIV-1 replication than single-agent regimens and that the effectiveness increases with the number of drugs used (306).

An interesting combination is that of hydroxyurea or other hydroxamates (i.e., D-aspartic acid  $\beta$ -hydroxamate) and ddI: this combination leads to a synergistic inhibitory effect on HIV-1 replication without increasing toxicity (288a, 296a). This synergistic action would result from the inhibitory effect of the hydroxamates on ribonucleotide reductase and, consequently, the decrease in the intracellular pool of deoxynucleoside triphosphates, including dATP, with which ddATP, the active metabolite of ddI, has to compete at the HIV RT level.

However, not all combinations lead to synergistic anti-HIV activity. For example, acyclovir and AZT show only additive to antagonistic effects against HIV *in vitro* (427) (although *in vivo*, in patients with AIDS, cotherapy of AZT with acyclovir results in a significant improvement in survival [102] [possibly due to the suppressive effect of acyclovir on herpesviruses that may act as cofactors, stimulating HIV replication]). Antagonism was observed if rsCD4 was combined with dextran sulfate (205). Also, ganciclovir antagonizes the anti-HIV activity of AZT and ddI, while increasing their toxicity (312). Conversely, AZT antagonizes the inhibitory activity of ganciclovir against CMV infection (157). Ribavirin shows an ambivalent behavior: it antagonizes the anti-HIV activity of AZT (469) but enhances the anti-HIV activity of the purine 2',3'-dideoxyribosides ddAdo, ddGuo (25), and ddIno (ddI) (46). This potentiating effect of ribavirin on the antiretroviral activity of purine 2',3'-dideoxyribosides (i.e., ddI) was not accompanied by an increase in toxicity, as has also been confirmed *in vivo* (37, 50). Thus, the combination of ddI with ribavirin seems to be an



attractive strategy that should be further pursued in the treatment of AIDS patients.

In fact, the biochemical basis for the potentiating effect of ribavirin on the anti-HIV activity of ddI has been well established (45, 199). Being an inhibitor of IMP dehydrogenase (leading to the conversion of IMP to XMP, which is then further converted to GMP, GDP, and GTP), ribavirin causes an increase in the intracellular IMP pool levels. IMP is then used as a phosphate donor by 5'-nucleotidase to convert ddI to ddIMP, which will then finally be converted to its antivirally active metabolite ddATP. On the other hand, ribavirin causes a depletion of the GTP pools; GTP serves as an obligatory cofactor in the conversion of IMP to succinyl AMP, which is then further converted to AMP, ADP, ATP, and, via ADP → dADP, to dATP. Hence, ribavirin may enhance the anti-HIV activity of ddI by facilitating its conversion to ddATP and, at the same time, suppressing the formation of dATP, the direct competitor of ddATP at the HIV RT level. A similar mechanism may be invoked to explain the potentiating effect of ribavirin on the anti-HIV activity of the 2'-fluoro "up"-analogs of ddAdo, ddIno, and ddGuo (231).

### VIRUS-DRUG RESISTANCE

The potential of HIV to become resistant to anti-HIV drugs has become an increasing concern since it was first reported that HIV variants isolated from patients following prolonged AZT therapy show reduced susceptibility to AZT (272). The following mutations in the HIV-1 RT were found to confer high-level resistance to AZT: 67 Asp → Asn, 70 Lys → Arg, 215 Thr → Phe/Tyr, and 219 Lys → Gln (274). Later, a fifth mutation, 41 Met → Leu, was found to contribute to the high level of HIV AZT resistance (247). The 215 Thr → Tyr mutation has been most frequently detected among AZT-resistant HIV-1 isolates from patients under prolonged AZT therapy (305). In AZT-resistant HIV strains selected by passage in cell culture, an additional novel mutation (219 Lys → Glu) was observed (271). Resistance to ddI is induced by the 74 Leu → Val mutation in HIV-1 RT (433). The mutation 69 Thr → Asp decreases the susceptibility to ddC (162, 163) and the mutation 184 Met → Val reduces the susceptibility to both ddC and ddI, but not AZT (187). The 184 Met → Val mutation is also responsible for resistance to the (-) enantiomeric 2',3'-dideoxy-3'-thiacytidine 3TC (172, 452) and its 5-fluoro-substituted counterpart FTC (407). The 65 Lys → Arg mutation confers resistance to ddC, 3TC, and ddI, but not AZT (186, 498), and the 75 Val → Thr mutation imparts resistance to D4T (263a).

In HIV-1-infected patients treated with AZT, mutations conferring resistance to AZT seem to occur in an ordered fashion (i.e., 41 → 41/215 → 41/67/215 → 41/67/70/215 → 41/67/70/215/219), each step leading to accruing resistance (248). The combination of ddC with AZT does not appreciably delay the emergence of AZT resistance (389); in fact, alternating therapy of AZT with ddC leads to the selection of virus resistant to both drugs (161). Although AZT-resistant HIV strains should, in principle, not exhibit cross-resistance to ddI or ddC, some reduction in susceptibility to ddI and ddC was noted with AZT-resistant HIV-1 isolates from five cohorts (about a twofold decrease in ddI or ddC susceptibility for each and a 10-fold decrease in AZT susceptibility [304]).

HIV-1 resistance to the HIV-1-specific RT inhibitors (NNRTIs) rapidly arises following passage of the virus in cell culture in the presence of these compounds. The mutation 181 Tyr → Cys is associated with resistance, or reduced susceptibility, to most of the NNRTIs (i.e., TIBO, HEPT, nevirapine, pyridi-

none, BHAP, TSAO, and α-APA), as already mentioned above. The mutation 188 Tyr → His is associated with resistance to TIBO and other compounds (43) but not nevirapine (400). The mutation 100 Leu → Ile is associated mainly with resistance to TIBO (43, 80, 314); the mutation 103 Lys → Asn is associated with resistance to TIBO, nevirapine, pyridinone, and BHAP (42, 43, 80, 355); the mutation 106 Val → Ala mainly leads to resistance to nevirapine and HEPT (40, 42, 80); the mutation 138 Glu → Lys is responsible for resistance to TSAO (44, 61); the mutation 190 Gly → Glu accounts for resistance to quinoxaline S-2720 (251, 252); and the mutation 236 Pro → Leu is associated with resistance to BHAP (148). Notably, the 190 Gly → Glu mutation leads to a dramatic reduction in RT activity (252). Although the different locations of the mutations conferring resistance to the different RT inhibitors should in the first place be interpreted to mean that these different RT inhibitors bind to different sites of the enzyme, it is likely that the secondary structure of the RNA coding for HIV-1 RT also contributes to the location of these mutations (408). Indeed, mutations may occur more readily at "unstable" nonhelical regions (i.e., loops, bulges, and bends) which could therefore be regarded as mutation prone (408).

Resistance development is not an exclusive property of the HIV-1 RT and HIV-1 RT inhibitors, whether nucleosides or non-nucleosides. HIV-1 resistance to the protease inhibitor Ro 31-8959 has been obtained after five passages of HIV-1 in cell culture in the presence of the compound (138). Resistance to C<sub>2</sub> symmetric inhibitors of HIV-1 protease has also been described (360), and in this case, resistance was due to Val → Ala mutation at position 82 of the protease.

Although the clinical significance of AZT resistance development, or for that matter resistance to any other anti-HIV drug, has not yet been settled (458), the rapid emergence of drug-resistant HIV-1 mutants under selective pressure of the HIV-1-specific RT inhibitors has been generally viewed as a limitation for, if not an argument against, the clinical usefulness of these compounds. Yet, a number of points should be kept in mind when assessing the relevance of HIV-drug resistance (126).

First, resistance should be considered a parameter of specificity, which means that the more specific the compound in its antiviral action, the greater the likelihood that it leads to resistance in the shortest possible time. This also means that, vice versa, compounds that do not lead to resistance may fail to do so because they are targeted at cellular (rather than viral) proteins and thus bound to be cytotoxic.

Second, drug-resistant virus variants might be less pathogenic than the wild-type variants. Otherwise, they would not be overgrown by the wild type in the absence of the drugs and only show up under continuous pressure of the drug. In fact, drug-resistant virus variants may be present in the virus pool of patients who never received the drug (336). Future clinical studies should address the role of these drug-resistant variants in disease progression.

Third, although AZT-resistant HIV-1 mutants may persist for a long time (i.e., 1 year) after withdrawal of the drug before reverting to the wild type (5, 71, 72, 267), it has not been determined how long it takes for NNRTI-resistant HIV-1 mutants to revert to the wild type: e.g., for pyridinone, L-697,661 resistance in the patient develops within 12 weeks of treatment (110), but as HIV-1 resistance to NNRTIs generally depends on one mutation, the time it needs to revert to the wild phenotype following withdrawal of the drug may not be as long as for the AZT resistance phenotype.

Fourth, because of their handicap relative to the wild type, drug-resistant virus strains may be less readily transmitted

from one person to another. In fact, there are few documented cases of transmission of drug-resistant virus (i.e., AZT-resistant HIV-1 [11, 153]), although this issue remains to be followed up by further epidemiological studies.

Fifth, if resistance to one of the NNRTIs develops, treatment could be switched to any of the other NNRTIs to which the virus has retained susceptibility. For example, 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide (480) is still active against those HIV-1 strains that, because of the 103 Lys → Asn or 181 Tyr → Cys mutation, have acquired resistance to other NNRTIs (i.e., TIBO, nevirapine, pyridinone, and BHAP). The  $\alpha$ -APA derivative R89439 is very active against the 100 Leu → Ile mutant, which is highly resistant to TIBO R82913 and R86183 (371). Within the TIBO class, a minor chemical modification, i.e., shifting the chlorine from the 9-position (R82913) to the 8-position (R86183), suffices to restore activity against the 181 Tyr → Cys mutant (367). Similarly, pyridinone L-702,019, which differs from its predecessor L-696,229 only by the addition of two chlorine atoms (in the benzene ring) and substitution of sulfur for oxygen (in the pyridine ring), is markedly inhibitory to HIV-1 mutants containing the 103 Lys → Asn or 181 Tyr → Cys mutation (181).

Sixth, in some instances, resistance to one of the NNRTIs may even be accompanied by hypersensitivity to others. For example, the 236 Pro → Leu mutation causing resistance to BHAP confers a 10-fold increased susceptibility to TIBO, nevirapine, and pyridinone (148). Also, the 236 Pro → Leu mutation, in combination with the 181 Tyr → Cys mutation, partially restores the susceptibility of the HIV-1 RT toward TIBO, nevirapine, and pyridinone.

Seventh, the 181 Tyr → Cys mutation, causing resistance to most NNRTIs, has been found to suppress the 215 mutation (Thr → Phe/Tyr) conferring resistance to AZT (270), and vice versa, the 181 Tyr → Cys mutation can be suppressed by AZT, which thus means that the NNRTI mutation at position 181 and the AZT mutation at position 215 seem to be mutually exclusive. Still other mutations have proved to counteract each other: 236 Pro → Leu versus 138 Glu → Lys; 215 Thr → Phe/Tyr versus 184 Met → Val; and 215 Thr → Phe/Tyr versus 74 Leu → Val (126). In addition to the 181 Tyr → Cys mutation, the 100 Leu → Ile mutation was also found to suppress resistance to AZT when coexpressed with AZT-specific mutations (79a). On the basis of mutations that seem to counteract each other (126), combinations of different drugs could be envisaged that, if combined, may suppress emergence of resistance to one another: e.g., combinations of AZT with either TIBO,  $\alpha$ -APA, HEPT, nevirapine, or pyridinone (to which BHAP and/or TSAO may be added).

Eighth, the triple combination of AZT, ddI, and pyridinone (or nevirapine) has been proposed as an example of "convergent combination therapy," which would restrict "multidrug resistance development because of evolutionary limitations" (94a). At the drug concentrations used (0.3  $\mu$ M AZT, 10  $\mu$ M ddI, and 0.09  $\mu$ M pyridinone), the combination was indeed found to prevent HIV-1 breakthrough. The authors (94a) surmised that this happened because a triple drug-resistant virus would be unable to replicate *per se*. This assumption has proved to be faulty, as has also been recognized by the authors (94), since HIV-1 coresistant to AZT, ddI, and an NNRTI (such as nevirapine) can be readily selected in cell culture (273). In fact, an HIV-1 variant with the RT mutations 74 (Leu → Val), 103 (Lys → Asn), 215 (Thr → Tyr), and 219 (Lys → Gln) is still viable (151) and retains susceptibility to AZT and pyridinone L-697,661 at concentrations (<1  $\mu$ M) that are therapeutically attainable in human plasma.

Ninth, what would seem a straightforward approach to pre-

vent drug-resistant HIV strains from arising is using "knocking out" concentrations of the NNRTIs (41). If NNRTIs, such as BHAP U-88204 or BHAP U-90152, are used from the start at a sufficiently high concentration (1 or 3  $\mu$ M, respectively), they completely suppress virus replication (147, 466), so that the virus is "knocked out" and does not have the opportunity to become resistant. If U-90152 is combined with AZT, the concentrations of the individual drugs can be lowered to achieve total virus clearance (147). Various NNRTIs, i.e., TIBO, HEPT, nevirapine, pyridinone, and BHAP, have been shown to knock out HIV-1 in cell culture when used at concentrations (1 to 10  $\mu$ g/ml) that are nontoxic to the cells (41). That the virus was really knocked out, and the cell culture cleared ("sterilized") from the virus infection, was ascertained by PCR analysis of the infected cell cultures: even with two successive 35-cycle PCR rounds, no proviral DNA could be detected in the HIV-1-infected cell cultures that had been treated from the start with the knocking out concentrations. In contrast with the NNRTIs, AZT proved unable to clear (or sterilize) the cell cultures from HIV infection at a concentration of 3  $\mu$ M (41, 147), and even at a concentration of 25  $\mu$ M, AZT did not prevent resumption of virus production, so that even in the continued presence of the drug, the HIV-1-infected cell cultures eventually produced as much virus as did untreated infected cells (426).

Tenth, when used at knocking out concentrations, the NNRTIs may be expected to lead to a long-lasting suppression of HIV-1 replication. This knocking out phenomenon could be achieved at lower concentrations if the NNRTIs are combined with each other or with any of the dideoxynucleoside analogs (i.e., AZT, ddI, or ddC), and such drug combinations could be particularly advantageous if based on the premise of mutually suppressive resistance (126). Also, with a four-drug combination consisting of AZT, ddI, ddC, and IFN- $\alpha$ , virus breakthrough could be delayed for a much longer time than with the one-, two-, or three-drug treatment regimens (306).

All of these considerations should somehow help to alleviate the concerns that have been raised with regard to the development of HIV resistance to the various RT inhibitors, whether nucleosides or non-nucleosides. Of course, the problem of virus-drug resistance would not have to be raised if the compounds were to be used only prophylactically, that is, to prevent HIV infection following occasional exposure to the virus, e.g., through sexual contact or needle stick or other injury, or to prevent perinatal HIV transmission at the time of delivery.

## CONCLUSION

Despite the enormous progress that has been made, and the wealth of selective anti-HIV agents that are now available, outsiders will keep insisting that there is still "no cure for AIDS." Yet, as discussed above, there are plenty of compounds that have proved to specifically interact with one or another target of the HIV replication cycle. There are also targets for which specific inhibitors still need to be found, as there are compounds for which the target(s) has not yet been found. Not for all compounds have the target proteins or target site been identified with as much unambiguity as for the polyanionic substances (virus adsorption), dideoxynucleoside analogs (substrate binding site of the viral RT), TIBO-like compounds (nonsubstrate binding site of the viral RT), or protease inhibitors (specific cleavage sites of the viral precursor proteins).

Antiviral agents in general, and antiretroviral agents in particular, could be seen as the following: an arc-shaped distribu-

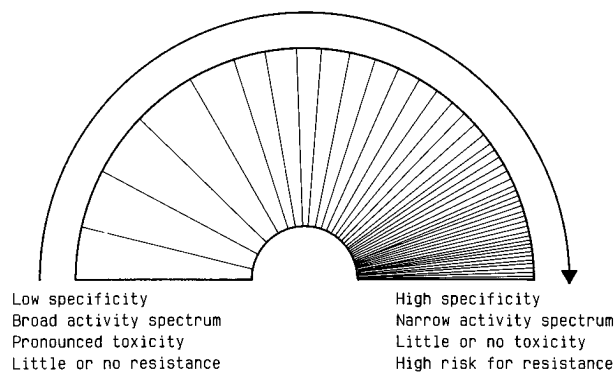


FIG. 20. Characteristics of antiviral agents, also extending to the antiretroviral agents.

tion with, at the extremes, at  $0^\circ$ , the compounds that are non-specific showing a broad spectrum of antiviral activity and not leading to resistance but proving toxic to the host, and at  $180^\circ$ , the compounds that are highly specific showing a very narrow spectrum of antiviral activity and not exhibiting toxicity but promptly leading to resistance. Depending on the target with which they interact, all anti-HIV agents can be positioned on such a graduated arc (Fig. 20). Those compounds that interact with common cellular or viral proteins will be close to  $0^\circ$ , whereas the compounds interacting with specific viral targets will be close to  $180^\circ$ . It also follows that the high specificity of the latter compounds cannot be acquired without the risk of resistance development.

In addition to the different stages of the HIV replicative cycle, other events outside this replicative cycle may be considered possible targets for anti-HIV chemotherapy. For example, cyclosporin A and FK506 have been reported to inhibit HIV replication, and this inhibition has been ascribed to an inhibitory effect of the compounds on the expression of tumor necrosis factor alpha, a known activator of HIV replication (178). In another study, cyclosporin A and FK506 were found to prevent the formation of the T-cell transcription factor NF-AT (nuclear factor of activated T cells), and on the basis of this coincidence, NF-AT has now also been regarded as a target for anti-HIV therapy (244).

When discovering new compounds that are active against HIV, or uncovering new targets that are amenable to anti-HIV therapy, quite often a syllogistic reasoning is followed. If (a), a compound is inhibitory to HIV replication, and if (b), the compound is found to interact with a specific viral target, then (c), the compound must inhibit HIV replication by acting at that particular target. A case in point is the aromatic C-nitroso compounds, which, on the one hand, inhibit HIV-1 infectivity and, on the other hand, eject zinc from the HIV-1 capsid zinc fingers and, therefore, are postulated to achieve their anti-HIV activity through zinc ejection (385). This relationship may be causal, indeed, but it may also be coincidental. In general, (a) plus (b) does not necessarily yield (c) and, thus, caution should be exercised when proposing new targets for anti-HIV chemotherapy and, even more so, when speculating on new therapeutic approaches that achieve their anti-HIV activity through interaction with such targets.

The chemotherapy of HIV infections, as for other chronic infections and malignant diseases, is moving into the direction of multiple drug combinations. The rationale for such drug combinations is threefold: to get synergistic (or at least, additive) activity, to lower the doses (and thus toxicity) of the

individual compounds, and to reduce the risk of drug resistance development. Given the wealth of promising anti-HIV agents that are now available, the number of two-, three-, or four-drug combinations that could be envisaged should be almost astronomical. As a guideline to selecting the appropriate drug combination before administering it to the patient, it may be useful to first evaluate, in experimental cell systems, whether the drugs, at therapeutically attainable concentrations, are able to completely suppress virus replication, knock out the virus, and thus prevent resistance from emerging. This strategy should provide the rationale for judiciously choosing the right compounds, at the right doses, to give the right answers in the clinical setting.

#### ACKNOWLEDGMENTS

My original investigations were supported in part by the Biomedical Research Programme of the European Community, the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek, the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek, the Belgian Geconcentreerde Onderzoeksacties, and the Janssen Research Foundation.

I thank C. Callebaut for excellent editorial assistance.

#### REFERENCES

- Abrams, D. I., A. I. Goldman, C. Launer, J. A. Korvick, J. D. Neaton, L. R. Crane, M. Grodesky, S. Wakefield, K. Muth, S. Kornegay, D. L. Cohn, A. Harris, R. Luskin-Hawk, N. Markowitz, J. H. Sampson, M. Thompson, L. Dayton, and the Terry Bein Community Programs for Clinical Research on AIDS. 1994. A comparative trial of didanosine or zalcitabine after treatment with zidovudine in patients with human immunodeficiency virus infection. *N. Engl. J. Med.* **330**:657-662.
- Abrams, D. I., S. Kuno, R. Wong, K. Jeffords, M. Nash, J. B. Molaghan, R. Gorter, and R. Ueno. 1989. Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Ann. Intern. Med.* **110**:183-188.
- Agrawal, S., and J. Y. Tang. 1992. GEM 91—an antisense oligonucleotide phosphorothioate as a therapeutic agent for AIDS. *Antisense Res. Dev.* **2**:261-266.
- Alam, M., C. M. Bechtold, A. K. Patick, M. T. Skoog, T. G. Gant, R. J. Colonna, A. I. Meyers, H. Li, J. Trimble, and P.-F. Lin. 1993. Substituted naphthalenones as a new structural class of HIV-1 reverse transcriptase inhibitors. *Antiviral Res.* **22**:131-141.
- Albert, J., J. Wahlberg, J. Lundeberg, S. Cox, E. Sandström, B. Wahren, and M. Uhlen. 1992. Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. *J. Virol.* **66**:5627-5630.
- Althaus, I. W., J. J. Chou, A. J. Gonzales, M. R. Deibel, K.-C. Chou, F. J. Kezdy, D. L. Romero, P. A. Aristoff, W. G. Tarpley, and F. Reusser. 1993. Steady-state kinetic studies with the non-nucleoside HIV-1 reverse transcriptase inhibitor U-87201E. *J. Biol. Chem.* **268**:6119-6124.
- Andersen, D. O., N. D. Weber, S. G. Wood, B. G. Hughes, B. K. Murray, and J. A. North. 1991. In vitro virucidal activity of selected anthraquinones and anthraquinone derivatives. *Antiviral Res.* **16**:185-196.
- Andrei, G., and E. De Clercq. 1993. Molecular approaches for the treatment of hemorrhagic fever virus infections. *Antiviral Res.* **22**:45-75.
- Animashaun, T., N. Mahmood, A. J. Hay, and R. C. Hughes. 1993. Inhibitory effects of novel mannose-binding lectins on HIV-infectivity and syncytium formation. *Antiviral Chem. Chemother.* **4**:145-153.
- Ankel, H., O. Turriziani, and G. Antonelli. 1991. Prostaglandin A inhibits replication of human immunodeficiency virus during acute infection. *J. Gen. Virol.* **72**:2797-2800.
- Anonymous. 1993. HIV seroconversion after occupational exposure despite early prophylactic zidovudine therapy. *Lancet* **341**:1077-1078.
- Antonelli, G., F. Dianzani, D. Bellarosa, O. Turriziani, E. Riva, and A. Gentile. 1994. Drug combination of AZT and ddI: synergism of action and prevention of appearance of AZT-resistance. *Antiviral Chem. Chemother.* **5**:51-55.
- Arnold, E., A. Jacobo-Molina, R. G. Nanni, R. L. Williams, X. Lu, J. Ding, A. D. Clark, Jr., A. Zhang, A. L. Ferris, P. Clark, A. Hizi, and S. H. Hughes. 1992. Structure of HIV-1 reverse transcriptase/DNA complex at 7 Å resolution showing active site locations. *Nature (London)* **357**:85-89.
- Artico, M., S. Massa, A. Mai, M. E. Marongiu, G. Piras, E. Tramontano, and P. La Colla. 1993. 3,4-Dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs): a new class of specific inhibitors of human immunodeficiency virus type 1. *Antiviral Chem. Chemother.* **4**:361-368.
- Ashkenazi, A., D. H. Smith, S. A. Marsters, L. Riddle, T. J. Gregory, D. D. Ho, and D. J. Capon. 1991. Resistance of primary isolates of human immunodeficiency virus type 1 to soluble CD4 is independent of CD4-rgp120

- binding affinity. Proc. Natl. Acad. Sci. USA **88**:7056-7060.
16. Ashorn, P., G. Englund, M. A. Martin, B. Moss, and E. A. Berger. 1991. Anti-HIV activity of CD4-*Pseudomonas* exotoxin on infected primary human lymphocytes and monocyte/macrophages. J. Infect. Dis. **163**:703-709.
  17. Ashorn, P., B. Moss, J. N. Weinstein, V. K. Chaudhary, D. J. Fitzgerald, I. Pastan, and E. A. Berger. 1990. Elimination of infectious human immunodeficiency virus from human T-cell cultures by synergistic action of CD4-*Pseudomonas* exotoxin and reverse transcriptase inhibitors. Proc. Natl. Acad. Sci. USA **87**:8889-8893.
  18. Aullo, P., J. Alcamí, M. R. Popoff, D. R. Klatzmann, J. R. Murphy, and P. Boquet. 1992. A recombinant diphtheria toxin related human CD4 fusion protein specifically kills HIV infected cells which express gp120 but selects fusion toxin resistant cells which carry HIV. EMBO J. **11**:575-583.
  19. Baba, M., E. De Clercq, D. Schols, R. Pauwels, R. Snoeck, C. Van Boeckel, G. Van Dedem, N. Kraaijeveld, P. Hobbelen, H. Ottenheijm, and F. Den Hollander. 1990. Novel sulfated polysaccharides: dissociation of anti-human immunodeficiency virus activity from antithrombin activity. J. Infect. Dis. **161**:208-213.
  20. Baba, M., E. De Clercq, H. Tanaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, K. Umezū, H. Nakashima, S. Mori, S. Shigeta, R. T. Walker, and T. Miyasaka. 1991. Potent and selective inhibition of human immunodeficiency virus type 1 (HIV-1) by 5-ethyl-6-phenylthiouracil derivatives through their interaction with the HIV-1 reverse transcriptase. Proc. Natl. Acad. Sci. USA **88**:2356-2360.
  21. Baba, M., E. De Clercq, H. Tanaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, K. Umezū, R. T. Walker, S. Mori, S. Shigeta, and T. Miyasaka. 1991. Highly potent and selective inhibition of human immunodeficiency virus type 1 by a novel series of 6-substituted acyclouridine derivatives. Mol. Pharmacol. **39**:805-810.
  22. Baba, M., M. Ito, S. Shigeta, H. Tanaka, T. Miyasaka, M. Ubasawa, K. Umezū, R. T. Walker, and E. De Clercq. 1991. Synergistic inhibition of human immunodeficiency virus type 1 replication by 5-ethyl-1-ethoxymethyl-6-(phenylthio)uracil (E-EPU) and azidothymidine *in vitro*. Antimicrob. Agents Chemother. **35**:1430-1433.
  23. Baba, M., T. Kira, S. Shigeta, T. Matsumoto, and H. Sawada. 1994. Selective inhibition of human immunodeficiency virus type 1 replication by novel fluoroalkylated oligomers *in vitro*. J. Acquired Immune Defic. Syndr. **7**:24-30.
  24. Baba, M., K. Konno, S. Shigeta, A. Wickramasinghe, and P. Mohan. 1993. Selective inhibition of human cytomegalovirus replication by naphthalenedisulfonic acid derivatives. Antiviral Res. **20**:223-233.
  25. Baba, M., R. Pauwels, J. Balzarini, P. Herdewijn, E. De Clercq, and J. Desmyter. 1987. Ribavirin antagonizes inhibitory effects of pyrimidine 2',3'-dideoxynucleosides but enhances inhibitory effects of purine 2',3'-dideoxynucleosides on replication of human immunodeficiency virus *in vitro*. Antimicrob. Agents Chemother. **31**:1613-1617.
  26. Baba, M., R. Pauwels, P. Herdewijn, E. De Clercq, J. Desmyter, and M. Vandeputte. 1987. Both 2',3'-dideoxythymidine and its 2',3'-unsaturated derivative (2',3'-dideoxythymidine) are potent and selective inhibitors of human immunodeficiency virus replication *in vitro*. Biochem. Biophys. Res. Commun. **142**:128-134.
  27. Baba, M., D. Schols, E. De Clercq, R. Pauwels, M. Nagy, J. Györgyi-Edelényi, M. Löw, and S. Görög. 1990. Novel sulfated polymers as highly potent and selective inhibitors of human immunodeficiency virus replication and giant cell formation. Antimicrob. Agents Chemother. **34**:134-138.
  28. Baba, M., D. Schols, P. Mohan, E. De Clercq, and S. Shigeta. 1993. Inhibition of HIV-1-induced cytopathogenicity, syncytium formation, and virus-cell binding by naphthalene-disulphonic acids through interaction with the viral envelope gp120 glycoprotein. Antiviral Chem. Chemother. **4**:229-234.
  29. Baba, M., D. Schols, R. Pauwels, H. Nakashima, and E. De Clercq. 1990. Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: a new strategy towards AIDS chemotherapy. J. Acquired Immune Defic. Syndr. **3**:493-499.
  30. Baba, M., S. Shigeta, S. Yuasa, H. Takashima, K. Sekiya, M. Ubasawa, H. Tanaka, T. Miyasaka, R. T. Walker, and E. De Clercq. 1994. Preclinical evaluation of MKC-442, a highly potent and specific inhibitor of human immunodeficiency virus type 1 *in vitro*. Antimicrob. Agents Chemother. **38**:688-692.
  31. Baba, M., H. Tanaka, E. De Clercq, R. Pauwels, J. Balzarini, D. Schols, H. Nakashima, C.-F. Perno, R. T. Walker, and T. Miyasaka. 1989. Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. Biochem. Biophys. Res. Commun. **165**:1375-1381.
  32. Baba, M., S. Yuasa, T. Niwa, M. Yamamoto, S. Yabuuchi, H. Takashima, M. Ubasawa, H. Tanaka, T. Miyasaka, R. T. Walker, J. Balzarini, E. De Clercq, and S. Shigeta. 1993. Effect of human serum on the *in vitro* anti-HIV-1 activity of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) derivatives as related to their lipophilicity and serum protein binding. Biochem. Pharmacol. **45**:2507-2512.
  33. Baboonian, C., A. Dagleish, L. Bountiff, J. Gross, S. Oroszlan, G. Rickett, C. Smith-Burchnell, P. Troke, and J. Merson. 1991. HIV-1 proteinase is required for synthesis of pro-viral DNA. Biochem. Biophys. Res. Commun. **179**:17-24.
  34. Bader, J. P., J. B. McMahon, R. J. Schultz, V. L. Narayanan, J. B. Pierce, W. A. Harrison, O. S. Weislow, C. F. Midelfort, S. F. Stinson, and M. R. Boyd. 1991. Oxathiin carboxanilide, a potent inhibitor of human immunodeficiency virus reproduction. Proc. Natl. Acad. Sci. USA **88**:6740-6744.
  35. Balzarini, J., Z. Hao, P. Herdewijn, D. G. Johns, and E. De Clercq. 1991. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. Proc. Natl. Acad. Sci. USA **88**:1499-1503.
  36. Balzarini, J., P. Herdewijn, and E. De Clercq. 1989. Differential patterns of intracellular metabolism of 2',3'-dideoxy-2',3'-dideoxythymidine and 3'-azido-2',3'-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. J. Biol. Chem. **264**:6127-6133.
  37. Balzarini, J., P. Herdewijn, and E. De Clercq. 1989. Potentiating effect of ribavirin on the antiretrovirus activity of 3'-azido-2,6-diaminopurine-2',3'-dideoxyriboside *in vitro* and *in vivo*. Antiviral Res. **11**:161-172.
  38. Balzarini, J., A. Holy, J. Jindrich, H. Dvorakova, Z. Hao, R. Snoeck, P. Herdewijn, D. G. Johns, and E. De Clercq. 1991. 9-[(2RS)-3-fluoro-2-phosphonylmethoxypropyl] derivatives of purines: a class of highly selective antiretroviral agents *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. USA **88**:4961-4965.
  39. Balzarini, J., A. Holy, J. Jindrich, L. Naesens, R. Snoeck, D. Schols, and E. De Clercq. 1993. Differential antitherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective *in vitro* and *in vivo* antiretrovirus activity of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine. Antimicrob. Agents Chemother. **37**:332-338.
  40. Balzarini, J., A. Karlsson, and E. De Clercq. 1993. Human immunodeficiency virus type 1 drug-resistance patterns with different 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine derivatives. Mol. Pharmacol. **44**:694-701.
  41. Balzarini, J., A. Karlsson, M.-J. Pérez-Pérez, M.-J. Camarasa, and E. De Clercq. 1993. Knocking-out concentrations of HIV-1-specific inhibitors completely suppress HIV-1 infection and prevent the emergence of drug-resistant virus. Virology **196**:576-585.
  42. Balzarini, J., A. Karlsson, M.-J. Pérez-Pérez, M.-J. Camarasa, W. G. Tarpley, and E. De Clercq. 1993. Treatment of human immunodeficiency virus type 1 (HIV-1)-infected cells with combinations of HIV-1-specific inhibitors results in a different resistance pattern than does treatment with single-drug therapy. J. Virol. **67**:5353-5359.
  43. Balzarini, J., A. Karlsson, M.-J. Pérez-Pérez, L. Vrang, J. Walbers, H. Zhang, B. Öberg, A.-M. Vandamme, M.-J. Camarasa, and E. De Clercq. 1993. HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. Virology **192**:246-253.
  44. Balzarini, J., A. Karlsson, A.-M. Vandamme, M.-J. Pérez-Pérez, H. Zhang, L. Vrang, B. Öberg, K. Bäckbro, T. Uge, A. San-Félix, S. Velazquez, M.-J. Camarasa, and E. De Clercq. 1993. Human immunodeficiency virus type 1 (HIV-1) strains selected for resistance against the HIV-1-specific [2',5'-bis-O-(tert-butylidimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)]-β-D-pentofuranosyl (TSAO) nucleoside analogues retain sensitivity to HIV-1-specific nonnucleoside inhibitors. Proc. Natl. Acad. Sci. USA **90**:6952-6956.
  45. Balzarini, J., C.-K. Lee, P. Herdewijn, and E. De Clercq. 1991. Mechanism of the potentiating effect of ribavirin on the activity of 2',3'-dideoxyinosine against human immunodeficiency virus. J. Biol. Chem. **266**:21509-21514.
  46. Balzarini, J., C.-K. Lee, D. Schols, and E. De Clercq. 1991. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) and 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) markedly potentiate the inhibitory effect of 2',3'-dideoxyinosine on human immunodeficiency virus in peripheral blood lymphocytes. Biochem. Biophys. Res. Commun. **178**:563-569.
  47. Balzarini, J., H. Mitsuya, E. De Clercq, and S. Broder. 1986. Aurintricarboxylic acid and Evans blue represent two different classes of anionic compounds which selectively inhibit the cytopathogenicity of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. Biochem. Biophys. Res. Commun. **136**:64-71.
  48. Balzarini, J., L. Naesens, and E. De Clercq. 1990. Anti-retrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) *in vivo* increases when it is less frequently administered. Int. J. Cancer **46**:337-340.
  49. Balzarini, J., L. Naesens, P. Herdewijn, I. Rosenberg, A. Holy, R. Pauwels, M. Baba, D. G. Johns, and E. De Clercq. 1989. Marked *in vivo* antiretrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine, a selective anti-human immunodeficiency virus agent. Proc. Natl. Acad. Sci. USA **86**:332-336.
  50. Balzarini, J., L. Naesens, M. J. Robins, and E. De Clercq. 1990. Potentiating effect of ribavirin on the *in vitro* and *in vivo* antiretrovirus activities of 2',3'-dideoxyinosine and 2',3'-dideoxy-2,6-diaminopurine riboside. J. Acquired Immune Defic. Syndr. **3**:1140-1147.
  51. Balzarini, J., L. Naesens, J. Slachmuylders, H. Niphuis, I. Rosenberg, A. Holy, H. Schellekens, and E. De Clercq. 1991. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication *in vitro* and simian immunodeficiency virus infection in Rhesus monkeys. AIDS **5**:21-28.

52. Balzarini, J., J. Neyts, D. Schols, M. Hosoya, E. Van Damme, W. Peumans, and E. De Clercq. 1992. The mannose-specific plant lectins from *Cymbidium* hybrid and *Epipactis helleborine* and the (*N*-acetylglucosamine)<sub>n</sub>-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication in vitro. *Antiviral Res.* **18**:191–207.
53. Balzarini, J., R. Pauwels, P. Herdewijn, E. De Clercq, D. A. Cooney, G.-J. Kang, M. Dalal, D. G. Johns, and S. Broder. 1986. Potent and selective anti-HTLV-III/LAV activity of 2',3'-dideoxycytidine, the 2',3'-unsaturated derivative of 2',3'-dideoxycytidine. *Biochem. Biophys. Res. Commun.* **140**:735–742.
54. Balzarini, J., M.-J. Pérez-Pérez, A. San-Félix, M.-J. Camarasa, I. C. Bathurst, P. J. Barr, and E. De Clercq. 1992. Kinetics of inhibition of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase by the novel HIV-1-specific nucleoside analogue [2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)thymine (TSAO-T). *J. Biol. Chem.* **267**:11831–11838.
55. Balzarini, J., M.-J. Pérez-Pérez, A. San-Félix, D. Schols, C.-F. Perno, A.-M. Vandamme, M.-J. Camarasa, and E. De Clercq. 1992. 2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)pyrimidine (TSAO) nucleoside analogues: highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **89**:4392–4396.
56. Balzarini, J., M.-J. Pérez-Pérez, A. San-Félix, S. Velazquez, M.-J. Camarasa, and E. De Clercq. 1992. [2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide) (TSAO) derivatives of purine and pyrimidine nucleosides as potent and selective inhibitors of human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **36**:1073–1080.
57. Balzarini, J., C.-F. Perno, D. Schols, and E. De Clercq. 1991. Activity of acyclic nucleoside phosphonate analogues against human immunodeficiency virus in monocyte/macrophages and peripheral blood lymphocytes. *Biochem. Biophys. Res. Commun.* **178**:329–335.
58. Balzarini, J., D. Schols, J. Neyts, E. Van Damme, W. Peumans, and E. De Clercq. 1991. α-(1-3)- and α-(1-6)-D-Mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections in vitro. *Antimicrob. Agents Chemother.* **35**:410–416.
59. Balzarini, J., H. Sobis, L. Naesens, M. Vandeputte, and E. De Clercq. 1990. Inhibitory effects of 9-(2-phosphonylmethoxyethyl)adenine and 3'-azido-2',3'-dideoxythymidine on tumor development in mice inoculated intracerebrally with Moloney murine sarcoma virus. *Int. J. Cancer* **45**:486–489.
60. Balzarini, J., A. Van Aerschot, R. Pauwels, M. Baba, D. Schols, P. Herdewijn, and E. De Clercq. 1989. 5-Halogeno-3'-fluoro-2',3'-dideoxyuridines as inhibitors of human immunodeficiency virus (HIV): potent and selective anti-HIV activity of 3'-fluoro-2',3'-dideoxy-5-chlorouridine. *Mol. Pharmacol.* **35**:571–577.
61. Balzarini, J., S. Velazquez, A. San-Félix, A. Karlsson, M.-J. Pérez-Pérez, M.-J. Camarasa, and E. De Clercq. 1993. Human immunodeficiency virus type 1-specific [2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)-purine analogues show a resistance spectrum that is different from that of the human immunodeficiency virus type 1-specific non-nucleoside analogues. *Mol. Pharmacol.* **43**:109–114.
62. Bartolucci, C., L. Cellai, P. Di Filippo, A. Segre, M. Brufani, L. Filocamo, A. D. Bianco, M. Guiso, V. Brizzi, A. Benedetto, A. Di Caro, and G. Elia. 1992. Rifamycins as inhibitors of retroviral reverse transcriptase from M-MULV, RAV-2, and HIV-1. *Il Farmaco* **47**:1367–1383.
63. Bârzu, T., M. Level, M. Petitou, J.-C. Lormeau, J. Choay, D. Schols, M. Baba, R. Pauwels, M. Witvrouw, and E. De Clercq. 1993. Preparation and anti-HIV activity of *O*-acylated heparin and dermatan sulfate derivatives with low anticoagulant effect. *J. Med. Chem.* **36**:3546–3555.
64. Batinic, D., and F. A. Robey. 1992. The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides. *J. Biol. Chem.* **267**:6664–6671.
65. Berger, E. A., K. A. Clouse, V. K. Chaudhary, S. Chakrabarti, D. J. Fitzgerald, I. Pastan, and B. Moss. 1989. CD4-*Pseudomonas* exotoxin hybrid protein blocks the spread of human immunodeficiency virus infection *in vitro* and is active against cells expressing the envelope glycoproteins from diverse primate immunodeficiency retroviruses. *Proc. Natl. Acad. Sci. USA* **86**:9539–9543.
66. Beutler, J. A., T. C. McKee, R. W. Fuller, M. Tischler, J. H. Cardellina II, K. M. Snader, T. G. McCloud, and M. R. Boyd. 1993. Frequent occurrence of HIV-inhibitory sulphated polysaccharides in marine invertebrates. *Antiviral Chem. Chemother.* **4**:167–172.
67. Boiziau, C., N. T. Thuong, and J.-J. Toulmé. 1992. Mechanisms of the inhibition of reverse transcription by antisense oligonucleotides. *Proc. Natl. Acad. Sci. USA* **89**:768–772.
68. Bone, R., J. P. Vacca, P. S. Anderson, and M. K. Holloway. 1991. X-ray crystal structure of the HIV protease complex with L-700,417, an inhibitor with pseudo C<sub>2</sub> symmetry. *J. Am. Chem. Soc.* **113**:9382–9384.
69. Bordier, B., C. Hélène, P. J. Barr, S. Litvak, and L. Sarih-Cottin. 1992. *In vitro* effect of antisense oligonucleotides on human immunodeficiency virus type 1 reverse transcription. *Nucleic Acids Res.* **20**:5999–6006.
70. Bos, O. J. M., L. M. Vansterkenburg, J. P. C. I. Boon, M. J. E. Fischer, J. Wiltling, and L. H. M. Janssen. 1990. Location and characterization of the suramin binding sites of human serum albumin. *Biochem. Pharmacol.* **40**:1595–1599.
71. Boucher, C. A. B., E. O'Sullivan, J. W. Mulder, C. Ramautarsing, P. Kellam, G. Darby, J. M. A. Lange, J. Goudsmit, and B. A. Larder. 1992. Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus-positive subjects. *J. Infect. Dis.* **165**:105–110.
72. Boucher, C. A. B., R. van Leeuwen, P. Kellam, P. Schipper, J. Tijnagel, J. M. A. Lange, and B. A. Larder. 1993. Effects of discontinuation of zidovudine treatment on zidovudine sensitivity of human immunodeficiency virus type 1 isolates. *Antimicrob. Agents Chemother.* **37**:1525–1530.
73. Boyer, P. L., M. J. Currens, J. B. McMahon, M. R. Boyd, and S. H. Hughes. 1993. Analysis of non-nucleoside drug-resistant variants of human immunodeficiency virus type 1 reverse transcriptase. *J. Virol.* **67**:2412–2420.
- 73a. Boyer, P. L., J. Ding, E. Arnold, and S. H. Hughes. 1994. Subunit specificity of mutations that confer resistance to nonnucleoside inhibitors in human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **38**:1909–1914.
74. Broder, S., R. Yarchoan, J. M. Collins, H. C. Lane, P. D. Markham, R. W. Klecker, R. R. Redfield, H. Mitsuya, D. F. Hoth, E. Gelmann, J. E. Groopman, L. Resnick, R. C. Gallo, C. E. Myers, and A. S. Fauci. 1985. Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS-related complex: clinical pharmacology and suppression of virus replication in vivo. *Lancet* **ii**:627–630.
75. Bryant, M. L., L. Ratner, R. J. Duronio, N. S. Kishore, B. Devadas, S. P. Adams, and J. I. Gordon. 1991. Incorporation of 12-methoxydodecanoate into the human immunodeficiency virus 1 gag polyprotein precursor inhibits its proteolytic processing and virus production in a chronically infected human lymphoid cell line. *Proc. Natl. Acad. Sci. USA* **88**:2055–2059.
- 75a. Buckheit, R. W., Jr., V. Fliakas-Boltz, W. D. Decker, J. L. Roberson, C. A. Pyle, E. L. White, B. J. Bowdon, J. B. McMahon, M. R. Boyd, J. P. Bader, D. G. Nickell, H. Barth, and T. K. Antonucci. 1994. Biological and biochemical anti-HIV activity of the benzothiadiazine class of nonnucleoside reverse transcriptase inhibitors. *Antiviral Res.* **25**:43–56.
76. Buckheit, R. W., Jr., M. G. Hollingshead, J. Germany-Decker, E. L. White, J. B. McMahon, L. B. Allen, L. J. Ross, W. D. Decker, L. Westbrook, W. M. Shannon, O. Weislow, J. P. Bader, and M. R. Boyd. 1993. Thiazolobenzimidazole: biological and biochemical anti-retroviral activity of a new nonnucleoside reverse transcriptase inhibitor. *Antiviral Res.* **21**:247–265.
77. Buckheit, R. W., Jr., E. L. White, J. Germany-Decker, L. B. Allen, L. J. Ross, W. M. Shannon, P. A. Janssen, and M. A. Chirigos. 1994. Cell-based and biochemical analysis of the anti-HIV activity of combinations of 3'-azido-3'-deoxythymidine and analogues of TIBO. *Antiviral Chem. Chemother.* **5**:35–42.
78. Bushman, F. D., and R. Craigie. 1991. Activities of human immunodeficiency virus (HIV) integration protein *in vitro*: specific cleavage and integration of HIV DNA. *Proc. Natl. Acad. Sci. USA* **88**:1339–1343.
79. Busso, M. E., and L. Resnick. 1990. Anti-human immunodeficiency virus effects of dextran sulfate are strain dependent and synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrob. Agents Chemother.* **34**:1991–1995.
- 79a. Byrnes, V. W., E. A. Emimi, W. A. Schleif, J. H. Condra, C. L. Schneider, W. J. Long, J. A. Wolfgang, D. J. Graham, L. Gotlib, A. J. Schlabach, B. S. Wolanski, O. M. Blahy, J. C. Quintero, A. Rhodes, E. Roth, D. L. Titus, and V. V. Sardana. 1994. Susceptibilities of human immunodeficiency virus type 1 enzyme and viral variants expressing multiple resistance-engendering amino acid substitutions to reverse transcriptase inhibitors. *Antimicrob. Agents Chemother.* **38**:1404–1407.
80. Byrnes, V. W., V. V. Sardana, W. A. Schleif, J. H. Condra, J. A. Waterbury, J. A. Wolfgang, W. J. Long, C. L. Schneider, A. J. Schlabach, B. S. Wolanski, D. J. Graham, L. Gotlib, A. Rhodes, D. L. Titus, E. Roth, O. M. Blahy, J. C. Quintero, S. Staszewski, and E. A. Emimi. 1993. Comprehensive mutant enzyme and viral variant assessment of human immunodeficiency virus type 1 reverse transcriptase resistance to non-nucleoside inhibitors. *Antimicrob. Agents Chemother.* **37**:1576–1579.
81. Caliò, R., N. Villani, E. Balestra, F. Sesa, A. Holy, J. Balzarini, E. De Clercq, C. F. Perno, and V. Del Gobbo. 1994. Enhancement of natural killer activity and interferon induction by different acyclic nucleoside phosphonates. *Antiviral Res.* **23**:77–89.
82. Callahan, L. N., M. Phelan, M. Mallinson, and M. A. Norcross. 1991. Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. *J. Virol.* **65**:1543–1550.
83. Cammack, N., P. Rouse, C. L. P. Marr, P. J. Reid, R. E. Boehme, J. A. V. Coates, C. R. Penn, and J. M. Cameron. 1992. Cellular metabolism of (–)-enantiomeric 2'-deoxy-3'-thiacytidine. *Biochem. Pharmacol.* **43**:2059–2064.
84. Campbell, T. B., R. K. Young, J. J. Eron, R. T. D'Aquila, W. G. Tarpley, and D. R. Kuritzkes. 1993. Inhibition of human immunodeficiency virus type 1

- recombination in vitro by the bisheteroaryl piperazine atevirdine (U-87201E) in combination with zidovudine or didanosine. *J. Infect. Dis.* **168**:318–326.
85. Cantor, G. H., T. F. McElwain, T. A. Birkebak, and G. H. Palmer. 1993. Ribozyme cleaves *rex1tax* mRNA and inhibits bovine leukemia virus expression. *Proc. Natl. Acad. Sci. USA* **90**:10932–10936.
  86. Capon, D. J., S. M. Chamow, J. Mordenti, S. A. Marsters, T. Gregory, H. Mitsuya, R. A. Byrn, C. Lucas, F. M. Wurm, J. E. Groopman, S. Broder, and D. H. Smith. 1989. Designing CD4 immunoadhesins for AIDS therapy. *Nature (London)* **337**:525–531.
  87. Cardin, A. D., P. L. Smith, L. Hyde, D. T. Blankenship, T. L. Bowlin, K. Schroeder, K. A. Stauderman, D. L. Taylor, and A. S. Tynms. 1991. Stilbene disulfonic acids. CD4 antagonists that block human immunodeficiency virus type-1 growth at multiple stages of the virus life cycle. *J. Biol. Chem.* **266**:13355–13363.
  88. Chatterjee, S., P. R. Johnson, and K. K. Wong, Jr. 1992. Dual-target inhibition of HIV-1 in vitro by means of an adeno-associated virus antisense vector. *Science* **258**:1485–1488.
  89. Cheeseman, S. H., S. E. Hattox, M. M. McLaughlin, R. A. Koup, C. Andrews, C. A. Bova, J. W. Pav, T. Roy, J. L. Sullivan, and J. J. Keirns. 1993. Pharmacokinetics of nevirapine: initial single-rising dose study in humans. *Antimicrob. Agents Chemother.* **37**:178–182.
  90. Chimirri, A., S. Grasso, A.-M. Monforte, P. Monforte, and M. Zappalà. 1991. Anti-HIV agents. I. Synthesis and in vitro anti-HIV evaluation of novel 1H,3H-thiazolo[3,4-a]benzimidazoles. *II Farmaco* **46**:817–823.
  91. Chimirri, A., S. Grasso, A.-M. Monforte, P. Monforte, and M. Zappalà. 1991. Anti-HIV agents. II. Synthesis and in vitro anti-HIV activity of novel 1H,3H-thiazolo[3,4-a]benzimidazoles. *II Farmaco* **46**:925–933.
  92. Chong, K.-T., P. J. Pagano, and R. R. Hinshaw. 1994. Bisheteroaryl piperazine reverse transcriptase inhibitor in combination with 3'-azido-3'-deoxythymidine or 2',3'-dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 replication in vitro. *Antimicrob. Agents Chemother.* **38**:288–293.
  93. Chong, K.-T., M. J. Ruwart, R. R. Hinshaw, K. F. Wilkinson, B. D. Rush, M. F. Yancey, J. W. Strohbach, and S. Thaisrivongs. 1993. Peptidomimetic HIV protease inhibitors: phosphate prodrugs with improved biological activities. *J. Med. Chem.* **36**:2575–2577.
  94. Chow, Y.-K., M. S. Hirsch, J. C. Kaplan, and R. T. D'Aquila. 1993. HIV-1 error revealed. *Nature (London)* **364**:679.
  - 94a. Chow, Y.-K., M. S. Hirsch, D. P. Merrill, L. J. Bechtel, J. J. Eron, J. C. Kaplan, and R. T. D'Aquila. 1993. Use of evolutionary limitations of HIV-1 multidrug resistance to optimize therapy. *Nature (London)* **361**:650–654.
  95. Ciomei, M., W. Pastori, M. Mariani, F. Sola, M. Grandi, and N. Mongelli. 1994. New sulfonated distamycin A derivatives with bFGF complexing activity. *Biochem. Pharmacol.* **47**:295–302.
  96. Clanton, D. J., R. A. Moran, J. B. McMahon, O. S. Weislow, R. W. Buckheit, Jr., M. G. Hollingshead, V. Ciminale, B. K. Felber, G. N. Pavlakis, and J. P. Bader. 1992. Sulfonic acid dyes: inhibition of the human immunodeficiency virus and mechanism of action. *J. Acquired Immune Defic. Syndr.* **5**:771–781.
  97. Cloyd, M. W., W. S. Lynn, K. Ramsey, and S. Baron. 1989. Inhibition of human immunodeficiency virus (HIV-1) infection by diphenylhydantoin (Dilantin) implicates role of cellular calcium in virus life cycle. *Virology* **173**:581–590.
  98. Cohen, K. A., J. Hopkins, R. H. Ingraham, C. Pargellis, J. C. Wu, D. E. H. Palladino, P. Kinkade, T. C. Warren, S. Rogers, J. Adams, P. R. Farina, and P. M. Grob. 1991. Characterization of the binding site for nevirapine (BI-RG-587), a nonnucleoside inhibitor of human immunodeficiency virus type-1 reverse transcriptase. *J. Biol. Chem.* **266**:14670–14674.
  99. Condra, J. H., E. A. Emini, L. Gotlib, D. J. Graham, A. J. Schlabach, J. A. Wolfgang, R. J. Colonna, and V. V. Sardana. 1992. Identification of the human immunodeficiency virus reverse transcriptase residues that contribute to the activity of diverse nonnucleoside inhibitors. *Antimicrob. Agents Chemother.* **36**:1441–1446.
  100. Connell, E. V., M.-C. Hsu, and D. D. Richman. 1994. Combinative interactions of a human immunodeficiency virus (HIV) *tat* antagonist with HIV reverse transcriptase inhibitors and an HIV protease inhibitor. *Antimicrob. Agents Chemother.* **38**:348–352.
  101. Constantoulakis, P., M. Campbell, B. K. Felber, G. Nasioulas, E. Afonina, and G. N. Pavlakis. 1993. Inhibition of Rev-mediated HIV-1 expression by an RNA binding protein encoded by the interferon-inducible 9-27 gene. *Science* **259**:1314–1317. [Retracted. *Science* **264**:492, 1994.]
  102. Cooper, D. A., P. O. Pehrson, C. Pedersen, M. Moroni, E. Oksenhendler, W. Rozenbaum, N. Clumeck, V. Faber, W. Stille, B. Hirschel, C. Farthing, R. Doherty, J. M. Yeo, and a European-Australian Collaborative Group. 1993. The efficacy and safety of zidovudine alone or as cotherapy with acyclovir for the treatment of patients with AIDS and AIDS-related complex: a double-blind, randomized trial. *AIDS* **7**:197–207.
  103. Cox, S. W., J. Albert, K. Aperia, and B. Wahren. 1993. Synergistic inhibition of primary isolates of human immunodeficiency virus type 1 by combinations of 3'-fluoro-3'-deoxythymidine and 2',3'-dideoxyinosine. *Antiviral Chem. Chemother.* **4**:241–244.
  104. Craig, J. C., I. B. Duncan, L. Whittaker, and N. A. Roberts. 1990. Antiviral synergy between inhibitors of HIV proteinase and reverse transcriptase. *Antiviral Chem. Chemother.* **4**:161–166.
  105. Craig, J. C., L. Whittaker, I. B. Duncan, and N. A. Roberts. 1993. *In vitro* resistance to an inhibitor of HIV proteinase (Ro 31-8959) relative to inhibitors of reverse transcriptase (AZT and TIBO). *Antiviral Chem. Chemother.* **4**:335–339.
  106. Cundy, K. C., J.-P. Shaw, and W. A. Lee. 1994. Oral, subcutaneous, and intramuscular bioavailabilities of the antiviral nucleotide analog 9-(2-phosphonylmethoxyethyl)adenine in cynomolgus monkeys. *Antimicrob. Agents Chemother.* **38**:365–368.
  - 106a. Cushman, M., W. M. Golebiewski, J. B. McMahon, R. W. Buckheit, Jr., D. J. Clanton, O. Weislow, R. D. Haugwitz, J. P. Bader, L. Graham, and W. G. Rice. 1994. Design, synthesis, and biological evaluation of cosalane, a novel anti-HIV agent which inhibits multiple features of virus reproduction. *J. Med. Chem.* **37**:3040–3050.
  107. Cushman, M., P. Wang, S. H. Chang, C. Wild, E. De Clercq, D. Schols, M. E. Goldman, and J. A. Bowen. 1991. Preparation and anti-HIV activities of aurintricarboxylic acid fractions and analogues: direct correlation of antiviral potency with molecular weight. *J. Med. Chem.* **34**:329–337.
  108. Cushman, M., P. Wang, J. G. Stowell, D. Schols, and E. De Clercq. 1992. Structural investigation and anti-HIV activities of high molecular weight ATA polymers. *J. Org. Chem.* **57**:7241–7248.
  109. Daar, E. S., X. L. Li, T. Moudgil, and D. D. Ho. 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. *Proc. Natl. Acad. Sci. USA* **87**:6574–6578.
  - 109a. Daluge, S. M., D. J. M. Purifoy, P. M. Savina, M. H. St. Clair, N. R. Parry, I. K. Dev, P. Novak, K. M. Ayers, J. E. Reardon, G. B. Roberts, J. A. Fyfe, M. R. Blum, D. R. Averett, R. E. Dornsife, B. A. Domin, R. Ferone, D. A. Lewis, and T. A. Krenitsky. 1994. 5-Chloro-2',3'-dideoxy-3'-fluorouridine (935U83), a selective anti-human immunodeficiency virus agent with an improved metabolic and toxicological profile. *Antimicrob. Agents Chemother.* **38**:1590–1603.
  110. Davey, R. T., Jr., R. L. Dewar, G. F. Reed, M. B. Vasudevachari, M. A. Polis, J. A. Kovacs, J. Falloon, R. E. Walker, H. Masur, S. E. Hanevich, D. G. O'Neill, M. R. Decker, J. A. Metcalf, M. A. Deloria, O. L. Laskin, N. Salzman, and H. C. Lane. 1993. Plasma viremia as a sensitive indicator of the antiretroviral activity of L-697,661. *Proc. Natl. Acad. Sci. USA* **90**:5608–5612.
  111. Debyser, Z., K. De Vreese, R. Pauwels, N. Yamamoto, J. Anné, E. De Clercq, and J. Desmyter. 1992. Differential inhibitory effects of TIBO derivatives on different strains of simian immunodeficiency virus. *J. Gen. Virol.* **73**:1799–1804.
  112. Debyser, Z., R. Pauwels, K. Andries, J. Desmyter, Y. Engelborghs, P. A. J. Janssen, and E. De Clercq. 1992. Allosteric inhibition of human immunodeficiency virus type 1 reverse transcriptase by tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione compounds. *Mol. Pharmacol.* **41**:203–208.
  113. Debyser, Z., R. Pauwels, K. Andries, J. Desmyter, M. Kukla, P. A. J. Janssen, and E. De Clercq. 1991. An antiviral target on reverse transcriptase of human immunodeficiency virus type 1 revealed by tetrahydroimidazo-[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione derivatives. *Proc. Natl. Acad. Sci. USA* **88**:1451–1455.
  114. Debyser, Z., R. Pauwels, M. Baba, J. Desmyter, and E. De Clercq. 1992. Common features in the interaction of tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione and 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine derivatives with the human immunodeficiency virus type 1 reverse transcriptase. *Mol. Pharmacol.* **41**:963–968.
  115. Debyser, Z., A.-M. Vandamme, R. Pauwels, M. Baba, J. Desmyter, and E. De Clercq. 1992. Kinetics of inhibition of endogenous human immunodeficiency virus type 1 reverse transcription by 2',3'-dideoxynucleoside 5'-triphosphate, tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione, and 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine derivatives. *J. Biol. Chem.* **267**:11769–11776.
  116. De Clercq, E. 1986. Chemotherapeutic approaches to the treatment of the acquired immune deficiency syndrome (AIDS). *J. Med. Chem.* **29**:1561–1569.
  117. De Clercq, E. 1987. Suramin in the treatment of AIDS: mechanism of action. *Antiviral Res.* **7**:1–10.
  118. De Clercq, E. 1989. New acquisitions in the development of anti-HIV agents. *Antiviral Res.* **12**:1–20.
  119. De Clercq, E. 1989. New promising inhibitors of the human immunodeficiency virus. *Curr. Opin. Infect. Dis.* **2**:401–410.
  120. De Clercq, E. 1990. Targets and strategies for the antiviral chemotherapy of AIDS. *Trends Biochem. Sci.* **11**:198–205.
  121. De Clercq, E. 1991. Basic approaches to anti-retroviral treatment. *J. Acquired Immune Defic. Syndr.* **4**:207–218.
  122. De Clercq, E. 1992. HIV inhibitors targeted at the reverse transcriptase. *AIDS Res. Hum. Retroviruses* **8**:119–134.
  123. De Clercq, E. 1992. Human immunodeficiency virus inhibitors targeted at virus-cell fusion and/or viral uncoating. *Int. J. Immunother.* **8**:115–123.
  124. De Clercq, E. 1993. Anti-HIV activity of sulfated polysaccharides, p. 87–

100. *In M. Yalpani* (ed.), Carbohydrates and carbohydrate polymers, analysis, biotechnology, modification, antiviral, biomedical and other applications. ATL Press, Mt. Prospect, Ill.
125. **De Clercq, E.** 1993. HIV-1-specific RT inhibitors: highly selective inhibitors of human immunodeficiency virus type 1 that are specifically targeted at the viral reverse transcriptase. *Med. Res. Rev.* **13**:229–258.
126. **De Clercq, E.** 1994. HIV resistance to reverse transcriptase inhibitors. *Biochem. Pharmacol.* **47**:155–169.
127. **De Clercq, E., and J. Balzarini.** 1985. In search of specific inhibitors of retrovirus replication. *Antiviral Res.* **1**(Suppl.):89–94.
128. **De Clercq, E., A. Van Aerschot, P. Herdewijn, M. Baba, R. Pauwels, and J. Balzarini.** 1989. Anti-HIV-1 activity of 2',3'-dideoxynucleoside analogues: structure-activity relationship. *Nucleosides Nucleotides* **8**:659–671.
129. **De Clercq, E., N. Yamamoto, R. Pauwels, M. Baba, D. Schols, H. Nakashima, J. Balzarini, Z. Debyser, B. A. Murrer, D. Schwartz, D. Thornton, G. Bridger, S. Fricker, G. Henson, M. Abrams, and D. Picker.** 1992. Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. *Proc. Natl. Acad. Sci. USA* **89**:5286–5290.
130. **De Clercq, E., N. Yamamoto, R. Pauwels, J. Balzarini, M. Witvrouw, K. De Vreese, Z. Debyser, B. Rosenwirth, P. Peichl, R. Datema, D. Thornton, R. Skerlj, F. Gaul, S. Padmanabhan, G. Bridger, G. Henson, and M. Abrams.** 1994. Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. *Antimicrob. Agents Chemother.* **38**:668–674.
131. **Dedera, D., N. Vander Heyden, and L. Ratner.** 1990. Attenuation of HIV-1 infectivity by an inhibitor of oligosaccharide processing. *AIDS Res. Hum. Retroviruses* **6**:785–794.
132. **De Luca, G. V., and M. J. Otto.** 1992. Synthesis and anti-HIV activity of pyrrolo-[1,2-d]-(1,4)-benzodiazepin-6-ones. *Bioorg. Med. Chem. Lett.* **2**:1639–1644.
133. **de Solms, S. J., E. A. Giuliani, J. P. Guare, J. P. Vacca, W. M. Sanders, S. L. Graham, J. M. Wiggins, P. L. Darke, I. S. Sigal, J. A. Zugay, E. A. Emini, W. A. Schleif, J. C. Quintero, P. S. Anderson, and J. R. Huff.** 1991. Design and synthesis of HIV protease inhibitors. Variations of the carboxy terminus of the HIV protease inhibitor L-682,679. *J. Med. Chem.* **34**:2852–2857.
134. **Devadas, B., T. Lu, A. Katoh, N. S. Kishore, A. C. Wade, P. P. Mehta, D. A. Rudnick, M. L. Bryant, S. P. Adams, Q. Li, G. W. Gokel, and J. I. Gordon.** 1992. Substrate specificity of *Saccharomyces cerevisiae* myristoyl-CoA:protein *N*-myristoyl-transferase. *J. Biol. Chem.* **267**:7224–7239.
135. **De Vreese, K., Z. Debyser, A.-M. Vandamme, R. Pauwels, J. Desmyter, E. De Clercq, and J. Anné.** 1992. Resistance of human immunodeficiency virus type 1 reverse transcriptase to TIBO derivatives induced by site-directed mutagenesis. *Virology* **188**:900–904.
136. **De Wit, S., P. Hermans, B. Sommereijns, E. O'Doherty, R. Westenborghs, V. Van De Velde, G. F. M. J. Cauwenbergh, and N. Clumeck.** 1992. Pharmacokinetics of R82913 in AIDS patients: a phase I dose-finding study of oral administration compared with intravenous infusion. *Antimicrob. Agents Chemother.* **36**:2661–2663.
137. **De Witte, P., P. Agostinis, J. Van Lint, W. Merlevede, and J. R. Vandenhede.** 1993. Inhibition of epidermal growth factor receptor tyrosine kinase activity by hypericin. *Biochem. Pharmacol.* **46**:1929–1936.
138. **Dianzani, F., G. Antonelli, O. Turriziani, E. Riva, G. Dong, and D. Belarosa.** 1993. *In vitro* selection of human immunodeficiency virus type 1 resistant to Ro 31-8959 proteinase inhibitor. *Antiviral Chem. Chemother.* **4**:329–333.
139. **Diringer, H., and B. Ehlers.** 1991. Chemoprophylaxis of scrapie in mice. *J. Gen. Virol.* **72**:457–460.
140. **Doi, J. T., Q.-F. Ma, G. L. Rowley, and G. L. Kenyon.** 1991. The synthesis of *N*-hydroxyphosphoramidate, a potential antiviral agent: inhibition of HIV-1 reverse transcriptase. *Med. Chem. Res.* **1**:226–229.
141. **Doong, S.-L., C.-H. Tsai, R. F. Schinazi, D. C. Liotta, and Y.-C. Cheng.** 1991. Inhibition of the replication of hepatitis B virus *in vitro* by 2',3'-dideoxy-3'-thiacytidine and related analogues. *Proc. Natl. Acad. Sci. USA* **88**:8495–8499.
142. **Dormont, D., B. Spire, F. Barré-Sinoussi, L. Montagnier, and J. C. Chermann.** 1985. Inhibition of RNA-dependent DNA polymerases of AIDS and SAIDS retroviruses by HPA-23 (ammonium-21-tungsto-9-antimoniate). *Ann. Inst. Pasteur Virol.* **136E**:75–83.
143. **Dornsife, R. E., M. H. St. Clair, A. T. Huang, T. J. Panella, G. W. Koszalka, C. L. Burns, and D. R. Averett.** 1991. Anti-human immunodeficiency virus synergism by zidovudine (3'-azidothymidine) and didanosine (dideoxyinosine) contrasts with their additive inhibition of normal human marrow progenitor cells. *Antimicrob. Agents Chemother.* **35**:322–328.
144. **Droplific, B., N. H. Lin, M. A. Martin, and K.-T. Jeang.** 1992. Functional characterization of a U5 ribozyme: intracellular suppression of human immunodeficiency virus type 1 expression. *J. Virol.* **66**:1432–1441.
145. **Duckworth, D. M., M. R. Harnden, R. M. Perkins, and D. N. Planterose.** 1991. Antiviral 9-[2-(phosphonomethoxy)alkoxy]purines. *Antiviral Chem. Chemother.* **2**:229–241.
146. **Dueweke, T. J., F. J. Keady, G. A. Waszak, M. R. Deibel, Jr., and W. G. Tarpley.** 1992. The binding of a novel bisheteroaryl piperazine mediates inhibition of human immunodeficiency virus type 1 reverse transcriptase. *J. Biol. Chem.* **267**:27–30.
147. **Dueweke, T. J., S. M. Poppe, D. L. Romero, S. M. Swaney, A. G. So, K. M. Downey, I. W. Althaus, F. Reusser, M. Busso, L. Resnick, D. L. Mayers, J. Lane, P. A. Aristoff, R. C. Thomas, and W. G. Tarpley.** 1993. U-90152, a potent inhibitor of human immunodeficiency virus type 1 replication. *Antimicrob. Agents Chemother.* **37**:1127–1131.
148. **Dueweke, T. J., T. Pushkarskaya, S. M. Poppe, S. M. Swaney, J. Q. Zhao, I. S. Y. Chen, M. Stevenson, and W. G. Tarpley.** 1993. A novel mutation in bisheteroaryl piperazine-resistant HIV-1 reverse transcriptase confers increased sensitivity to other nonnucleoside inhibitors. *Proc. Natl. Acad. Sci. USA* **90**:4713–4717.
149. **Duran, N., and P.-S. Song.** 1986. Hypericin and its photodynamic action. *Photochem. Photobiol.* **43**:677–680.
150. **Egberink, H., M. Borst, H. Niphuis, J. Balzarini, H. Neu, H. Schellekens, E. De Clercq, M. Horzinek, and M. Koolen.** 1990. Suppression of feline immunodeficiency virus infection *in vivo* by 9-(2-phosphonomethoxyethyl)adenine. *Proc. Natl. Acad. Sci. USA* **87**:3087–3091.
151. **Emini, E. A., D. J. Graham, L. Gottlieb, J. H. Condra, V. W. Byrnes, and W. A. Schleif.** 1993. HIV and multidrug resistance. *Nature (London)* **364**:679.
152. **Erice, A., H. H. Balfour, Jr., D. E. Myers, V. L. Leske, K. J. Sannerud, V. Kuebelbeck, J. D. Irvin, and F. M. Uckun.** 1993. Anti-human immunodeficiency virus type 1 activity of an anti-CD4 immunoconjugate containing pokeweed antiviral protein. *Antimicrob. Agents Chemother.* **37**:835–838.
153. **Erice, A., D. L. Mayers, D. G. Strike, K. J. Sannerud, F. E. McCutchan, K. Henry, and H. H. Balfour, Jr.** 1993. Brief report: primary infection with zidovudine-resistant human immunodeficiency virus type 1. *N. Engl. J. Med.* **328**:1163–1165.
154. **Erickson, J., D. J. Neidhart, J. VanDrie, D. J. Kempf, X. C. Wang, D. W. Norbeck, J. J. Plattner, J. W. Rittenhouse, M. Turon, N. Wideburg, W. E. Kohlbrener, R. Simmer, R. Helfrich, D. A. Paul, and M. Knigge.** 1990. Design, activity, and 2.8 Å crystal structure of a C<sub>2</sub> symmetric inhibitor complexed to HIV-1 protease. *Science* **249**:527–533.
155. **Eriksson, B. F. H., and R. F. Schinazi.** 1989. Combinations of 3'-azido-3'-deoxythymidine (zidovudine) and phosphonoformate (foscarnet) against human immunodeficiency virus type 1 and cytomegalovirus replication *in vitro*. *Antimicrob. Agents Chemother.* **33**:663–669.
- 155a. **Faraj, A., L. A. Agrofoglio, J. K. Wakefield, S. McPherson, C. D. Morrow, G. Gosselin, C. Mathe, J.-L. Imbach, R. F. Schinazi, and J.-P. Sommadossi.** 1994. Inhibition of human immunodeficiency virus type 1 reverse transcriptase by the 5'-triphosphate β enantiomers of cytidine analogs. *Antimicrob. Agents Chemother.* **38**:2300–2305.
156. **Faulds, D., and R. N. Brogden.** 1992. Didanosine. A review of its antiviral activity, pharmacokinetic properties and therapeutic potential in human immunodeficiency virus infection. *Drugs* **44**:94–116.
157. **Feng, J. S., J. Y. Crouch, P. Y. Tian, H. L. Lucia, and G. D. Hsiung.** 1993. Zidovudine antagonizes the antiviral effects of ganciclovir against cytomegalovirus infection in cultured cells and in guinea pigs. *Antiviral Chem. Chemother.* **4**:19–25.
158. **Fenouillet, E., and J.-C. Gluckman.** 1991. Effect of a glucosidase inhibitor on the bioactivity and immunoreactivity of human immunodeficiency virus type 1 envelope glycoprotein. *J. Gen. Virol.* **72**:1919–1926.
- 158a. **Fenster, S. D., R. W. Wagner, B. C. Froehler, and D. J. Chin.** 1994. Inhibition of human immunodeficiency virus type 1 *env* expression by C-5 propyne oligonucleotides specific for Rev-response element Stem-Loop V. *Biochemistry* **33**:8391–8398.
159. **Ferrari, P., M.-A. Trabaud, M. Rommain, E. Mandine, R. Zaliz, C. Desgranges, and P. Smets.** 1991. Toxicity and activity of purified trichosanthin. *AIDS* **5**:865–870.
160. **Finberg, R. W., D. C. Diamond, D. B. Mitchell, Y. Rosenstein, G. Soman, T. C. Norman, S. L. Schreiber, and S. J. Burakoff.** 1990. Prevention of HIV-1 infection and preservation of CD4 function by the binding of CPFS to gp120. *Science* **249**:287–291.
161. **Fischl, M. A., L. Resnick, R. Coombs, A. B. Kremer, J. C. Pottage, Jr., R. J. Fass, K. H. Fife, W. G. Powderly, A. C. Collier, R. L. Aspinall, S. L. Smith, K. G. Kowalski, and C.-B. Wallemark.** 1994. The safety and efficacy of combination *N*-butyl-deoxyjirimycin (SC-48334) and zidovudine in patients with HIV-1 infection and 200–500 CD4 cells/mm<sup>3</sup>. *J. Acquired Immune Defic. Syndr.* **7**:139–147.
162. **Fitzgibbon, J. E., A. E. Farnham, S. J. Sperber, H. Kim, and D. T. Dubin.** 1993. Human immunodeficiency virus type 1 *pol* gene mutations in an AIDS patient treated with multiple antiretroviral drugs. *J. Virol.* **67**:7271–7275.
163. **Fitzgibbon, J. E., R. M. Howell, C. A. Haberzettl, S. J. Sperber, D. J. Gocke, and D. T. Dubin.** 1992. Human immunodeficiency virus type 1 *pol* gene mutations which cause decreased susceptibility to 2',3'-dideoxycytidine. *Antimicrob. Agents Chemother.* **36**:153–157.
164. **Flexner, C., P. A. Barditch-Crovo, D. M. Kornhauser, H. Farzadegan, L. J. Nerhood, R. E. Chaisson, K. M. Bell, K. J. Lorentsen, C. W. Hendrix, B. G. Petty, and P. S. Leitman.** 1991. Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. *Antimicrob. Agents Chemother.* **35**:2544–2550.

165. Frank, K. B., G. J. Noll, E. V. Connell, and I. S. Sim. 1991. Kinetic interaction of human immunodeficiency virus type 1 reverse transcriptase with the antiviral tetrahydroimidazo[4,5,1-jk][1,4]-benzodiazepine-2-(1*H*)-thione compound, R82150. *J. Biol. Chem.* **266**:14232-14236.
166. Friedman, S. H., D. L. DeCamp, R. P. Sijbesma, G. Srdanov, F. Wudl, and G. L. Kenyon. 1993. Inhibition of the HIV-1 protease by fullerene derivatives: model building studies and experimental verification. *J. Am. Chem. Soc.* **115**:6506-6509.
167. Fukuma, M., Y. Seto, and T. Yamase. 1991. In vitro antiviral activity of polyoxotungstate (PM-19) and other polyoxometalates against herpes simplex virus. *Antiviral Res.* **16**:327-339.
168. Furman, P. A., M. Davis, D. C. Liotta, M. Paff, L. W. Frick, D. J. Nelson, R. E. Dornisfe, J. A. Wurster, L. J. Wilson, J. A. Fyfe, J. V. Tuttle, W. H. Miller, L. Condreay, D. R. Averett, R. F. Schinazi, and G. R. Painter. 1992. The anti-hepatitis B virus activities, cytotoxicities, and anabolic profiles of the (-) and (+) enantiomers of *cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. *Antimicrob. Agents Chemother.* **36**:2686-2692.
169. Furman, P. A., J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, and D. W. Barry. 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **83**:8333-8337.
170. Galpin, S., N. A. Roberts, T. O'Connor, D. J. Jeffries, and D. Kinchington. 1994. Antiviral properties of the HIV-1 proteinase inhibitor Ro 31-8959. *Antiviral Chem. Chemother.* **5**:43-45.
171. Gargemi, J. D., R. M. Cozens, E. De Clercq, J. Balzarini, and H.-K. Hochkeppel. 1989. 9-(2-Phosphorylmethoxyethyl)adenine in the treatment of murine acquired immunodeficiency disease and opportunistic herpes simplex virus infections. *Antimicrob. Agents Chemother.* **33**:1864-1868.
172. Gao, Q., Z. Gu, M. A. Parniak, J. Cameron, N. Cammack, C. Boucher, and M. A. Wainberg. 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high-level resistance to the (-) enantiomer of 2',3'-dideoxy-3'-thiacytidine. *Antimicrob. Agents Chemother.* **37**:1390-1392.
173. Gao, W.-Y., F.-S. Han, C. Storm, W. Egan, and Y.-C. Cheng. 1992. Phosphorothioate oligonucleotides are inhibitors of human DNA polymerases and RNase H: implications for antisense technology. *Mol. Pharmacol.* **41**:223-229.
174. Gatti, G., J. O. Kahn, J. Lifson, R. Williams, L. Turin, P. A. Volberding, and J. G. Gambertoglio. 1991. Pharmacokinetics of GLQ223 in rats, monkeys, and patients with AIDS or AIDS-related complex. *Antimicrob. Agents Chemother.* **35**:2531-2537.
175. Getman, D. P., G. A. DeCrescenzo, R. M. Heintz, K. L. Reed, J. J. Talley, M. L. Bryant, M. Clare, K. A. Houseman, J. J. Marr, R. A. Mueller, M. L. Vazquez, H.-S. Shieh, W. C. Stallings, and R. A. Stegeman. 1993. Discovery of a novel class of potent HIV-1 protease inhibitors containing the (R)-(hydroxyethyl)urea isostere. *J. Med. Chem.* **36**:288-291.
176. Ghosh, A. K., W. J. Thompson, H. Y. Lee, S. P. McKee, P. M. Munson, T. T. Duong, P. L. Darke, J. A. Zugay, E. A. Emini, W. A. Schleif, J. R. Huff, and P. S. Anderson. 1993. Cyclic sulfonates as novel and high affinity P<sub>2</sub> ligands for HIV-1 protease inhibitors. *J. Med. Chem.* **36**:924-927.
177. Ghosh, A. K., W. J. Thompson, S. P. McKee, T. T. Duong, T. A. Lyle, J. C. Chen, P. L. Darke, J. A. Zugay, E. A. Emini, W. A. Schleif, J. R. Huff, and P. S. Anderson. 1993. 3-Tetrahydrofuran and pyran urethanes as high-affinity P<sub>2</sub>-ligands for HIV-1 protease inhibitors. *J. Med. Chem.* **36**:292-294.
178. Goldfeld, A. E., E. K. Flemington, V. A. Boussiotis, C. M. Theodos, R. G. Titus, J. L. Strominger, and S. H. Speck. 1992. Transcription of the tumor necrosis factor  $\alpha$  gene is rapidly induced by anti-immunoglobulin and blocked by cyclosporin A and FK506 in human B cells. *Proc. Natl. Acad. Sci. USA* **89**:12198-12201.
179. Goldman, M. E., J. H. Nunberg, J. A. O'Brien, J. C. Quintero, W. A. Schleif, K. F. Freund, S. L. Gaul, W. S. Saari, J. S. Wai, J. M. Hoffman, P. S. Anderson, D. J. Hupe, E. A. Emini, and A. M. Stern. 1991. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. *Proc. Natl. Acad. Sci. USA* **88**:6863-6867.
180. Goldman, M. E., J. A. O'Brien, T. L. Ruffing, J. H. Nunberg, W. A. Schleif, J. C. Quintero, P. K. S. Siegl, J. M. Hoffman, A. M. Smith, and E. A. Emini. 1992. L-696,229 specifically inhibits human immunodeficiency virus type 1 reverse transcriptase and possesses antiviral activity in vitro. *Antimicrob. Agents Chemother.* **36**:1019-1023.
181. Goldman, M. E., J. A. O'Brien, T. L. Ruffing, W. A. Schleif, V. V. Sardana, V. W. Byrnes, J. H. Condra, J. M. Hoffman, and E. A. Emini. 1993. A nonnucleoside reverse transcriptase inhibitor active on human immunodeficiency virus type 1 isolates resistant to related inhibitors. *Antimicrob. Agents Chemother.* **37**:947-949.
182. Goldman, M. E., G. S. Salituro, J. A. Bowen, J. M. Williamson, D. L. Zink, W. A. Schleif, and E. A. Emini. 1990. Inhibition of human immunodeficiency virus-1 reverse transcriptase activity by rubromycins: competitive interaction at the template primer site. *Mol. Pharmacol.* **38**:20-25.
183. Gosh, A. K., W. J. Thompson, M. K. Holloway, S. P. McKee, T. T. Duong, H. Y. Lee, P. M. Munson, A. M. Smith, J. M. Wai, P. L. Darke, J. A. Zugay, E. A. Emini, W. A. Schleif, J. R. Huff, and P. S. Anderson. 1993. Potent HIV protease inhibitors: the development of tetrahydrofuranylglycines as novel P<sub>2</sub>-ligands and pyrazine amides as P<sub>3</sub>-ligands. *J. Med. Chem.* **36**:2300-2310.
- 183a. Gosselin, G., R. F. Schinazi, J.-P. Sommadossi, C. Mathé, M.-C. Bergogne, A.-M. Aubertin, A. Kirn, and J.-L. Imbach. 1994. Anti-human immunodeficiency virus activities of the  $\beta$ -L enantiomer of 2',3'-dideoxycytidine and its 5-fluoro derivative in vitro. *Antimicrob. Agents Chemother.* **38**:1292-1297.
184. Göttlinger, H. G., J. G. Sodroski, and W. A. Haseltine. 1989. Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **86**:5781-5785.
185. Gruters, R. A., J. J. Neefjes, M. Tersmette, R. E. Y. de Goede, A. Tulp, H. G. Huisman, F. Miedema, and H. L. Ploegh. 1987. Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. *Nature (London)* **330**:74-77.
186. Gu, Z., Q. Gao, H. Fang, H. Salomon, M. A. Parniak, E. Goldberg, J. Cameron, and M. A. Wainberg. 1994. Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase that encodes human immunodeficiency virus resistance to 2',3'-dideoxycytidine and 2',3'-dideoxy-3'-thiacytidine. *Antimicrob. Agents Chemother.* **38**:275-281.
187. Gu, Z., Q. Gao, X. Li, M. A. Parniak, and M. A. Wainberg. 1992. Novel mutation in the human immunodeficiency virus type 1 reverse transcriptase gene that encodes cross-resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine. *J. Virol.* **66**:7128-7135.
- 187a. Guo, L., N. K. Heinzinger, M. Stevenson, L. M. Schopfer, and J. M. Salhany. 1994. Inhibition of gp120-CD4 interaction and human immunodeficiency virus type 1 infection in vitro by pyridoxal 5'-phosphate. *Antimicrob. Agents Chemother.* **38**:2483-2487.
188. Gupta, P., R. Balachandran, P. Thampatty, C. Rinaldo, and M. Ho. 1990. Oxophenarsine, an antisyphilis drug inhibits HIV-1-specific protein synthesis in acutely and persistently infected lymphocytes. *AIDS Res. Hum. Retroviruses* **6**:1417-1423.
189. Hafkemeyer, P., K. Neffel, R. Hobi, A. Pfaltz, H. Lutz, K. Lüthi, F. Foehrer, S. Spadari, and U. Hübscher. 1991. HP 0.35, a cephalosporin degradation product is a specific inhibitor of lentiviral RNAses H. *Nucleic Acids Res.* **19**:4059-4065.
190. Hamamoto, Y., H. Nakashima, T. Matsui, A. Matsuda, T. Ueda, and N. Yamamoto. 1987. Inhibitory effect of 2',3'-didehydro-2',3'-dideoxynucleosides on infectivity, cytopathic effects, and replication of human immunodeficiency virus. *Antimicrob. Agents Chemother.* **31**:907-910.
191. Handa, A., H. Hoshino, K. Nakajima, M. Adachi, K. Ikeda, K. Achiwa, T. Itoh, and Y. Suzuki. 1991. Inhibition of infection with human immunodeficiency virus type 1 by sulfated gangliosides. *Biochem. Biophys. Res. Commun.* **175**:1-9.
192. Hao, Z., D. A. Cooney, D. Farquhar, C. F. Perno, K. Zhang, R. Masood, Y. Wilson, N. R. Hartman, J. Balzarini, and D. G. Johns. 1990. Potent DNA chain termination activity and selective inhibition of human immunodeficiency virus reverse transcriptase by 2',3'-dideoxyuridine-5'-triphosphate. *Mol. Pharmacol.* **37**:157-163.
193. Hao, Z., D. A. Cooney, N. R. Hartman, C. F. Perno, A. Fridland, A. L. DeVico, M. G. Sarngadharan, S. Broder, and D. G. Johns. 1988. Factors determining the activity of 2',3'-dideoxynucleosides in suppressing human immunodeficiency virus *in vitro*. *Mol. Pharmacol.* **34**:431-435.
194. Harakeh, S., R. J. Jariwalla, and L. Pauling. 1990. Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. *Proc. Natl. Acad. Sci. USA* **87**:7245-7249.
195. Harmenberg, J., A. Åkesson-Johansson, L. Vrang, and S. Cox. 1990. Synergistic inhibition of human immunodeficiency virus replication in vitro by combinations of 3'-azido-3'-deoxythymidine and 3'-fluoro-3'-deoxythymidine. *AIDS Res. Hum. Retroviruses* **6**:1197-1202.
196. Harnden, M. R., and L. J. Jennings. 1993. Antiviral 9-[2-(phosphonomethylthio)alkoxy]purines. *Antiviral Chem. Chemother.* **4**:215-227.
197. Harrington, J. A., J. E. Reardon, and T. Spector. 1993. 3'-Azido-3'-deoxythymidine (AZT) monophosphate: an inhibitor of exonucleolytic repair of AZT-terminated DNA. *Antimicrob. Agents Chemother.* **37**:918-920.
198. Harrison, G. S., C. J. Long, F. Maxwell, L. M. Glode, and I. H. Maxwell. 1992. Inhibition of HIV production in cells containing an integrated, HIV-regulated diphtheria toxin A chain gene. *AIDS Res. Hum. Retroviruses* **8**:39-45.
199. Hartman, N. R., G. S. Ahluwalia, D. A. Cooney, H. Mitsuya, S. Kageyama, A. Fridland, S. Broder, and D. G. Johns. 1991. Inhibitors of IMP dehydrogenase stimulate the phosphorylation of the anti-human immunodeficiency virus nucleosides 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine. *Mol. Pharmacol.* **40**:118-124.
200. Hartman, N. R., D. G. Johns, and H. Mitsuya. 1990. Pharmacokinetic analysis of dextran sulfate in rats as pertains to its clinical usefulness for therapy of HIV infection. *AIDS Res. Hum. Retroviruses* **6**:805-812.
201. Hartmann, K., J. Balzarini, J. Higgins, E. De Clercq, and N. C. Pedersen. 1994. *In vitro* activity of acyclic nucleoside phosphonate derivatives against feline immunodeficiency virus in Crandell feline kidney cells and feline peripheral blood lymphocytes. *Antiviral Chem. Chemother.* **5**:13-19.



202. Hartshorn, K. L., E. G. Sandstrom, D. Neumeyer, T. J. Paradis, T.-C. Chou, R. T. Schooley, and M. S. Hirsch. 1986. Synergistic inhibition of human T-cell lymphotropic virus type III replication in vitro by phosphonoformate and recombinant alpha-A interferon. *Antimicrob. Agents Chemother.* **30**:189-191.
203. Hartshorn, K. L., M. W. Vogt, T.-C. Chou, R. S. Blumberg, R. Byington, R. T. Schooley, and M. S. Hirsch. 1987. Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha A interferon. *Antimicrob. Agents Chemother.* **31**:168-172.
204. Hatanaka, K., T. Yoshida, T. Uryu, O. Yoshida, H. Nakashima, N. Yamamoto, T. Mimura, and Y. Kaneko. 1989. Synthesis of an inhibitor of human immunodeficiency virus infection. *Jpn. J. Cancer Res.* **80**:95-98.
205. Hayashi, S., R. L. Fine, T.-C. Chou, M. J. Currens, S. Broder, and H. Mitsuya. 1990. In vitro inhibition of the infectivity and replication of human immunodeficiency virus type 1 by combination of antiretroviral 2',3'-dideoxynucleosides and virus-binding inhibitors. *Antimicrob. Agents Chemother.* **34**:82-88.
206. Heidenreich, O., F. Benseler, A. Fahrenholz, and F. Eckstein. 1994. High activity and stability of hammerhead ribozymes containing 2'-modified pyrimidine nucleosides and phosphorothioates. *J. Biol. Chem.* **269**:2131-2138.
207. Heidenreich, O., and F. Eckstein. 1992. Hammerhead ribozyme-mediated cleavage of the long terminal repeat RNA of human immunodeficiency virus type 1. *J. Biol. Chem.* **267**:1904-1909.
208. Heijntink, R. A., G. A. De Wilde, J. Kruijning, L. Berk, J. Balzarini, E. De Clercq, A. Holy, and S. W. Schalm. 1993. Inhibitory effect of 9-(2-phosphorylmethoxyethyl)adenine (PMEA) on human and duck hepatitis B virus infection. *Antiviral Res.* **21**:141-153.
209. Herrmann, C. H., and A. P. Rice. 1993. Specific interaction of the human immunodeficiency virus Tat proteins with a cellular protein kinase. *Virology* **197**:601-608.
210. Hill, C. L., M. S. Weeks, and R. F. Schinazi. 1990. Anti-HIV-1 activity, toxicity, and stability studies of representative structural families of polyoxometalates. *J. Med. Chem.* **33**:2767-2772.
211. Hizi, A., R. Tal, M. Shaharabany, M. J. Currens, M. R. Boyd, S. H. Hughes, and J. B. McMahon. 1993. Specific inhibition of the reverse transcriptase of human immunodeficiency virus type 1 and the chimeric enzymes of human immunodeficiency virus type 1 and type 2 by nonnucleoside inhibitors. *Antimicrob. Agents Chemother.* **37**:1037-1042.
212. Ho, H.-T., and M. J. M. Hitchcock. 1989. Cellular pharmacology of 2',3'-dideoxy-2',3'-didehydrothymidine, a nucleoside analog active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **33**:844-849.
213. Holmes, D. S., R. C. Bethell, N. Cammack, I. R. Clemens, J. Kitchin, P. McMeekin, C. L. Mo, D. C. Orr, B. Patel, I. L. Paternoster, and R. Storer. 1993. Synthesis and structure-activity relationships of a series of penicillin-derived HIV proteinase inhibitors containing a stereochemically unique peptide isostere. *J. Med. Chem.* **36**:3129-3136.
214. Hoover, E. A., J. P. Ebner, N. S. Zeidner, and J. I. Mullins. 1991. Early therapy of feline leukemia virus infection (FeLV-FAIDS) with 9-(2-phosphorylmethoxyethyl)adenine (PMEA). *Antiviral Res.* **16**:77-92.
215. Hosoya, M., J. Balzarini, S. Shigeta, and E. De Clercq. 1991. Differential inhibitory effects of sulfated polysaccharides and polymers on the replication of various myxoviruses and retroviruses, depending on the composition of the target amino acid sequences of the viral envelope glycoproteins. *Antimicrob. Agents Chemother.* **35**:2515-2520.
216. Hsu, M.-C., U. Dhingra, J. V. Earley, M. Holly, D. Keith, C. M. Nalin, A. R. Richou, A. D. Schutt, S. Y. Tam, M. J. Potash, D. J. Volsky, and D. D. Richman. 1993. Inhibition of type 1 human immunodeficiency virus replication by a Tat antagonist to which the virus remains sensitive after prolonged exposure *in vitro*. *Proc. Natl. Acad. Sci. USA* **90**:6395-6399.
217. Hsu, M.-C., A. D. Schutt, M. Holly, L. W. Slice, M. I. Sherman, D. D. Richman, M. J. Potash, and D. J. Volsky. 1991. Inhibition of HIV replication in acute and chronic infections in vitro by a tat antagonist. *Science* **254**:1799-1802.
218. Huang, P., D. Farquhar, and W. Plunkett. 1990. Selective action of 3'-azido-3'-deoxythymidine 5'-triphosphate on viral reverse transcriptases and human DNA polymerases. *J. Biol. Chem.* **265**:11914-11918.
219. Hudson, J. B., I. Lopez-Bazzocchi, and G. H. N. Towers. 1991. Antiviral activities of hypericin. *Antiviral Res.* **15**:101-112.
220. Humber, D. C., M. J. Bamford, R. C. Bethell, N. Cammack, K. Copley, D. N. Evans, N. M. Gray, M. M. Hann, D. C. Orr, J. Saunders, B. E. V. Shenoy, R. Storer, G. G. Weingarten, and P. G. Wyatt. 1993. A series of penicillin derived C<sub>2</sub>-symmetric inhibitors of HIV-1 proteinase: synthesis, mode of interaction, and structure-activity relationship. *J. Med. Chem.* **36**:3120-3128.
221. Ikeda, S., J. Neyts, N. Yamamoto, B. Murrer, B. Theobald, G. Bossard, G. Henson, M. Abrams, D. Picker, and E. De Clercq. 1993. *In vitro* activity of a novel series of polyoxosilicotungstates against human myxo-, herpes- and retroviruses. *Antiviral Chem. Chemother.* **4**:253-262.
222. Ikeda, S., S. Nishiya, A. Yamamoto, T. Yamase, C. Nishimura, and E. De Clercq. 1994. Antiviral activity of a Keggin polyoxotungstate PM-19 against herpes simplex virus in mice. *Antiviral Chem. Chemother.* **5**:47-50.
223. Inouye, Y., Y. Tokutake, T. Yoshida, Y. Seto, H. Hujita, K. Dan, A. Yamamoto, S. Nishiya, T. Yamase, and S. Nakamura. 1993. *In vitro* antiviral activity of polyoxomolybdates. Mechanism of inhibitory effect of PM-104 (NH<sub>4</sub>)<sub>12</sub>H<sub>2</sub>(EU<sub>4</sub>(MoO<sub>4</sub>)(H<sub>2</sub>O)<sub>16</sub>(Mo<sub>7</sub>O<sub>24</sub>)<sub>4</sub>). 13H<sub>2</sub>O on human immunodeficiency virus type 1. *Antiviral Res.* **20**:317-331.
224. Ito, M., M. Baba, S. Shigeta, E. De Clercq, R. T. Walker, H. Tanaka, and T. Miyasaka. 1991. Synergistic inhibition of human immunodeficiency virus type 1 (HIV-1) replication in vitro by 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (HEPT) and recombinant alpha interferon. *Antiviral Res.* **15**:323-330.
225. Jacob, G. S. 1993. Aminosugar inhibitors as anti-HIV agents, p. 74-86. *In M. Yalpani (ed.), Carbohydrates and carbohydrate polymers, analysis, biotechnology, modification, antiviral, biomedical and other applications.* ATL Press, Mt. Prospect, Ill.
226. Jacob, G. S., P. Scudder, T. D. Butters, J. Jones, and D. C. Tiemeier. 1992. Aminosugar attenuation of HIV infection, p. 137-152. *In C. K. Chu, and H. G. Cutler (ed.), Natural products as antiviral agents.* Plenum Press, New York.
227. Jacobo-Molina, A., J. Ding, R. G. Nanni, A. D. Clark, Jr., X. Lu, C. Tantillo, R. L. Williams, G. Kamer, A. L. Ferris, P. Clark, A. Hizi, S. H. Hughes, and E. Arnold. 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA. *Proc. Natl. Acad. Sci. USA* **90**:6320-6324.
228. Jansen, R. W., G. Molema, R. Pauwels, D. Schols, E. De Clercq, and D. K. F. Meijer. 1991. Potent *in vitro* anti-human immunodeficiency virus-1 activity of modified human serum albumins. *Mol. Pharmacol.* **39**:818-823.
229. Jansen, R. W., D. Schols, R. Pauwels, E. De Clercq, and D. K. F. Meijer. 1993. Novel, negatively charged, human serum albumins display potent and selective *in vitro* anti-human immunodeficiency virus type 1 activity. *Mol. Pharmacol.* **44**:1003-1007.
230. Jentsch, K. D., G. Hunsmann, H. Hartmann, and P. Nickel. 1987. Inhibition of human immunodeficiency virus type 1 reverse transcriptase by suramin-related compounds. *J. Gen. Virol.* **68**:2183-2192.
231. Johns, D. G., G. S. Ahluwalia, D. A. Cooney, H. Mitsuya, and J. S. Driscoll. 1993. Enhanced stimulation by ribavirin of the 5'-phosphorylation and anti-human immunodeficiency virus activity of purine 2'-β-fluoro-2',3'-dideoxynucleosides. *Mol. Pharmacol.* **44**:519-523.
232. Johnson, V. A., M. A. Barlow, T.-C. Chou, R. A. Fisher, B. D. Walker, M. S. Hirsch, and R. T. Schooley. 1989. Synergistic inhibition of human immunodeficiency virus type 1 (HIV-1) replication in vitro by recombinant soluble CD4 and 3'-azido-3'-deoxythymidine. *J. Infect. Dis.* **159**:837-844.
233. Johnson, V. A., M. A. Barlow, D. P. Merrill, T.-C. Chou, and M. S. Hirsch. 1990. Three-drug synergistic inhibition of HIV-1 replication in vitro by zidovudine, recombinant soluble CD4, and recombinant interferon-alpha A. *J. Infect. Dis.* **161**:1059-1067.
234. Johnson, V. A., D. P. Merrill, J. A. Videler, T.-C. Chou, R. E. Byington, J. J. Eron, R. T. D'Aquila, and M. S. Hirsch. 1991. Two-drug combinations of zidovudine, didanosine, and recombinant interferon-α A inhibit replication of zidovudine-resistant human immunodeficiency virus type 1 synergistically in vitro. *J. Infect. Dis.* **164**:646-655.
235. Johnson, V. A., B. D. Walker, M. A. Barlow, T. J. Paradis, T.-C. Chou, and M. S. Hirsch. 1989. Synergistic inhibition of human immunodeficiency virus type 1 and type 2 replication in vitro by castanospermine and 3'-azido-3'-deoxythymidine. *Antimicrob. Agents Chemother.* **33**:53-57.
236. Johnston, M. I., and D. F. Hoth. 1993. Present status and future prospects for HIV therapies. *Science* **260**:1286-1293.
- 236a. Jonckheere, H., J.-M. Taymans, J. Balzarini, S. Velázquez, M.-J. Camarasa, J. Desmyter, E. De Clercq, and J. Anné. 1994. Resistance of HIV-1 reverse transcriptase against [2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-3'-spiro-5'-(4'-amino-1'',2''-oxathiole-2'',2''-dioxide)] (TSAO) derivatives is determined by the mutation Glu<sup>138</sup> → Lys on the p51 subunit. *J. Biol. Chem.* **269**:25255-25258.
237. Jurkiewicz, E., R. Jansen, B. Kunze, W. Trowitzsch-Kienast, E. Forche, H. Reichenbach, G. Höfle, and G. Hunsmann. 1992. Three new potent HIV-1 inhibitors from myxobacteria. *Antiviral Chem. Chemother.* **3**:189-193.
238. Kageyama, S., T. Mimoto, Y. Murakawa, M. Nomizu, H. Ford, Jr., T. Shirasaka, S. Gulnik, J. Erickson, K. Takada, H. Hayashi, S. Broder, Y. Kiso, and H. Mitsuya. 1993. In vitro anti-human immunodeficiency virus (HIV) activities of transition state mimetic HIV protease inhibitors containing allophenylnorstatine. *Antimicrob. Agents Chemother.* **37**:810-817.
239. Kageyama, S., J. N. Weinstein, T. Shirasaka, D. J. Kempf, D. W. Norbeck, J. J. Plattner, J. Erickson, and H. Mitsuya. 1992. In vitro inhibition of human immunodeficiency virus (HIV) type 1 replication by C<sub>2</sub> symmetry-based HIV protease inhibitors as single agents or in combinations. *Antimicrob. Agents Chemother.* **36**:926-933.
240. Kahn, J. O., S. W. Lagakos, D. D. Richman, A. Cross, C. Pettinelli, S.-H. Liou, M. Brown, P. A. Volberding, C. S. Crumpacker, G. Beall, H. S. Sacks, T. C. Merigan, M. Beltangady, L. Smaldone, R. Dolin, and the NIAID AIDS Clinical Trials Group. 1992. A controlled trial comparing continued zidovudine with didanosine in human immunodeficiency virus infection. *N. Engl. J. Med.* **327**:581-587.
241. Kalebic, T., A. Kinter, G. Poli, M. E. Anderson, A. Meister, and A. S. Fauci. 1991. Suppression of human immunodeficiency virus expression in chron-

- ically infected monocyctic cells by glutathione, glutathione ester, and N-acetylcysteine. *Proc. Natl. Acad. Sci. USA* **88**:986-990.
242. **Karn, J., and M. A. Graeble.** 1992. New insights into the mechanism of HIV-1 *trans*-activation. *Trends Genet.* **8**:365-368.
  243. **Karpas, A., G. W. J. Fleet, R. A. Dwek, S. Petrusson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob, and T. W. Rademacher.** 1988. Aminosugar derivatives as potential anti-human immunodeficiency virus agents. *Proc. Natl. Acad. Sci. USA* **85**:9229-9233.
  244. **Karpas, A., M. Lowdell, S. K. Jacobson, and F. Hill.** 1992. Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK506. *Proc. Natl. Acad. Sci. USA* **89**:8351-8355.
  245. **Kashman, Y., K. R. Gustafson, R. W. Fuller, J. H. Cardellina, J. B. McMahon, M. J. Currens, R. W. Buckheit, Jr., S. H. Hughes, G. M. Cragg, and M. R. Boyd.** 1992. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. *J. Med. Chem.* **35**:2735-2743.
  246. **Kawasaki, A. M., M. D. Casper, S. M. Freier, E. A. Lesnik, M. C. Zounes, L. L. Cummins, C. Gonzalez, and P. D. Cook.** 1993. Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets. *J. Med. Chem.* **36**:831-841.
  247. **Kellam, P., C. A. B. Boucher, and B. A. Larder.** 1992. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. *Proc. Natl. Acad. Sci. USA* **89**:1934-1938.
  248. **Kellam, P., C. A. B. Boucher, J. M. G. H. Tijnagel, and B. A. Larder.** 1994. Zidovudine treatment results in the selection of human immunodeficiency virus type 1 variants whose genotypes confer increasing levels of drug resistance. *J. Gen. Virol.* **75**:341-351.
  249. **Kempf, D. J., K. C. Marsh, D. A. Paul, M. F. Knigge, D. W. Norbeck, W. E. Kohlbrenner, L. Codacovi, S. Vasavanonda, P. Bryant, X. C. Wang, N. E. Wideburg, J. J. Clement, J. J. Plattner, and J. Erickson.** 1991. Antiviral and pharmacokinetic properties of C<sub>2</sub> symmetric inhibitors of the human immunodeficiency virus type 1 protease. *Antimicrob. Agents Chemother.* **35**:2209-2214.
  250. **Kempf, D. J., D. W. Norbeck, L. M. Codacovi, X. C. Wang, W. E. Kohlbrenner, N. E. Wideburg, D. A. Paul, M. F. Knigge, S. Vasavanonda, A. Craig-Kennard, A. Saldivar, W. Rosenbrook, Jr., J. J. Clement, J. J. Plattner, and J. Erickson.** 1990. Structure-based, C<sub>2</sub> symmetric inhibitors of HIV protease. *J. Med. Chem.* **33**:2687-2689.
  251. **Kleim, J.-P., R. Bender, U.-M. Billhardt, C. Meichsner, G. Riess, M. Rösner, I. Winkler, and A. Paessens.** 1993. Activity of a novel quinoxaline derivative against human immunodeficiency virus type 1 reverse transcriptase and viral replication. *Antimicrob. Agents Chemother.* **37**:1659-1664.
  252. **Kleim, J.-P., R. Bender, R. Kirsch, C. Meichsner, A. Paessens, and G. Riess.** 1994. Mutational analysis of residue 190 of human immunodeficiency virus type 1 reverse transcriptase. *Virology* **200**:696-701.
  253. **Kleinert, H. D., S. H. Rosenberg, W. R. Baker, H. H. Stein, V. Klinghofer, J. Barlow, K. Spina, J. Polakowski, P. Kovar, J. Cohen, and J. Denissen.** 1992. Discovery of a peptide-based renin inhibitor with oral bioavailability and efficacy. *Science* **257**:1940-1943.
  254. **Kohl, N. E., E. A. Emini, W. A. Schleif, L. J. Davis, J. C. Heimbach, R. A. F. Dixon, E. M. Scolnick, and I. S. Sigal.** 1988. Active human immunodeficiency virus protease is required for viral infectivity. *Proc. Natl. Acad. Sci. USA* **85**:4686-4690.
  255. **Kohlstaedt, L. A., J. Wang, J. M. Friedman, R. A. Rice, and T. A. Steitz.** 1992. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **256**:1783-1790.
  256. **Koizumi, N., H. Sakagami, A. Utsumi, S. Fujinaga, M. Takeda, K. Asano, I. Sugawara, S. Ichikawa, H. Kondo, S. Mori, K. Miyatake, Y. Nakano, H. Nakashima, T. Murakami, N. Miyano, and N. Yamamoto.** 1993. Anti-HIV (human immunodeficiency virus) activity of sulfated paramylon. *Antiviral Res.* **21**:1-14.
  257. **Kong, X.-B., Q.-Y. Zhu, R. M. Ruprecht, K. A. Watanabe, J. M. Zeidler, J. W. M. Gold, B. Polsky, D. Armstrong, and T.-C. Chou.** 1991. Synergistic inhibition of human immunodeficiency virus type 1 replication *in vitro* by two-drug and three-drug combinations of 3'-azido-3'-deoxythymidine, phosphonoformate, and 2',3'-dideoxythymidine. *Antimicrob. Agents Chemother.* **35**:2003-2011.
  258. **Kopp, E. B., J. J. Miglietta, A. G. Shrutkowski, C.-K. Shih, P. M. Grob, and M. T. Skoog.** 1991. Steady state kinetics and inhibition of HIV-1 reverse transcriptase by a non-nucleoside dipyrindiazepinone, BI-RG-587, using a heteropolymeric template. *Nucleic Acids Res.* **19**:3035-3039.
  259. **Kort, J. J., J. A. Bilello, G. Bauer, and G. L. Drusano.** 1993. Preclinical evaluation of antiviral activity and toxicity of Abbott A77003, an inhibitor of the human immunodeficiency virus type 1 protease. *Antimicrob. Agents Chemother.* **37**:115-119.
  260. **Koshida, R., S. Cox, J. Harmenberg, G. Gilljam, and B. Wahren.** 1989. Structure-activity relationships of fluorinated nucleoside analogs and their synergistic effect in combination with phosphonoformate against human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **33**:2083-2088.
  261. **Koshida, R., L. Vrang, G. Gilljam, J. Harmenberg, B. Öberg, and B. Wahren.** 1989. Inhibition of human immunodeficiency virus *in vitro* by combinations of 3'-azido-3'-deoxythymidine and foscarnet. *Antimicrob. Agents Chemother.* **33**:778-780.
  262. **Koup, R. A., V. J. Merluzzi, K. D. Hargrave, J. Adams, K. Grozinger, R. J. Eckner, and J. L. Sullivan.** 1991. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by the dipyrindiazepinone BI-RG-587. *J. Infect. Dis.* **163**:966-970.
  263. **Kozlowski, M. R., and A. Watson.** 1992. Inhibition of gp120 binding to the CD4 antigen by dyes: mechanism of effect and contribution to anti-HIV activity. *Antiviral Chem. Chemother.* **3**:49-53.
  - 263a. **Lacey, S. F., and B. A. Larder.** 1994. Novel mutation (V75T) in human immunodeficiency virus type 1 reverse transcriptase confers resistance to 2',3'-dideoxy-2',3'-dideoxythymidine in cell culture. *Antimicrob. Agents Chemother.* **38**:1428-1432.
  264. **Lai, M.-H. T., J. Tang, V. Wroblewski, A. G. Dee, N. Margolin, C. Vlahos, B. Bowdon, R. Buckheit, J. Colacino, and K. Y. Hui.** 1993. Impeded progression of Friend disease in mice by an inhibitor of retroviral proteases. *J. Acquired Immune Defic. Syndr.* **6**:24-31.
  265. **Lam, P. Y. S., P. K. Jadhav, C. J. Eyermann, C. N. Hodge, Y. Ru, L. T. Bachelier, J. L. Meek, M. J. Otto, M. M. Rayner, Y. N. Wong, C.-H. Chang, P. C. Weber, D. A. Jackson, T. R. Sharpe, and S. Erickson-Viitanen.** 1994. Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. *Science* **263**:380-384.
  266. **Lambert, D. M., S. R. Petteway, Jr., C. E. McDaniel, T. K. Hart, J. J. Leary, G. B. Dreyer, T. D. Meek, P. J. Bugelski, D. P. Bolognesi, B. W. Metcalf, and T. J. Matthews.** 1992. Human immunodeficiency virus type 1 protease inhibitors irreversibly block infectivity of purified virions from chronically infected cells. *Antimicrob. Agents Chemother.* **36**:982-988.
  267. **Land, S., C. McGavin, R. Lucas, and C. Birch.** 1992. Incidence of zidovudine-resistant human immunodeficiency virus isolated from patients before, during, and after therapy. *J. Infect. Dis.* **166**:1139-1142.
  268. **Langner, K.-D., M. Niedrig, P. Fultz, D. Anderson, G. Reiner, H. Repke, H. Gelderblom, B. Seed, J. Hilfenhaus, and G. Zettlmeissl.** 1993. Antiviral effects of different CD4-immunoglobulin constructs against HIV-1 and SIV: immunological characterization, pharmacokinetic data and *in vivo* experiments. *Arch. Virol.* **130**:157-170.
  269. **Langtry, H. D., and D. M. Campoli-Richards.** 1989. Zidovudine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* **37**:408-450.
  270. **Larder, B. A.** 1992. 3'-Azido-3'-deoxythymidine resistance suppressed by a mutation conferring human immunodeficiency virus type 1 resistance to nonnucleoside reverse transcriptase inhibitors. *Antimicrob. Agents Chemother.* **36**:2664-2669.
  271. **Larder, B. A., K. E. Coates, and S. D. Kemp.** 1991. Zidovudine-resistant human immunodeficiency virus selected by passage in cell culture. *J. Virol.* **65**:5232-5236.
  272. **Larder, B. A., G. Darby, and D. D. Richman.** 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**:1731-1734.
  273. **Larder, B. A., P. Kellam, and S. D. Kemp.** 1993. Convergent combination therapy can select viable multidrug-resistant HIV-1 *in vitro*. *Nature (London)* **365**:451-453.
  274. **Larder, B. A., and S. D. Kemp.** 1989. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science* **246**:1155-1158.
  275. **Lasarte, J. J., P. Sarobe, J. Golvano, I. Prieto, M. P. Civeira, A. Gullón, P. S. Sarin, J. Prieto, and F. Borrás-Cuesta.** 1994. CD4-modified synthetic peptides containing phenylalanine inhibit HIV-1 infection *in vitro*. *J. Acquired Immune Defic. Syndr.* **7**:129-134.
  276. **Lavie, G., F. Valentine, B. Levin, Y. Mazur, G. Gallo, D. Lavie, D. Weiner, and D. Meruelo.** 1989. Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. *Proc. Natl. Acad. Sci. USA* **86**:5963-5967.
  277. **Lee-Huang, S., P. L. Huang, H.-F. Kung, B.-Q. Li, P. L. Huang, P. Huang, H. I. Huang, and H.-C. Chen.** 1991. TAP 29: an anti-human immunodeficiency virus protein from *Trichosanthes kirilowii* that is nontoxic to intact cells. *Proc. Natl. Acad. Sci. USA* **88**:6570-6574.
  278. **Lenard, J., A. Rabson, and R. Vanderoef.** 1993. Photodynamic inactivation of infectivity of human immunodeficiency virus and other enveloped viruses using hypericin and rose bengal: inhibition of fusion and syncytia formation. *Proc. Natl. Acad. Sci. USA* **90**:158-162.
  279. **Li, C. J., L. J. Zhang, B. J. Dezube, C. S. Crumpacker, and A. B. Pardee.** 1993. Three inhibitors of type 1 human immunodeficiency virus long terminal repeat-directed gene expression and virus replication. *Proc. Natl. Acad. Sci. USA* **90**:1839-1842.
  280. **Lin, T.-S., M.-Z. Luo, M.-C. Liu, S. B. Pai, G. E. Dutschman, and Y.-C. Cheng.** 1994. Antiviral activity of 2',3'-dideoxy-β-L-5-fluorocytidine (β-L-FddC) and 2',3'-dideoxy-β-L-cytidine (β-L-ddC) against hepatitis B virus and human immunodeficiency virus type 1 *in vitro*. *Biochem. Pharmacol.* **47**:171-174.

281. Lin, T.-S., R. F. Schinazi, M. S. Chen, E. Kinney-Thomas, and W. H. Prusoff. 1987. Antiviral activity of 2',3'-dideoxycytidin-2'-ene (2',3'-dideoxy-2',3'-didehydrocytidine) against human immunodeficiency virus *in vitro*. *Biochem. Pharmacol.* **36**:311-316.
282. Lin, T.-S., R. F. Schinazi, B. P. Griffith, E. M. August, B. F. H. Eriksson, D.-K. Zheng, L. Huang, and W. H. Prusoff. 1989. Selective inhibition of human immunodeficiency virus type 1 replication by the (-) but not the (+) enantiomer of gossypol. *Antimicrob. Agents Chemother.* **33**:2149-2151.
283. Lin, T.-S., R. F. Schinazi, and W. H. Prusoff. 1987. Potent and selective *in vitro* activity of 3'-deoxythymidin-2'-ene (3'-deoxy-2',3'-didehydrothymidine) against human immunodeficiency virus. *Biochem. Pharmacol.* **36**:2713-2718.
284. Lin, T.-S., R. F. Schinazi, J. Zhu, E. Birks, R. Carbone, Y. Si, K. Wu, L. Huang, and W. H. Prusoff. 1993. Anti-HIV-1 activity and cellular pharmacology of various analogs of gossypol. *Biochem. Pharmacol.* **46**:251-255.
285. Lisiewicz, J., D. Sun, M. Klotman, S. Agrawal, P. Zamecnik, and R. Gallo. 1992. Specific inhibition of human immunodeficiency virus type 1 replication by antisense oligonucleotides: an *in vitro* model for treatment. *Proc. Natl. Acad. Sci. USA* **89**:11209-11213.
286. Livermore, D. G. H., R. C. Bethell, N. Cammack, A. P. Hancock, M. M. Hann, D. V. S. Green, R. B. Lamont, S. A. Noble, D. C. Orr, J. J. Payne, M. V. J. Ramsay, A. H. Shingler, C. Smith, R. Storer, C. Williamson, and T. Willson. 1993. Synthesis and anti-HIV-1 activity of a series of imidazo[1,5-*b*]pyridazines. *J. Med. Chem.* **36**:3784-3794.
287. Lo, K. M. S., M. A. Biasolo, G. Dehni, G. Palú, and W. A. Haseltine. 1992. Inhibition of replication of HIV-1 by retroviral vectors expressing *tat*-antisense and anti-*tat* ribozyme RNA. *Virology* **190**:176-183.
288. Lorentsen, K. J., C. W. Hendrix, J. M. Collins, D. M. Kornhauser, B. G. Petty, R. W. Klecker, C. Flexner, R. H. Eckel, and P. S. Lietman. 1989. Dextran sulfate is poorly absorbed after oral administration. *Ann. Intern. Med.* **111**:561-566.
- 288a. Lori, F., A. Malykh, A. Cara, D. Sun, J. N. Weinstein, J. Lisiewicz, and R. C. Gallo. 1994. Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. *Science* **266**:801-805.
289. Loya, S., and A. Hizi. 1990. The inhibition of human immunodeficiency virus type 1 reverse transcriptase by avarol and avarone derivatives. *FEBS Lett.* **269**:131-134.
290. Loya, S., and A. Hizi. 1993. The interaction of illimaquinone, a selective inhibitor of the RNase H activity, with the reverse transcriptases of human immunodeficiency and murine leukemia retroviruses. *J. Biol. Chem.* **268**:9323-9328.
291. Loya, S., R. Tal, Y. Kashman, and A. Hizi. 1990. Illimaquinone, a selective inhibitor of the RNase H activity of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **34**:2009-2012.
292. Lyle, T. A., C. M. Wiscourt, J. P. Guare, W. J. Thompson, P. S. Anderson, P. L. Darke, J. A. Zugay, E. A. Emin, W. A. Schleif, J. C. Quintero, R. A. F. Dixon, I. S. Sigal, and J. R. Huff. 1991. Benzocycloalkyl amines as novel C-termini for HIV protease inhibitors. *J. Med. Chem.* **34**:1228-1230.
- 292a. Lynch, G., L. Low, S. Li, A. Sloane, S. Adams, C. Parish, B. Kemp, and A. L. Cunningham. 1994. Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD4-gp120 interaction, but do not inhibit monocyte infection. *J. Leukocyte Biol.* **56**:266-272.
- 292b. Maag, H., J. T. Nelson, J. L. Rios Steiner, and E. J. Prisbe. 1994. Solid-state and solution conformations of the potent HIV inhibitor, 4'-azidothymidine. *J. Med. Chem.* **37**:431-438.
293. Maass, G., U. Immendorfer, B. Koenig, U. Leser, B. Mueller, R. Goody, and E. Pfaff. 1993. Viral resistance to the thiazolo-iso-indolinones, a new class of nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **37**:2612-2617.
294. Mahmood, N., P. S. Moore, N. De Tommasi, F. De Simone, S. Colman, A. J. Hay, and C. Pizarro. 1993. Inhibition of HIV infection by caffeoylquinic acid derivatives. *Antiviral Chem. Chemother.* **4**:235-240.
295. Mahmood, N., C. Pizarro, R. Aquino, N. De Tommasi, S. Piacente, S. Colman, A. Burke, and A. J. Hay. 1993. Inhibition of HIV infection by flavanoids. *Antiviral Res.* **22**:189-199.
296. Mahoney, C. W., A. Azzi, and K.-P. Huang. 1990. Effects of suramin, an anti-human immunodeficiency virus reverse transcriptase agent, on protein kinase C. *J. Biol. Chem.* **265**:5424-5428.
- 296a. Malley, S. D., J. M. Grange, F. Hamed-Sangsari, and J. R. Vila. 1994. Synergistic anti-human immunodeficiency virus type 1 effect of hydroxamate compounds with 2',3'-dideoxyinosine in infected resting human lymphocytes. *Proc. Natl. Acad. Sci. USA* **91**:11017-11021.
297. Margolis, D. M., J. M. Ostrove, and S. E. Straus. 1993. HSV-1 activation of HIV-1 transcription is augmented by a cellular protein that binds near the initiator element. *Virology* **192**:370-374.
298. Marshall, W. S., and M. H. Caruthers. 1993. Phosphordithioate DNA as a potential therapeutic drug. *Science* **259**:1564-1570.
299. Mathes, L. E., K. A. Hayes, C. L. Swenson, P. J. Polas, S. E. Weisbrode, and G. J. Kociba. 1991. Evaluation of antiviral activity and toxicity of dextran sulfate in feline leukemia virus-infected cats. *Antimicrob. Agents Chemother.* **35**:2147-2150.
300. Matsui, T., S. Kobayashi, O. Yoshida, S.-I. Ishii, Y. Abe, and N. Yamamoto. 1990. Effects of succinylated concanavalin A on infectivity and syncytial formation of human immunodeficiency virus. *Med. Microb. Immunol.* **179**:225-235.
301. Matsukura, M., K. Shinozuka, G. Zon, H. Mitsuya, M. Reitz, J. S. Cohen, and S. Broder. 1987. Phosphorothioate analogs of oligodeoxynucleotides: inhibitors of replication and cytopathic effects of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **84**:7706-7710.
302. Matsukura, M., G. Zon, K. Shinozuka, M. Robert-Guroff, T. Shimada, C. A. Stein, H. Mitsuya, F. Wong-Staal, J. S. Cohen, and S. Broder. 1989. Regulation of viral expression of human immunodeficiency virus *in vitro* by an antisense phosphorothioate oligodeoxynucleotide against *rev* (*art/hrs*) in chronically infected cells. *Proc. Natl. Acad. Sci. USA* **86**:4244-4248.
303. Mayaux, J.-F., A. Bousseau, R. Pauwels, T. Huet, Y. Hélin, N. Dereu, M. Evers, F. Soler, C. Poujade, E. De Clercq, and J.-B. Le Pecq. 1994. Triterpene derivatives that block entry of human immunodeficiency virus type 1 into cells. *Proc. Natl. Acad. Sci. USA* **91**:3564-3568.
304. Mayers, D. L., A. J. Japour, J.-M. Arduino, S. M. Hammer, R. Reichman, K. F. Wagner, R. Chung, J. Lane, C. S. Crumpacker, G. X. McLeod, L. A. Beckett, C. R. Roberts, D. Winslow, D. Burke, and the RV43 Study Group. 1994. Dideoxynucleoside resistance emerges with prolonged zidovudine monotherapy. *Antimicrob. Agents Chemother.* **38**:307-314.
305. Mayers, D. L., F. E. McCutchan, E. E. Sanders-Buell, L. I. Merritt, S. Dilworth, A. K. Fowler, C. A. Marks, N. M. Ruiz, D. Richman, C. R. Roberts, and D. S. Burke. 1992. Characterization of HIV isolates arising after prolonged zidovudine therapy. *J. Acquired Immune Defic. Syndr.* **5**:749-759.
306. Mazzulli, T., S. Rusconi, D. P. Merrill, R. T. D'Aquila, M. Moonis, T.-C. Chou, and M. S. Hirsch. 1994. Alternating versus continuous drug regimens in combination chemotherapy of human immunodeficiency virus type 1 infection *in vitro*. *Antimicrob. Agents Chemother.* **38**:656-661.
307. McGrath, M. S., K. M. Hwang, S. E. Caldwell, I. Gaston, K.-C. Luk, P. Wu, V. L. Ng, S. Crowe, J. Daniels, J. Marsh, T. Deinhardt, P. V. Lekas, J. C. Vennari, H.-W. Yeung, and J. D. Lifson. 1989. GLQ223: an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. *Proc. Natl. Acad. Sci. USA* **86**:2844-2848.
308. McGuigan, C., R. N. Pathirana, J. Balzarini, and E. De Clercq. 1993. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.* **36**:1048-1052.
309. McMahon, J. B., R. J. Gulakowski, O. S. Weislow, R. J. Schultz, V. L. Narayanan, D. J. Clanton, R. Pedemonte, F. W. Wassmundt, R. W. Buckheit, Jr., W. D. Decker, E. L. White, J. P. Bader, and M. R. Boyd. 1993. Diarylsulfones, a new chemical class of nonnucleoside antiviral inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **37**:754-760.
310. McQuade, T. J., A. G. Tomasselli, L. Liu, V. Karacostas, B. Moss, T. K. Sawyer, R. L. Heinrikson, and W. G. Tarpley. 1990. A synthetic HIV-1 protease inhibitor with antiviral activity arrests HIV-like particle maturation. *Science* **247**:454-456.
311. McShan, W. M., R. D. Rossen, A. H. Laughter, J. Trial, D. J. Kessler, J. G. Zenguei, M. E. Hogan, and F. M. Orson. 1992. Inhibition of transcription of HIV-1 in infected human cells by oligodeoxynucleotides designed to form DNA triple helices. *J. Biol. Chem.* **267**:5712-5721.
312. Medina, D. J., G. D. Hsiung, and J. W. Mellors. 1992. Ganciclovir antagonizes the anti-human immunodeficiency virus type 1 activity of zidovudine and didanosine *in vitro*. *Antimicrob. Agents Chemother.* **36**:1127-1130.
313. Mellors, J. W., G. E. Dutschman, G.-J. Im, E. Tramontano, S. R. Winkler, and Y.-C. Cheng. 1992. *In vitro* selection and molecular characterization of human immunodeficiency virus-1 resistant to non-nucleoside inhibitors of reverse transcriptase. *Mol. Pharmacol.* **41**:446-451.
314. Mellors, J. W., G.-J. Im, E. Tramontano, S. R. Winkler, D. J. Medina, G. E. Dutschman, H. Z. Bazmi, G. Piras, C. J. Gonzalez, and Y.-C. Cheng. 1993. A single conservative amino acid substitution in the reverse transcriptase of human immunodeficiency virus-1 confers resistance to (+)-(5S)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione (TIBO R82150). *Mol. Pharmacol.* **43**:11-16.
315. Meng, T.-C., M. A. Fischl, A. M. Boota, S. A. Spector, D. Bennett, Y. Bassiakos, S. Lai, B. Wright, and D. D. Richman. 1992. Combination therapy with zidovudine and dideoxycytidine in patients with advanced human immunodeficiency virus infection. *Ann. Intern. Med.* **116**:13-20.
316. Mergny, J. L., G. Duval-Valentin, C. H. Nguyen, L. Perrouault, B. Faucon, M. Rougée, T. Montenay-Garestier, E. Bisagni, and C. Hélène. 1992. Triple helix-specific ligands. *Science* **256**:1681-1684.
317. Merluzzi, V. J., K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C.-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosenthal, R. Faanes, R. J. Eckner, R. A. Koup, and J. L. Sullivan. 1990. Inhibition of HIV-1 replication by a non-nucleoside reverse transcriptase inhibitor. *Science* **250**:1411-1413.
318. Mertens, A., H. Zilch, B. König, W. Schäfer, T. Poll, W. Kampe, H. Seidel, U. Leser, and H. Leinert. 1993. Selective non-nucleoside HIV-1 reverse transcriptase inhibitors. New 2,3-dihydrothiazolo[2,3-*a*]isoindol-5-(9*bH*)-ones and related compounds with anti-HIV-1 activity. *J. Med. Chem.* **36**:2526-2535.

319. Meruelo, D., G. Lavie, and D. Lavie. 1988. Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. *Proc. Natl. Acad. Sci. USA* **85**:5230-5234.
320. Meylan, P. R. A., J. C. Guatelli, J. R. Munis, D. D. Richman, and R. S. Kornbluth. 1993. Mechanisms for the inhibition of HIV replication by interferons- $\alpha$ , - $\beta$  and - $\gamma$  in primary human macrophages. *Virology* **193**:138-148.
321. Michne, W. F., J. D. Schroeder, T. R. Bailey, D. C. Young, J. V. Hughes, and F. J. Dutko. 1993. Keto/enol epoxy steroids: a new structural class of HIV-1 Tat inhibitors. *J. Med. Chem.* **36**:2701-2702.
322. Milligan, J. F., M. D. Matteucci, and J. C. Martin. 1993. Current concepts in antisense drug design. *J. Med. Chem.* **36**:1923-1937.
323. Mitsuya, H., and S. Broder. 1986. Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* **83**:1911-1915.
324. Mitsuya, H., and S. Broder. 1987. Strategies for antiviral therapy in AIDS. *Nature (London)* **325**:773-778.
325. Mitsuya, H., M. Popovic, R. Yarchoan, S. Matsushita, R. C. Gallo, and S. Broder. 1984. Suramin protection of T cells *in vitro* against infectivity and cytopathic effect of HTLV-III. *Science* **226**:172-174.
326. Mitsuya, H., K. J. Weinhold, P. A. Furman, M. St. Clair, S. Nusinoff Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder. 1985. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. USA* **82**:7096-7100.
327. Mitsuya, H., R. Yarchoan, and S. Broder. 1990. Molecular targets for AIDS therapy. *Science* **249**:1533-1544.
328. Miyasaka, T., H. Tanaka, M. Baba, H. Hayakawa, R. T. Walker, J. Balzarini, and E. De Clercq. 1989. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J. Med. Chem.* **32**:2507-2509.
329. Moelling, K., T. Schulze, and H. Diringer. 1989. Inhibition of human immunodeficiency virus type 1 RNase H by sulfated polyanions. *J. Virol.* **63**:5489-5491.
330. Mohan, P., A. J. Hopfinger, and M. Baba. 1991. Naphthalenesulfonic acid derivatives as potential anti-HIV-1 agents. Chemistry, biology and molecular modelling of their inhibition of reverse transcriptase. *Antiviral Chem. Chemother.* **2**:215-222.
- 330a. Mohan, P., S. Loya, O. Avidan, S. Verma, G. S. Dhindsa, M. F. Wong, P. P. Huang, M. Yashiro, M. Baba, and A. Hizi. 1994. Synthesis of naphthalenesulfonic acid small molecules as selective inhibitors of the DNA polymerase and ribonuclease H activities of HIV-1 reverse transcriptase. *J. Med. Chem.* **37**:2513-2519.
331. Mohan, P., D. Schols, M. Baba, and E. De Clercq. 1992. Sulfonic acid polymers as a new class of human immunodeficiency virus inhibitors. *Antiviral Res.* **18**:139-150.
332. Mohan, P., R. Singh, and M. Baba. 1991. Anti-HIV-1 and HIV-2 activity of naphthalenedisulfonic acid derivatives. Inhibition of cytopathogenesis, giant cell formation, and reverse transcriptase activity. *Biochem. Pharmacol.* **41**:642-646.
333. Mohan, P., R. Singh, and M. Baba. 1991. Potential anti-AIDS agents. Synthesis and antiviral activity of naphthalenesulfonic acid derivatives against HIV-1 and HIV-2. *J. Med. Chem.* **34**:212-217.
334. Mohan, P., R. Singh, J. Wepsiec, I. Gonzalez, D. K. Sun, and P. S. Sarin. 1990. Inhibition of HIV replication by naphthalenesulfonic acid derivatives and a bis naphthalenedisulfonic acid compound. *Life Sci.* **47**:993-999.
335. Mohan, P., M. F. Wong, S. Verma, P. P. Huang, A. Wickramasinghe, and M. Baba. 1993. Structure-activity relationship studies with symmetric naphthalenesulfonic acid derivatives. Synthesis and influence of spacer and naphthalenesulfonic acid moiety on anti-HIV-1 activity. *J. Med. Chem.* **36**:1996-2003.
336. Mohri, H., M. K. Singh, W. T. W. Ching, and D. D. Ho. 1993. Quantitation of zidovudine-resistant human immunodeficiency virus type 1 in the blood of treated and untreated patients. *Proc. Natl. Acad. Sci. USA* **90**:25-29.
337. Montefiori, D. C., W. E. Robinson, Jr., and W. M. Mitchell. 1988. Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **85**:9248-9252.
338. Moriya, T., H. Kurita, K. Matsumoto, T. Otake, H. Mori, M. Morimoto, N. Ueba, and N. Kunita. 1991. Potent inhibitory effect of a series of modified cyclodextrin sulfates (mCDS) on the replication of HIV-1 *in vitro*. *J. Med. Chem.* **34**:2301-2304.
339. Moriya, T., K. Saito, H. Kurita, K. Matsumoto, T. Otake, H. Mori, M. Morimoto, N. Ueba, and N. Kunita. 1993. A new candidate for an anti-HIV-1 agent: modified cyclodextrin sulfate (mCDS71). *J. Med. Chem.* **36**:1674-1677.
340. Müller, W. E. G., K. Renneisen, M. H. Kreuter, H. C. Schröder, and I. Winkler. 1988. The D-mannose-specific lectin from *Gerardia savaglia* blocks binding of human immunodeficiency virus type 1 to H9 cells and human lymphocytes *in vitro*. *J. Acquired Immune Defic. Syndr.* **1**:453-458.
341. Müller, W. E. G., B. E. Weiler, R. Charubala, W. Pfeleiderer, L. Leserman, R. W. Sobol, R. J. Suhadolnik, and H. C. Schröder. 1991. Cordycepin analogues of 2',5'-oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. *Biochemistry* **30**:2027-2033.
342. Naesens, L., J. Balzarini, and E. De Clercq. 1991. Single-dose administration of 9-(2-phosphorylmethoxyethyl)adenine (PMEA) and 9-(2-phosphorylmethoxyethyl)-2,6-diaminopurine (PMEDAP) in the prophylaxis of retrovirus infection *in vivo*. *Antiviral Res.* **16**:53-64.
343. Nakamura, S., S. Sakurada, S. Z. Salahuddin, Y. Osada, N. G. Tanaka, N. Sakamoto, M. Sekiguchi, and R. C. Gallo. 1992. Inhibition of development of Kaposi's sarcoma-related lesions by a bacterial cell wall complex. *Science* **255**:1437-1440.
344. Nakane, H., M. Arisawa, A. Fujita, S. Koshimura, and K. Ono. 1991. Inhibition of HIV-reverse transcriptase activity by some phloroglucinol derivatives. *FEBS Lett.* **286**:83-85.
345. Nakane, H., and K. Ono. 1990. Differential inhibitory effects of some catechin derivatives on the activities of human immunodeficiency virus reverse transcriptase and cellular deoxyribonucleic and ribonucleic acid polymerases. *Biochemistry* **29**:2841-2845.
346. Nakashima, H., M. Masuda, T. Murakami, Y. Koyanagi, A. Matsumoto, N. Fujii, and N. Yamamoto. 1992. Anti-human immunodeficiency virus activity of a novel synthetic peptide, T22 ([Tyr-5,12, Lys-7]polyphemusin II): a possible inhibitor of virus-cell fusion. *Antimicrob. Agents Chemother.* **36**:1249-1255.
347. Nakashima, H., T. Matsui, O. Yoshida, Y. Isowa, Y. Kido, Y. Motoki, M. Ito, S. Shigetate, T. Mori, and N. Yamamoto. 1987. A new anti-human immunodeficiency virus substance, glycyrrhizin sulfate: endowment of glycyrrhizin with reverse transcriptase-inhibitory activity by chemical modification. *Jpn. J. Cancer Res.* **78**:767-771.
348. Nanni, R. G., J. Ding, A. Jacobo-Molina, S. H. Hughes, and E. Arnold. 1993. Review of HIV-1 reverse transcriptase three-dimensional structure: implications for drug design. *Perspect. Drug Discovery Design* **1**:129-150.
349. Nasr, M., C. Litterst, and J. McGowan. 1990. Computer-assisted structure-activity correlations of dideoxynucleoside analogs as potential anti-HIV drugs. *Antiviral Res.* **14**:125-148.
350. Navia, M. A., P. M. D. Fitzgerald, B. M. McKeever, C.-T. Leu, J. C. Heimbach, W. K. Herber, I. S. Sigal, P. L. Darke, and J. P. Springer. 1989. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature (London)* **337**:615-620.
351. Neurath, A. R., P. Haberfield, B. Joshi, I. K. Hewlett, N. Strick, and S. Jiang. 1991. Rapid prescreening for antiviral agents against HIV-1 based on their inhibitory activity in site-directed immunoassays. I. The V3 loop of gp120 as target. *Antiviral Chem. Chemother.* **2**:303-312.
352. Newlander, K. A., J. F. Callahan, M. L. Moore, T. A. Tomaszek, Jr., and W. F. Huffman. 1993. A novel constrained reduced-amide inhibitor of HIV-1 protease derived from the sequential incorporation of  $\gamma$ -turn mimetics into a model substrate. *J. Med. Chem.* **36**:2321-2331.
353. Neyts, J., R. Snoeck, P. Wutzler, M. Cushman, R. Klöcking, B. Helbig, P. Wang, and E. De Clercq. 1992. Poly(hydroxy)carboxylates as selective inhibitors of cytomegalovirus and herpes simplex virus replication. *Antiviral Chem. Chemother.* **3**:215-222.
354. Nozaki-Renard, J., T. Iino, Y. Sato, Y. Marumoto, G. Ohta, and M. Furu-sawa. 1990. Fluoroquinolones protect the human lymphocyte CEM cell lines from HIV-1-mediated cytotoxicity. *Cell Struct. Funct.* **15**:295-299.
355. Nunberg, J. H., W. A. Schleif, E. J. Boots, J. A. O'Brien, J. C. Quintero, J. M. Hoffman, E. A. Emimi, and M. E. Goldman. 1991. Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. *J. Virol.* **65**:4887-4892.
356. Offensperger, W.-B., S. Offensperger, E. Walter, H. E. Blum, and W. Gerok. 1991. Sulfated polyanions do not inhibit duck hepatitis B virus infection. *Antimicrob. Agents Chemother.* **35**:2431-2433.
- 356a. Ojwang, J., A. Elbaggari, H. B. Marshall, K. Jayaraman, M. S. McGrath, and R. F. Rando. 1994. Inhibition of human immunodeficiency virus type 1 activity *in vitro* by oligonucleotides composed entirely of guanosine and thymidine. *J. Acquired Immune Defic. Syndr.* **7**:560-570.
357. Okada, T., B. K. Patterson, S.-Q. Ye, and M. E. Gurney. 1993. Aurothiols inhibit HIV-1 infectivity by gold(I) ligand exchange with a component of the virion surface. *Virology* **192**:631-642.
358. Otake, T., K. Miyano, H. Mori, M. Morimoto, N. Ueba, N. Kunita, H. Nakashima, and T. Kurimura. 1991. Anti-HIV-1 activity of sulfated amphotericin B *in vitro*. *Antiviral Res.* **16**:243-255.
359. Otake, T., D. Schols, M. Witvrouw, L. Naesens, H. Nakashima, T. Moriya, H. Kurita, K. Matsumoto, N. Ueba, and E. De Clercq. 1994. Modified cyclodextrin sulfates (mCDS11) have potent inhibitory activity against HIV and high oral bioavailability. *Antiviral Chem. Chemother.* **5**:155-161.
360. Otto, M. J., S. Garber, D. L. Winslow, C. D. Reid, P. Aldrich, P. K. Jadhav, C. E. Patterson, C. N. Hodge, and Y.-S. E. Cheng. 1993. *In vitro* isolation and identification of human immunodeficiency virus (HIV) variants with reduced sensitivity to C-2 symmetrical inhibitors of HIV type 1 protease. *Proc. Natl. Acad. Sci. USA* **90**:7543-7547.
361. Otto, M. J., C. D. Reid, S. Garber, P. Y.-S. Lam, H. Scarnati, L. T. Bacheler, M. M. Rayner, and D. L. Winslow. 1993. *In vitro* anti-human immunode-

- iciency virus (HIV) activity of XM323, a novel HIV protease inhibitor. *Antimicrob. Agents Chemother.* **37**:2606–2611.
362. **Pal, R., V. S. Kalyanaraman, G. M. Hoke, and M. G. Sarngadharan.** 1989. Processing and secretion of envelope glycoproteins of human immunodeficiency virus type 1 in the presence of trimming glucosidase inhibitor deoxyojirimycin. *Intervirology* **30**:27–35.
  363. **Pal, R., S. Mumbauer, G. Hoke, R. Larocca, C. Myers, M. G. Sarngadharan, and C. A. Stein.** 1991. Effect of Evans blue and trypan blue on syncytia formation and infectivity of human immunodeficiency virus type I and type II *in vitro*. *AIDS Res. Hum. Retroviruses* **7**:537–543.
  364. **Patel, M., M. Yanagishita, G. Roderiquez, D. C. Bou-Habib, T. Oravec, V. C. Hascall, and M. A. Norcross.** 1993. Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res. Hum. Retroviruses* **9**:167–174.
  365. **Patil, A. D., A. J. Freyer, D. S. Eggleston, R. C. Haltiwanger, M. F. Bean, P. B. Taylor, M. J. Caranfa, A. L. Breen, H. R. Bartus, R. K. Johnson, R. P. Hertzberg, and J. W. Westley.** 1993. The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, *Calophyllum inophyllum* Linn. *J. Med. Chem.* **36**:4131–4138.
  366. **Pätzold, S., J. Schneider, C. Rudolph, D. Marmé, and C. Schächtele.** 1993. Novel indolocarbazole protein kinase C inhibitors prevent reactivation of HIV-1 in latently infected cells. *Antiviral Res.* **22**:273–283.
  367. **Pauwels, R., K. Andries, Z. Debyser, M. J. Kukla, D. Schols, H. J. Breslin, R. Woestenborghs, J. Desmyter, M. A. C. Janssen, E. De Clercq, and P. A. J. Janssen.** 1994. New TIBO derivatives are potent inhibitors of HIV-1 replication and are synergistic with 2',3'-dideoxynucleoside analogs. Submitted for publication.
  368. **Pauwels, R., K. Andries, Z. Debyser, P. Van Daele, D. Schols, P. Stoffels, K. De Vreese, R. Woestenborghs, A.-M. Vandamme, C. G. M. Janssen, J. Anné, G. Cauwenbergh, J. Desmyter, J. Heykants, M. A. C. Janssen, E. De Clercq, and P. A. J. Janssen.** 1993. Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of  $\alpha$ -anilino-phenylacetamide derivatives targeted at HIV-1 reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **90**:1711–1715.
  369. **Pauwels, R., K. Andries, J. Desmyter, D. Schols, M.-J. Kukla, H. J. Breslin, A. Raeymaeckers, J. Van Gelder, R. Woestenborghs, J. Heykants, H. Schellekens, M. A. C. Janssen, E. De Clercq, and P. A. J. Janssen.** 1990. Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. *Nature (London)* **343**:470–474.
  370. **Pauwels, R., J. Balzarini, D. Schols, M. Baba, J. Desmyter, I. Rosenberg, A. Huly, and E. De Clercq.** 1988. Phosphonylmethoxyethyl purine derivatives, a new class of anti-human immunodeficiency virus agents. *Antimicrob. Agents Chemother.* **32**:1025–1030.
  371. **Pauwels, R., M. P. de Béthune, K. Andries, P. Stoffels, J. Desmyter, P. A. J. Janssen, and E. De Clercq.** 1994. Effects of combinations of HIV-1 specific RT inhibitors with complementary resistance profiles on the time and type of drug-resistant HIV-1 development. abstr. 140. Abstracts of the Seventh International Conference on Antiviral Research, Charleston, South Carolina, 27 February–4 March, 1994. *Antiviral Res.* **23**(Suppl. 1):109.
  372. **Peters, M., M. Witvrouw, E. De Clercq, and B. Ruf.** 1991. Pharmacokinetics of intravenous pentosan polysulphate (HOE/BAY 946) in HIV-positive patients. *AIDS* **5**:1534–1535.
  373. **Pialoux, G., M. Youle, B. Dupont, B. Gazzard, G. F. M. J. Cauwenbergh, P. A. M. Stoffels, S. Davies, J. De Saint Martin, and P. A. J. Janssen.** 1991. Pharmacokinetics of R82913 in patients with AIDS or AIDS-related complex. *Lancet* **338**:140–143.
  374. **Pieken, W. A., D. B. Olsen, F. Benseler, H. Aurup, and F. Eckstein.** 1991. Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. *Science* **253**:314–317.
  375. **Pluda, J. M., L. E. Shay, A. Foli, S. Tannenbaum, P. J. Cohen, B. R. Goldspiel, D. Adamo, M. R. Cooper, S. Broder, and R. Yarchoan.** 1993. Administration of pentosan polysulfate to patients with human immunodeficiency virus-associated Kaposi's sarcoma. *J. Natl. Cancer Inst.* **85**:1585–1592.
  376. **Poli, G., J. M. Orenstein, A. Kinter, T. M. Folks, and A. S. Fauci.** 1989. Interferon- $\alpha$  but not AZT suppresses HIV expression in chronically infected cell lines. *Science* **244**:575–577.
  377. **Popik, W., and P. M. Pitha.** 1992. Transcriptional activation of the tat-defective human immunodeficiency virus type-1 provirus: effect of interferon. *Virology* **189**:435–447.
  378. **Prochaska, H. J., Y. Yeh, P. Baron, and B. Polsky.** 1993. Olitpraz, an inhibitor of human immunodeficiency virus type 1 replication. *Proc. Natl. Acad. Sci. USA* **90**:3953–3957.
  379. **Puech, F., G. Gosselin, I. Lefebvre, A. Pompon, A.-M. Aubertin, A. Kirn, and J.-L. Imbach.** 1993. Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process. *Antiviral Res.* **22**:155–174.
  380. **Puglisi, J. D., R. Tan, B. J. Calnan, A. D. Frankel, and J. R. Williamson.** 1992. Conformation of the TAR RNA-arginine complex by NMR spectroscopy. *Science* **257**:76–80.
  381. **Pulliam, L., B. G. Herndier, and M. S. McGrath.** 1991. Purified trichosanthin (GLQ223) exacerbation of indirect HIV-associated neurotoxicity *in vitro*. *AIDS* **5**:1237–1242.
  382. **Qatsha, K. A., C. Rudolph, D. Marmé, C. Schächtele, and W. S. May.** 1993. Gö 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells *in vitro*. *Proc. Natl. Acad. Sci. USA* **90**:4674–4678.
  383. **Ratner, L., N. Vander Heyden, and D. Dederá.** 1991. Inhibition of HIV and SIV infectivity by blockage of  $\alpha$ -glucosidase activity. *Virology* **181**:180–192.
  384. **Rausch, D. M., J. D. Lifson, M. P. Padgett, B. Chandrasekhar, J. Lendvay, K. M. Hwang, and L. E. Eiden.** 1992. CD4(81-92)-based peptide derivatives. Structural requirements for blockade of HIV infection, blockade of HIV-induced syncytium formation, and virostatic activity *in vitro*. *Biochem. Pharmacol.* **43**:1785–1796.
  385. **Rice, W. G., C. A. Schaeffer, B. Harten, F. Villinger, T. L. South, M. F. Summers, L. E. Henderson, J. W. Bess Jr., L. O. Arthur, J. S. McDougal, S. L. Orloff, J. Mendeleyev, and E. Kun.** 1993. Inhibition of HIV-1 infectivity by zinc-ejecting aromatic C-nitroso compounds. *Nature (London)* **361**:473–475.
  386. **Rich, D. H., C.-Q. Sun, J. V. N. Vara Prasad, A. Pathiaseril, M. V. Toth, G. R. Marshall, M. Clare, R. A. Mueller, and K. Houseman.** 1991. Effect of hydroxyl group configuration in hydroxyethylamine dipeptide isosteres on HIV protease inhibition. Evidence for multiple binding modes. *J. Med. Chem.* **34**:1222–1225.
  387. **Richman, D., A. S. Rosenthal, M. Skoog, R. J. Eckner, T.-C. Chou, J. P. Sabo, and V. J. Merluzzi.** 1991. BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. *Antimicrob. Agents Chemother.* **35**:305–308.
  388. **Richman, D., C.-K. Shih, I. Lowy, J. Rose, P. Prodanovich, S. Goff, and J. Griffin.** 1991. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. *Proc. Natl. Acad. Sci. USA* **88**:11241–11245.
  389. **Richman, D. D., T.-C. Meng, S. A. Spector, M. A. Fischl, L. Resnick, and S. Lai.** 1994. Resistance to AZT and ddC during long-term combination therapy in patients with advanced infection with human immunodeficiency virus. *J. Acquired Immune Defic. Syndr.* **7**:135–138.
  390. **Rittner, K., and G. Sczakiel.** 1991. Identification and analysis of antisense RNA target regions of the human immunodeficiency virus type 1. *Nucleic Acids Res.* **19**:1421–1426.
  391. **Roberts, N. A., J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay, A. Kröhn, R. W. Lambert, J. H. Merrett, J. S. Mills, K. E. B. Parkes, S. Redshaw, A. J. Ritchie, D. L. Taylor, G. J. Thomas, and P. J. Machin.** 1990. Rational design of peptide-based HIV proteinase inhibitors. *Science* **248**:358–361.
  392. **Romero, D. L., M. Busso, C.-K. Tan, F. Reusser, J. R. Palmer, S. M. Poppe, P. A. Aristoff, K. M. Downey, A. G. So, L. Resnick, and W. G. Tarpley.** 1991. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. *Proc. Natl. Acad. Sci. USA* **88**:8806–8810.
  393. **Romero, D. L., R. A. Morge, M. J. Genin, C. Biles, M. Busso, L. Resnick, I. W. Althaus, F. Reusser, R. C. Thomas, and W. G. Tarpley.** 1993. Bis (heteroaryl)piperazine (BHAP) reverse transcriptase inhibitors: structure-activity relationships of novel substituted indole analogues and the identification of 1-[5-(methanesulfonamido-1*H*-indol-2-yl)carbonyl]-4-[3-[(1-methylethyl)amino]pyridinyl]piperazine monomethanesulfonate (U-90152S), a second-generation clinical candidate. *J. Med. Chem.* **36**:1505–1508.
  - 393a. **Rosenwirth, B., A. Billich, R. Datema, P. Donatsch, F. Hammerschmid, R. Harrison, P. Hiestand, H. Jaksche, P. Mayer, P. Peichl, V. Quesniaux, F. Schatz, H.-J. Schuurman, R. Traber, R. Wenger, B. Wolf, G. Zenke, and M. Zurini.** 1994. Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM 811, a nonimmunosuppressive cyclosporine analog. *Antimicrob. Agents Chemother.* **38**:1763–1772.
  394. **Rossi, J. J., E. M. Cantin, N. Sarver, and P. F. Chang.** 1991. The potential use of catalytic RNAs in therapy of HIV infection and other diseases. *Pharmacol. Ther.* **50**:245–254.
  395. **Rossi, J. J., D. Elkins, J. A. Zaia, and S. Sullivan.** 1992. Ribozymes as anti-HIV-1 therapeutic agents: principles, applications, and problems. *AIDS Res. Hum. Retroviruses* **8**:183–189.
  396. **Ruprecht, R. M., L. D. Bernard, R. Bronson, M. A. Gama Sosa, and S. Mullaney.** 1991. Castanospermine vs. its 6-*O*-butanoyl analog: a comparison of toxicity and antiviral activity *in vitro* and *in vivo*. *J. Acquired Immune Defic. Syndr.* **4**:48–55.
  397. **Ruprecht, R. M., S. Mullaney, J. Andersen, and R. Bronson.** 1989. *In vivo* analysis of castanospermine, a candidate antiretroviral agent. *J. Acquired Immune Defic. Syndr.* **2**:149–157.
  398. **Ruprecht, R. M., L. D. Rossoni, W. A. Haseltine, and S. Broder.** 1985. Suppression of retroviral propagation and disease by suramin in murine systems. *Proc. Natl. Acad. Sci. USA* **82**:7733–7737.
  399. **Saag, M. S., E. A. Emini, O. L. Laskin, J. Douglas, W. I. Lapidus, W. A. Schleif, R. J. Whitley, C. Hildebrand, V. W. Byrnes, J. C. Kappes, K. W. Anderson, F. E. Massari, G. M. Shaw, and the L-697,661 Working Group.** 1993. A short term clinical evaluation of L-697,661, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. *N. Engl. J. Med.* **329**:1065–1072.
  400. **Sardana, V. V., E. A. Emini, L. Gotlib, D. J. Graham, D. W. Lineberger,**

- W. J. Long, A. J. Schlabach, J. A. Wolfgang, and J. H. Condra. 1992. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple nonnucleoside inhibitors. *J. Biol. Chem.* **267**:17526-17530.
401. Sarin, P. S., S. Agrawal, M. P. Civeira, J. Goodchild, T. Ikeuchi, and P. C. Zamecnik. 1988. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. *Proc. Natl. Acad. Sci. USA* **85**:7448-7451.
402. Sarin, P. S., D. Sun, A. Thornton, and W. E. G. Müller. 1987. Inhibition of replication of the etiologic agent of acquired immune deficiency syndrome (human T-lymphotropic retrovirus/lymphadenopathy-associated virus) by avarol and avarone. *J. Natl. Cancer Inst.* **78**:663-666.
403. Sarver, N., E. M. Cantin, P. S. Chang, J. A. Zaia, P. A. Ladne, D. A. Stephens, and J. J. Rossi. 1990. Ribozymes as potential anti-HIV-1 therapeutic agents. *Science* **247**:1222-1225.
404. Schäfer, W., W.-G. Friebe, H. Leinert, A. Mertens, T. Poll, W. Von Der Saal, H. Zilch, B. Nuber, and M. L. Ziegler. 1993. Non-nucleoside inhibitors of HIV-1 reverse transcriptase: molecular modeling and X-ray structure investigations. *J. Med. Chem.* **36**:726-732.
405. Schinazi, R. F., F. D. Boudinot, S. S. Ibrahim, C. Manning, H. M. McClure, and D. C. Liotta. 1992. Pharmacokinetics and metabolism of racemic 2',3'-dideoxy-5-fluoro-3'-thiacytidine in rhesus monkeys. *Antimicrob. Agents Chemother.* **36**:2432-2438.
406. Schinazi, R. F., C. K. Chu, A. Peck, A. McMillan, R. Mathis, D. Cannon, L.-S. Jeong, J. W. Beach, W.-B. Choi, S. Yeola, and D. C. Liotta. 1992. Activities of the four optical isomers of 2',3'-dideoxy-3'-thiacytidine (BCH-189) against human immunodeficiency virus type 1 in human lymphocytes. *Antimicrob. Agents Chemother.* **36**:672-676.
407. Schinazi, R. F., R. M. Lloyd, Jr., M.-H. Nguyen, D. L. Cannon, A. McMillan, N. Ilksoy, C. K. Chu, D. C. Liotta, H. Z. Bazmi, and J. W. Mellors. 1993. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrob. Agents Chemother.* **37**:875-881.
408. Schinazi, R. F., R. M. Lloyd, Jr., C. S. Ramanathan, and E. W. Taylor. 1994. Antiviral drug resistance mutations in human immunodeficiency virus type 1 reverse transcriptase occur in specific RNA structural regions. *Antimicrob. Agents Chemother.* **38**:268-274.
409. Schinazi, R. F., A. McMillan, D. Cannon, R. Mathis, R. M. Lloyd, A. Peck, J.-P. Sommadossi, M. St. Clair, J. Wilson, P. A. Furman, G. Painter, W.-B. Choi, and D. C. Liotta. 1992. Selective inhibition of human immunodeficiency viruses by racemates and enantiomers of *cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. *Antimicrob. Agents Chemother.* **36**:2423-2431.
410. Schinazi, R. F., J. R. Mead, and P. M. Feorino. 1992. Insights into HIV chemotherapy. *AIDS Res. Hum. Retroviruses* **8**:963-990.
411. Schinazi, R. F., R. Sijbesma, G. Srdanov, C. L. Hill, and F. Wudl. 1993. Synthesis and virucidal activity of a water-soluble configurationally stable, derivatized C<sub>60</sub> fullerene. *Antimicrob. Agents Chemother.* **37**:1707-1710.
412. Schols, D., M. Baba, R. Pauwels, and E. De Clercq. 1989. Flow cytometric method to demonstrate whether anti-HIV-1 agents inhibit virion binding to T4<sup>+</sup> cells. *J. Acquired Immune Defic. Syndr.* **2**:10-15.
413. Schols, D., M. Baba, R. Pauwels, J. Desmyter, and E. De Clercq. 1989. Specific interaction of aurantricarboxylic acid with the human immunodeficiency virus/CD4 cell receptor. *Proc. Natl. Acad. Sci. USA* **86**:3322-3326.
414. Schols, D., E. De Clercq, J. Balzarini, M. Baba, M. Witvrouw, H. Hosoya, G. Andrei, R. Snoeck, J. Neyts, R. Pauwels, M. Nagy, J. Györgyi-Edelényi, R. Machovich, I. Horváth, M. Löw, and S. Görög. 1990. Sulphated polymers are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, respiratory syncytial virus, and toga-, arena- and retroviruses. *Antiviral Chem. Chemother.* **1**:233-240.
415. Schols, D., E. De Clercq, M. Witvrouw, H. Nakashima, R. Snoeck, R. Pauwels, A. Van Schepdael, and P. Claes. 1991. Sulphated cyclodextrins are potent anti-HIV agents acting synergistically with 2',3'-dideoxynucleoside analogues. *Antiviral Chem. Chemother.* **2**:45-53.
416. Schols, D., R. Pauwels, M. Witvrouw, J. Desmyter, and E. De Clercq. 1992. Differential activity of polyanionic compounds and castanospermine against HIV replication and HIV-induced syncytium formation depending on virus strain and cell type. *Antiviral Chem. Chemother.* **3**:23-29.
417. Schols, D., P. Wutzler, R. Klöcking, B. Helbig, and E. De Clercq. 1991. Selective inhibitory activity of polyhydroxycarboxylates derived from phenolic compounds against human immunodeficiency virus replication. *J. Acquired Immune Defic. Syndr.* **4**:677-685.
418. Shapira-Nahor, O., H. Golding, L. K. Vujcic, S. Resto-Ruiz, R. L. Fields, and F. A. Robey. 1990. CD4-Derived synthetic peptide blocks the binding of HIV-1 gp120 to CD4-bearing cells and prevents HIV-1 infection. *Cell. Immunol.* **128**:101-117.
419. Shibuya, H., K. Irie, J. Ninomiya-Tsuji, M. Goebel, T. Taniguchi, and K. Matsumoto. 1992. New human gene encoding a positive modulator of HIV Tat-mediated transactivation. *Nature (London)* **357**:700-702.
420. Shih, C.-K., J. M. Rose, G. L. Hansen, J. C. Wu, A. Bacolla, and J. A. Griffin. 1991. Chimeric human immunodeficiency virus type 1/type 2 reverse transcriptase display reversed sensitivity to nonnucleoside analog inhibitors. *Proc. Natl. Acad. Sci. USA* **88**:9878-9882.
421. Shimizu, H., H. Tsuchie, K. Yoshida, S. Morikawa, T. Tsuruoka, H. Yamamoto, H. Ushijima, and T. Kitamura. 1990. Inhibitory effect of novel 1-deoxynojirimycin derivatives on HIV-1 replication. *AIDS* **4**:975-979.
422. Shirazi, Y., and P. M. Pitha. 1993. Interferon  $\alpha$ -mediated inhibition of human immunodeficiency virus type 1 provirus synthesis in T-cells. *Virology* **193**:303-312.
423. Sijbesma, R., G. Srdanov, F. Wudl, J. A. Castoro, C. Wilkins, S. H. Friedman, D. L. DeCamp, and G. L. Kenyon. 1993. Synthesis of a fullerene derivative for the inhibition of HIV enzymes. *J. Am. Chem. Soc.* **115**:6510-6512.
424. Sioud, M., and K. Drlica. 1991. Prevention of human immunodeficiency virus type 1 integrase expression in *Escherichia coli* by a ribozyme. *Proc. Natl. Acad. Sci. USA* **88**:7303-7307.
425. Smith, M. S., E. L. Brian, E. De Clercq, and J. S. Pagano. 1989. Susceptibility of human immunodeficiency virus type 1 replication in vitro to acyclic adenosine analogs and synergy of the analogs with 3'-azido-3'-deoxythymidine. *Antimicrob. Agents Chemother.* **33**:1482-1486.
426. Smith, M. S., E. L. Brian, and J. S. Pagano. 1987. Resumption of virus production after human immunodeficiency virus infection of T lymphocytes in the presence of azidothymidine. *J. Virol.* **61**:3769-3773.
427. Smith, M. S., and J. S. Pagano. 1991. Inhibition of human immunodeficiency virus type 1 replication by guanosine analogues and lack of synergistic antiviral effect of acyclovir with 3'-azido-3'-deoxythymidine. *Antiviral Chem. Chemother.* **2**:29-34.
428. Smith, M. S., R. J. Thresher, and J. S. Pagano. 1991. Inhibition of human immunodeficiency virus type 1 morphogenesis in T cells by alpha interferon. *Antimicrob. Agents Chemother.* **35**:62-67.
429. Soudeyns, H., X.-J. Yao, Q. Gao, B. Belleau, J.-L. Kraus, N. Nguyen-Ba, B. Spira, and M. A. Wainberg. 1991. Anti-human immunodeficiency virus type 1 activity and in vitro toxicity of 2'-deoxy-3-thiacytidine (BCH-189), a novel heterocyclic nucleoside analog. *Antimicrob. Agents Chemother.* **35**:1386-1390.
430. Spector, S. A., D. Ripley, and K. Hsia. 1989. Human immunodeficiency virus inhibition is prolonged by 3'-azido-3'-deoxythymidine alternating with 2',3'-dideoxycytidine compared with 3'-azido-3'-deoxythymidine alone. *Antimicrob. Agents Chemother.* **33**:920-923.
431. Srinivas, R. V., B. L. Robbins, M. C. Connelly, Y.-F. Gong, N. Bischofberger, and A. Fridland. 1993. Metabolism and in vitro antiretroviral activities of bis(pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates. *Antimicrob. Agents Chemother.* **37**:2247-2250.
432. Starrett, J. E., Jr., D. R. Tortolani, M. J. M. Hitchcock, J. C. Martin, and M. M. Mansuri. 1992. Synthesis and in vitro evaluation of a phosphonate prodrug: bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine. *Antiviral Res.* **19**:267-273.
433. St. Clair, M. H., J. L. Martin, G. Tudor-Williams, M. C. Bach, C. L. Vavro, D. M. King, P. Kellam, S. D. Kemp, and B. A. Larder. 1991. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* **253**:1557-1559.
434. St. Clair, M. H., C. A. Richards, T. Spector, K. J. Weinhold, W. H. Miller, A. J. Langlois, and P. A. Furman. 1987. 3'-Azido-3'-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. *Antimicrob. Agents Chemother.* **31**:1972-1977.
435. Stevenson, N. R., and J. Lenard. 1993. Antiretroviral activities of hypericin and rose bengal: photodynamic effects on Friend leukemia virus infection of mice. *Antiviral Res.* **21**:119-127.
436. Sullenger, B. A., and T. R. Cech. 1993. Tethering ribozymes to a retroviral packaging signal for destruction of viral RNA. *Science* **262**:1566-1569.
437. Tachedjian, G., D. Tyssen, D. Jardine, S. Locarnini, and C. Birch. 1992. Synergistic inhibition of human immunodeficiency virus type 1 *in vitro* by interferon alpha and coumestrolin A1. *Antiviral Chem. Chemother.* **3**:183-188.
438. Takahashi, I., S. Nakanishi, E. Kobayashi, H. Nakano, K. Suzuki, and T. Tamaoki. 1989. Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their antiretroviral activity. *Biochem. Biophys. Res. Commun.* **165**:1207-1212.
439. Take, Y., Y. Tokutake, Y. Inouye, T. Yoshida, A. Yamamoto, T. Yamase, and S. Nakamura. 1991. Inhibition of proliferation of human immunodeficiency virus type 1 by novel heteropolyoxotungstates in vitro. *Antiviral Res.* **15**:113-124.
440. Tamura, Y., P. K. Lai, W. G. Bradley, K. Konno, A. Tanaka, and M. Nonoyama. 1991. A soluble factor induced by an extract from *Pinus parviflora* Sieb et Zucc can inhibit the replication of human immunodeficiency virus *in vitro*. *Proc. Natl. Acad. Sci. USA* **88**:2249-2253.
441. Tan, C.-K., R. Civil, A. M. Mian, A. G. So, and K. M. Downey. 1991. Inhibition of the RNase H activity of HIV reverse transcriptase by azidothymidylate. *Biochemistry* **30**:4831-4835.
442. Tan, G. T., A. D. Kinghorn, S. H. Hughes, and J. M. Pezzuto. 1991. Psychotrine and its *O*-methyl ether are selective inhibitors of human immunodeficiency virus-1 reverse transcriptase. *J. Biol. Chem.* **266**:23529-23536.

443. **Tanabe-Tochikura, A., H. Nakashima, T. Murakami, O. Tenmyo, T. Oki, and N. Yamamoto.** 1992. Anti-human immunodeficiency virus (HIV) activity of the novel antiviral antibiotic quartromicins which enhance inhibitory effect of 3'-azido-2',3'-dideoxythymidine (AZT) *in vitro*. *Antiviral Chem. Chemother.* **3**:345-349.
444. **Tanabe-Tochikura, A., T. S. Tochikura, O. Yoshida, T. Oki, and N. Yamamoto.** 1990. Pradimicin A inhibition of human immunodeficiency virus: attenuation by mannan. *Virology* **176**:467-473.
445. **Tang, J., J. M. Colacino, S. H. Larsen, and W. Spitzer.** 1990. Virucidal activity of hypericin enveloped and non-enveloped DNA and RNA viruses. *Antiviral Res.* **13**:313-326.
446. **Tang, J. Y., J. Tamsamani, and S. Agrawal.** 1993. Self-stabilized antisense oligodeoxynucleotide phosphorothioates: properties and anti-HIV activity. *Nucleic Acids Res.* **21**:2729-2735.
- 446a. **Tantillo, C., J. Ding, A. Jacobo-Molina, R. G. Nanni, P. L. Boyer, S. H. Hughes, R. Pauwels, K. Andries, P. A. J. Janssen, and E. Arnold.** 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. *J. Mol. Biol.* **243**:369-387.
447. **Tashiro, A., S. Shoji, and Y. Kubota.** 1989. Antimyrystoylation of the gag proteins in the human immunodeficiency virus-infected cells with N-myristoyl glycolic diethylacetal resulted in inhibition of virus production. *Biochem. Biophys. Res. Commun.* **165**:1145-1154.
448. **Ternansky, R. J., J. M. Morin, Jr., C. Lopez, C. J. Paget, Jr., F. W. Bell, A. S. Cantrell, S. R. Jaskunas, C. L. Jordan, M. D. Kinnick, J. A. Palkowitz, C. A. Parrish, P. Pranc, R. T. Vasileff, S. J. West, M. Hogberg, P. Lind, R. Noreen, C. Sahlberg, X. X. Zhou, L. Vrang, C. Rydergard, C. Ahgren, B. Öberg, and N. G. Johansson.** 1993. The discovery and general SAR studies of a novel class of potent non-nucleoside reverse transcriptase inhibitors, abstr. 43. Abstracts of the Sixth International Conference on Antiviral Research, Venice, Italy, April 25-30, 1993. *Antiviral Res.* **20**(Suppl. 1):68.
449. **Terrett, N. K., D. Bojanic, J. R. Merson, and P. T. Stephenson.** 1992. Imidazo[2',3':6,5]dipyrido[3,2-b:2',3'-e]-1,4-diazepines: non-nucleoside HIV-1 reverse transcriptase inhibitors with greater enzyme affinity than nevirapine. *Bioorg. Med. Chem. Lett.* **2**:1745-1750.
450. **Thaisrivongs, S., S. R. Turner, J. W. Strohbach, R. E. TenBrink, W. G. Tarpley, T. J. McQuade, R. L. Heinrichson, A. G. Tomasselli, J. O. Hui, and W. J. Howe.** 1993. Inhibitors of the protease from human immunodeficiency virus: synthesis, enzyme inhibition, and antiviral activity of a series of compounds containing the dihydroxyethylene transition-state isostere. *J. Med. Chem.* **36**:941-952.
451. **Thormar, H., J. Balzarini, A. Holy, J. Jindrich, I. Rosenberg, Z. Debyser, J. Desmyter, and E. De Clercq.** 1993. Inhibition of visna virus replication by 2',3'-dideoxynucleosides and acyclic nucleoside phosphonate analogs. *Antimicrob. Agents Chemother.* **37**:2540-2544.
452. **Tisdale, M., S. D. Kemp, N. R. Parry, and B. A. Larder.** 1993. Rapid *in vitro* selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **90**:5653-5656.
453. **Tochikura, T. S., H. Nakashima, and N. Yamamoto.** 1989. Antiviral agents with activity against human retroviruses. *J. Acquired Immune Defic. Syndr.* **2**:441-447.
454. **Tomasselli, A. G., J. O. Hui, T. K. Sawyer, D. J. Staples, C. Bannow, I. M. Reardon, J. Howe, D. L. DeCamp, C. S. Craik, and R. L. Heinrichson.** 1990. Specificity and inhibition of proteases from human immunodeficiency viruses 1 and 2. *J. Biol. Chem.* **265**:14675-14683.
455. **Tramontano, E., and Y.-C. Cheng.** 1992. HIV-1 reverse transcriptase inhibition by a dipyrindodiazepinone derivative: BI-RG-587. *Biochem. Pharmacol.* **43**:1371-1376.
456. **Tsai, C.-C., K. E. Follis, A. Sabo, R. F. Grant, C. Bartz, R. E. Nolte, R. E. Benveniste, and N. Bischofberger.** 1994. Preexposure prophylaxis with 9-(2-phosphonylmethoxyethyl)adenine against simian immunodeficiency virus infection in macaques. *J. Infect. Dis.* **169**:260-266.
457. **Tsuchihashi, Z., M. Khosla, and D. Herschlag.** 1993. Protein enhancement of hammerhead ribozyme catalysis. *Science* **262**:99-102.
- 457a. **Tucker, T. J., T. A. Lyle, C. M. Wiscourt, S. F. Britcher, S. D. Young, W. M. Sanders, W. C. Lumma, M. E. Goldman, J. A. O'Brien, R. G. Ball, C. F. Homnick, W. A. Schleif, E. A. Emini, J. R. Huff, and P. S. Anderson.** 1994. Synthesis of a series of 4-(arylethynyl)-6-chloro-4-cyclopropyl-3,4-dihydroquinazolin-2(1H)-ones as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. *J. Med. Chem.* **37**:2437-2444.
458. **Tudor-Williams, G., and V. C. Emery.** 1992. Development of *in vitro* resistance to zidovudine is likely to be clinically significant. *Rev. Med. Virol.* **2**:123-129.
459. **Tuerk, C., S. MacDougall, and L. Gold.** 1992. RNA pseudoknots that inhibit human immunodeficiency virus type 1 reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **89**:6988-6992.
460. **Tung, F. Y.-T., and M. D. Daniel.** 1993. Targeted inhibition of immunodeficiency virus replication in lymphocytes through retroviral mediated gene transfer. *Arch. Virol.* **133**:407-421.
461. **Uryu, T., N. Ikushima, K. Katsuraya, T. Shoji, N. Takahashi, T. Yoshida, K. Kanno, T. Murakami, H. Nakashima, and N. Yamamoto.** 1992. Sulfated alkyl oligosaccharides with potent inhibitory effects on human immunodeficiency virus infection. *Biochem. Pharmacol.* **43**:2385-2392.
462. **Vacca, J. P., J. P. Guare, S. J. deSolms, W. M. Sanders, E. A. Giuliani, S. D. Young, P. L. Darke, J. Zugay, I. S. Sigal, W. A. Schleif, J. C. Quintero, E. A. Emini, P. S. Anderson, and J. R. Huff.** 1991. L-687,908, a potent hydroxyethylene-containing HIV protease inhibitor. *J. Med. Chem.* **34**:1225-1228.
463. **Van Aerschoot, A., P. Herdewijn, J. Balzarini, R. Pauwels, and E. De Clercq.** 1989. 3'-Fluoro-2',3'-dideoxy-5-chlorouridine: most selective anti-HIV-1 agent among a series of new 2'- and 3' fluorinated 2',3'-dideoxynucleoside analogues. *J. Med. Chem.* **32**:1743-1749.
464. **Vandamme, A.-M., Z. Debyser, R. Pauwels, K. De Vreese, P. Goubau, M. Youle, B. Gazzard, P. A. Stoffels, G. F. Cauwenbergh, J. Anné, K. Andries, P. A. J. Janssen, J. Desmyter, and E. De Clercq.** 1994. Characterization of HIV-1 strains isolated from patients treated with TIBO R82913. *AIDS Res. Hum. Retroviruses* **10**:39-46.
465. **van Roey, P., W. A. Pangborn, R. F. Schinazi, G. Painter, and D. C. Liotta.** 1993. Absolute configuration of the antiviral agent (-)-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. *Antiviral Chem. Chemother.* **4**:369-375.
466. **Vasudevachari, M. B., C. Battista, H. C. Lane, M. C. Psallidopoulos, B. Zhao, J. Cook, J. R. Palmer, D. L. Romero, W. G. Tarpley, and N. P. Salzman.** 1992. Prevention of the spread of HIV-1 infection with non-nucleoside reverse transcriptase inhibitors. *Virology* **190**:269-277.
467. **Vink, C., D. C. van Gent, Y. Elgersma, and R. H. A. Plasterk.** 1991. Human immunodeficiency virus integrase protein requires a subterminal position of its viral DNA recognition sequence for efficient cleavage. *J. Virol.* **65**:4636-4644.
468. **Vogt, M. W., A. G. Durno, T.-C. Chou, L. A. Coleman, T. J. Paradis, R. T. Schooley, J. C. Kaplan, and M. S. Hirsch.** 1988. Synergistic interaction of 2',3'-dideoxycytidine and recombinant interferon- $\alpha$ -A on replication of human immunodeficiency virus type 1. *J. Infect. Dis.* **158**:378-385.
469. **Vogt, M. W., K. L. Hartshorn, P. A. Furman, T.-C. Chou, J. A. Fyfe, L. A. Coleman, C. Crumpacker, R. T. Schooley, and M. S. Hirsch.** 1987. Ribavirin antagonizes the effect of azidothymidine on HIV replication. *Science* **235**:1376-1379.
470. **Walker, B. D., M. Kowalski, W. C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W. A. Haseltine, and J. Sodroski.** 1987. Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc. Natl. Acad. Sci. USA* **84**:8120-8124.
471. **Ward, R. H. R., D. J. Capon, C. M. Jett, K. K. Murthy, J. Mordenti, C. Lucas, S. W. Frie, A. M. Prince, J. D. Green, and J. W. Eichberg.** 1991. Prevention of HIV-1 IIB infection in chimpanzees by CD4 immunoadhesin. *Nature (London)* **352**:434-436.
472. **Weaver, J. L., P. Gergely, P. S. Pine, E. Patzer, and A. Aszalos.** 1990. Polyionic compounds selectively alter availability of CD4 receptors for HIV coat protein gp120. *AIDS Res. Hum. Retroviruses* **6**:1125-1130.
473. **Weaver, J. L., P. S. Pine, R. Anand, S. Bell, and A. Aszalos.** 1992. Inhibition of the binding of HIV gp120 to CD4 by dyes. *Antiviral Chem. Chemother.* **3**:147-151.
474. **Weaver, J. L., P. S. Pine, G. Dutschman, Y.-C. Cheng, K.-H. Lee, and A. Aszalos.** 1992. Prevention of binding of gp120 by anti-HIV active tannins. *Biochem. Pharmacol.* **43**:2479-2480.
475. **Weeks, M. S., C. L. Hill, and R. F. Schinazi.** 1992. Synthesis, characterization, and anti-human immunodeficiency virus activity of water-soluble salts of polyoxotungstate anions with covalently attached organic groups. *J. Med. Chem.* **35**:1216-1221.
476. **Weerasinghe, M., S. E. Liem, S. Asad, S. E. Read, and S. Joshi.** 1991. Resistance to human immunodeficiency virus type 1 (HIV-1) infection in human CD4<sup>+</sup> lymphocyte-derived cell lines conferred by using retroviral vectors expressing an HIV-1 RNA-specific ribozyme. *J. Virol.* **65**:5531-5534.
477. **Weiler, B. E., H. C. Schröder, V. Stefanovich, D. Stewart, J. M. S. Forrest, L. B. Allen, B. J. Bowden, M. H. Kreuter, R. Voth, and W. E. G. Müller.** 1990. Sulphoeverman, a polyanionic polysaccharide, and the narcissus lectin potentially inhibit human immunodeficiency virus infection by binding to viral envelope protein. *J. Gen. Virol.* **71**:1957-1963.
478. **White, E. L., R. W. Buckheit, Jr., L. J. Ross, J. M. Germany, K. Andries, R. Pauwels, P. A. J. Janssen, W. M. Shannon, and M. A. Chirigos.** 1991. A TIBO derivative, R82913, is a potent inhibitor of HIV-1 reverse transcriptase with heteropolymer templates. *Antiviral Res.* **16**:257-266.
479. **Whittington, R., and R. N. Brogden.** 1992. Zalcitabine. A review of its pharmacology and clinical potential in acquired immunodeficiency syndrome (AIDS). *Drugs* **44**:656-683.
480. **Williams, T. M., T. M. Ciccarone, S. C. MacTough, C. S. Rooney, S. K. Balani, J. H. Condra, E. A. Emini, M. E. Goldman, W. J. Greenlee, L. R. Kauffman, J. A. O'Brien, V. V. Sardana, W. A. Schleif, A. D. Theoharides, and P. S. Anderson.** 1993. 5-Chloro-3-(phenylsulfonyl)indole-2-carboxamide: a novel, non-nucleoside inhibitor of HIV-1 reverse transcriptase. *J. Med. Chem.* **36**:1291-1294.
481. **Wilson, J. E., J. L. Martin, K. Borroto-Esoda, S. Hopkins, G. Painter, D. C. Liotta, and P. A. Furman.** 1993. The 5'-triphosphates of the (-) and (+) enantiomers of *cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolane-5-yl]cy-

- tosine equally inhibit human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **37**:1720–1722.
482. **Winkler, D. A., and G. Holan.** 1989. Design of potential anti-HIV agents. 1. Mannosidase inhibitors. *J. Med. Chem.* **32**:2084–2089.
483. **Witvrouw, M., R. Pauwels, A.-M. Vandamme, D. Schols, D. Reymen, N. Yamamoto, J. Desmyter, and E. De Clercq.** 1992. Cell type-specific anti-human immunodeficiency virus type 1 activity of the transactivation inhibitor Ro5-3335. *Antimicrob. Agents Chemother.* **36**:2628–2633.
484. **Witvrouw, M., D. Schols, G. Andrei, R. Snoeck, S. Ikeda, R. Pauwels, A. Van Schepdael, J. Arnout, P. Claes, J. Desmyter, and E. De Clercq.** 1992. New polyacetal polysulphate active against human immunodeficiency virus and other enveloped viruses. *Antiviral Chem. Chemother.* **3**:351–360.
485. **Wlodawer, A., and J. W. Erickson.** 1993. Structure-based inhibitors of HIV-1 protease. *Annu. Rev. Biochem.* **62**:543–585.
486. **Wu, J. C., T. C. Warren, J. Adams, J. Proudfoot, J. Skiles, P. Raghavan, C. Perry, I. Potocki, P. R. Farina, and P. M. Grob.** 1991. A novel dipyrididiazepinone inhibitor of HIV-1 reverse transcriptase acts through a nonsubstrate binding site. *Biochemistry* **30**:2022–2026.
487. **Yamamoto, N., D. Schols, E. De Clercq, Z. Debyser, R. Pauwels, J. Balzarini, H. Nakashima, M. Baba, M. Hosoya, R. Snoeck, J. Neyts, G. Andrei, B. A. Murrer, B. Theobald, G. Bossard, G. Henson, M. Abrams, and D. Picker.** 1992. Mechanism of anti-human immunodeficiency virus action of polyoxometalates, a class of broad-spectrum antiviral agents. *Mol. Pharmacol.* **42**:1109–1117.
488. **Yao, X.-J., M. A. Wainberg, and M. A. Parniak.** 1992. Mechanism of inhibition of HIV-1 infection *in vitro* by purified extract of *Prunella vulgaris*. *Virology* **187**:56–62.
489. **Yao, X.-J., M. A. Wainberg, M. Richard, and M. Pollak.** 1991. The ability of suramin to block CD4-gp120 binding is reversed in the presence of albumin. *Antimicrob. Agents Chemother.* **35**:2636–2638.
490. **Yarchoan, R., H. Mitsuya, and S. Broder.** 1993. Challenges in the therapy of HIV infection. *Trends Pharmacol. Sci.* **14**:196–202.
491. **Yeh, P., D. Landais, M. Lemaître, I. Maury, J.-Y. Crenne, J. Becquart, A. Murry-Brelrier, F. Boucher, G. Montay, R. Fleer, P.-H. Hirel, J.-F. Mayaux, and D. Klatzmann.** 1992. Design of yeast-secreted albumin derivatives for human therapy: biological and antiviral properties of a serum albumin-CD4 genetic conjugate. *Proc. Natl. Acad. Sci. USA* **89**:1904–1908.
492. **Yokota, T., K. Konno, E. Chonan, S. Mochizuki, K. Kojima, S. Shigeta, and E. De Clercq.** 1990. Comparative activities of several nucleoside analogs against duck hepatitis B virus *in vitro*. *Antimicrob. Agents Chemother.* **34**:1326–1330.
493. **Yokota, T., S. Mochizuki, K. Konno, S. Mori, S. Shigeta, and E. De Clercq.** 1991. Inhibitory effects of selected antiviral compounds on human hepatitis B virus DNA synthesis. *Antimicrob. Agents Chemother.* **35**:394–397.
494. **Yu, M., J. Ojwang, O. Yamada, A. Hampel, J. Rapaport, D. Looney, and F. Wong-Staal.** 1993. A hairpin ribozyme inhibits expression of diverse strains of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **90**:6340–6344.
495. **Yuasa, S., Y. Sadakata, H. Takashima, K. Sekiya, N. Inouye, M. Ubasawa, and M. Baba.** 1993. Selective and synergistic inhibition of human immunodeficiency virus type 1 reverse transcriptase by a non-nucleoside inhibitor, MKC-442. *Mol. Pharmacol.* **44**:895–900.
- 495a. **Yusa, K., T. Oh-hara, S. Tsukahara, K. Baba, M. Taniguchi, M. Kozawa, S. Takeuchi, H. Hara, and T. Tsuruo.** 1994. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by daphnodorins. *Antiviral Res.* **25**:57–66.
496. **Zamecnik, P. C., J. Goodchild, Y. Taguchi, and P. S. Sarin.** 1986. Inhibition of replication and expression of human T-cell lymphotropic virus type III in cultured cells by exogenous synthetic oligonucleotides complementary to viral RNA. *Proc. Natl. Acad. Sci. USA* **83**:4143–4146.
497. **Zarling, J. M., P. A. Moran, O. Haffar, J. Sias, D. D. Richman, C. A. Spina, D. E. Myers, V. Kuebelbeck, J. A. Ledbetter, and F. M. Uckun.** 1990. Inhibition of HIV replication by pokeweed antiviral protein targeted to CD4<sup>+</sup> cells by monoclonal antibodies. *Nature (London)* **347**:92–95.
498. **Zhang, D., A. M. Caliendo, J. J. Eron, K. M. DeVore, J. C. Kaplan, M. S. Hirsch, and R. T. D'Aquila.** 1994. Resistance to 2',3'-dideoxycytidine conferred by a mutation in codon 65 of the human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **38**:282–287.
499. **Zhang, H., L. Vrang, K. Bäckbro, T. Unge, R. Noréén, and B. Öberg.** 1994. Enzymatic properties and sensitivity to inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase with Glu138 → Arg and Tyr188 → His mutations. *Antiviral Res.* **24**:43–57.
500. **Zhang, H., L. Vrang, T. Unge, and B. Öberg.** 1993. Characterization of HIV reverse transcriptases with Tyr 181 → Cys and Leu 100 → Ile mutations. *Antiviral Chem. Chemother.* **4**:301–308.