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Review

Anti-AIDS Drug Development: Challenges and Strategies

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A myriad of chemical derivatives has been shown to inhibit *in vitro* replication of the AIDS virus at concentrations that are nontoxic to the host cells. The majority of these agents acts by either (i) inhibiting enzymes such as reverse transcriptase (RT), protease, or glucosidase, (ii) arresting expression of genes or gene products, or (iii) inhibiting viral processes such as giant cell (syncytia) formation or viral binding to the target cell. The nucleoside RT inhibitors are the most widely studied agents at both the preclinical and the clinical levels. Their inability to cure AIDS has stimulated the discovery of several novel nonnucleoside RT inhibitors, possessing varied structures and demonstrating activity at nanomolar concentrations. These agents demonstrate a unique mode of binding to RT and show a high specificity for HIV-1. Protease inhibitors, soluble CD4 derivatives, oligonucleotides, and many anionic derivatives also demonstrate potent anti-HIV-1 activities. These derivatives possess mechanisms of action different to the nucleosides and exhibit selectivity as exemplified by their high *in vitro* therapeutic indices. This article discusses the structural parameters that govern activity in these agents, the pros and cons regarding the development of these compounds as putative anti-AIDS agents, and the future promise of searching for newer agents directed at novel targets to inhibit the AIDS virus.

KEY WORDS: Anti-AIDS agents; human immunodeficiency virus 1 (HIV-1); development; inhibitors; mechanism; selectivity.

INTRODUCTION

Since the discovery of a retroviral cause for acquired immunedeficiency syndrome (AIDS) (1–3), many laboratories have actively pursued the search, design, and development of new anti-human immunodeficiency virus (HIV-1) agents. These studies have produced a wide array of agents possessing diverse structures that are capable of inhibiting different sites of the virus life cycle. The discovery of these agents has been the result of one or many approaches. These have included the random anti-HIV-1 screening of a wide inventory of chemical substances, the evaluation of substances with known antiviral activity against other viruses or inhibitory activity against specific targets, such as reverse transcriptase (RT), present in other species (e.g., animal, avian), and rational drug design. In general, the presently known inhibitors of HIV-1 inhibit either specific viral enzymes or viral processes and thereby inhibit viral multiplication.

The fact that the AIDS virus is endowed with unique viral enzymes and genes that are required for replication provides the medicinal chemist with attractive targets for drug design. In this respect, among the enzymes, RT, protease, and the glucosidases have been popular targets, for which there are known inhibitors. In considering viral processes, agents exist that have the ability to prevent viral binding to the target cell, arrest giant cell (syncytia) formation, or prevent gene expression. Broadly, these ongoing approaches continue both to produce new specific inhibitors and to contribute to the development of known inhibitors. The design of new specific enzyme inhibitors can be rationally strengthened if the relevant enzyme has been functionally characterized and its structural coordinates confirmed by X-ray analysis. However, it must be emphasized that until these data become available, it is still possible to design inhibitors. This is possible only after the discovery of a lead compound and has a greater chance for success if the mechanism of action of the lead compound has been determined. A pertinent example is the discovery of many potent nucleoside inhibitors of HIV-1 RT which were designed without X-ray crystallographic data on the enzyme. Indeed, the retroviral RT still remains a popular target for drug design and synthesis.

REVERSE TRANSCRIPTASE INHIBITORS

Nucleosides and Derivatives

Once it was established that an RNA virus was responsible for AIDS, research groups rushed to test randomly many nucleoside derivatives, among other compounds, for activity against HIV-1. The selection of compounds for antiviral evaluation was narrowed down in certain cases by considering nucleoside derivatives that have been known to possess antiviral activity against several animal and human viruses. In the United States, the first drug to be licensed by

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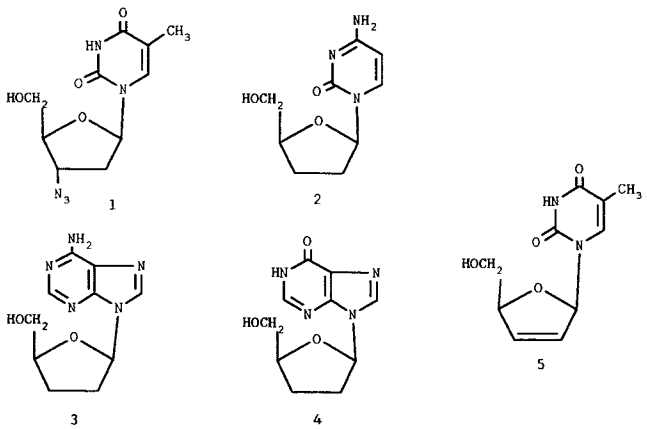
the FDA and used on a wide scale in AIDS patients was 3′-azido-2′,3′-dideoxythymidine (AZT) (1) (Scheme I). It is worthy to note that AZT was first synthesized as an antitumor agent in 1964 (4) and was later shown to have inhibitory activity against the Friend leukemia virus (5). In 1985, it was reported that AZT demonstrated *in vitro* inhibition of the cytopathogenic effect of the AIDS virus (6) and was subsequently administered to AIDS patients (7). The anti-HIV-1 activity of AZT stimulated the evaluation of a wide variety of nucleoside derivatives. For many research groups this was not a formidable task because they possessed a large inventory of already synthesized nucleoside derivatives. These and other investigations led to the discovery of activity in other nucleoside derivatives such as 2′,3′-dideoxycytidine (DDC) (2), 2′,3′-dideoxyadenosine (DDA) (3), 2′,3′-dideoxyinosine (DDI) (4), and 2′,3′-dideoxy-2′,3′-didehydrothymidine (D4T) (5) and their consequent introduction into clinical trials. Recently, DDI has also gained FDA approval.

The mechanism of action of these anti-HIV-1 nucleoside agents involves the sequential *in vivo* phosphorylation of the 5′-hydroxyl group to the active triphosphate species. The triphosphate inhibits the retroviral RT and also acts as a chain terminator (8). Therefore, for the nucleosides, the 5′-hydroxy group is a requirement for activity. One study has analyzed the preferred sugar conformations of certain anti-HIV-1 nucleosides and demonstrated that the location of the 5′-hydroxy group is important in relationship to the base (9). Structurally, AZT is unique in that it has a functionality rare in therapeutic drugs, an azido group in the 3′-position of the sugar ring. It has been postulated that the azido group functions to bind to both the polynucleotide binding site and the mononucleotide 5′-phosphate binding site of RT to contribute to DNA chain termination (10).

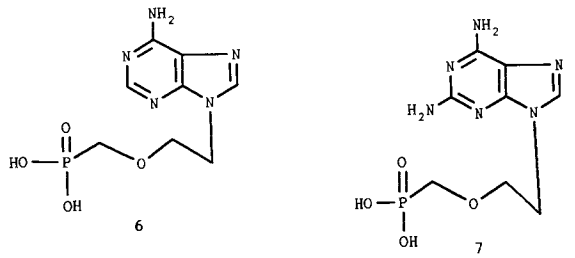
Structure–activity studies have revealed that modifications at the 3′-position of the sugar do allow the presence of a hydrogen (as in DDC, DDA, or DDI), or a fluorine in the α-orientation (erythro compound), or the presence of a 2′,3′-double bond (as in D4T). However, these sugar modifications must be matched with the appropriate nucleic acid base to ensure optimum activity. One problem with purine nucleosides (such as DDA and DDI) was their short half-life due to acid lability. This undesirable property was circumvented by preparing 2′-fluoro analogues, which were as active as the parent analogues but these derivatives also exhibited increased toxicity (11). The introduction of a 6-halo substituent into certain 2′,3′-dideoxypurine nucleoside derivatives has been shown to confer lipophilicity without decreasing the anti-HIV-1 activity of these compounds (12).

The administration of these nucleoside agents in the clinic has revealed various forms of toxicity. These include bone marrow suppression and anemia (AZT), peripheral neuropathy (DDC, DDI, and D4T), and pancreatitis (DDI) (13). Although these side effects have been shown to be dose-limiting, they may preclude the long-term use of these nucleosides as singular agents against AIDS. Resistance has become a matter of concern after the discovery of AZT-resistant HIV-1 mutants (14). Three amino acid mutations where Asp 67 → Asn, Lys 70 → Arg, Thr 215 → Phe or Tyr, have been shown to be common to several AZT-resistant isolates (15). Recently, it has been shown that it is possible to induce resistance to DDI and sensitivity to AZT by a mutation in HIV-1 reverse transcriptase (16). Nucleoside anti-RT analogues may still prove promising in anti-AIDS therapy if they are prescribed early in asymptomatic patients and/or are used in combination with other nucleosides or with derivatives possessing a different mechanism(s) of action. This type of regimen would have the advantage of reducing or eliminating the toxic effects seen with singular nucleoside agents, as well as delay or abrogate the emergence of nucleoside resistant mutants.

The shortcomings of toxicity and resistance associated with the clinically used nucleosides have provided the impetus for detailed structure–activity relationship studies in the anti-HIV nucleoside area (17). A pertinent structural modification in the development of nucleoside analogues has been the opening of the furanose sugar ring to prepare acyclic derivatives. This strategy is strongly substantiated by the success of the acyclic nucleoside derivative, Acyclovir, for the management of genital herpes. Phosphomethoxyalkyl derivatives of adenine, namely, 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (6) and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) (7), demonstrate anti-HIV-1 activity and are more potent than AZT in certain animal models (18,19) (Scheme II). The side chain of Acyclovir has a terminal hydroxy group, a required necessity to form the active triphosphate species. In PMEA and PMEDAP the terminal side-chain moiety is a phosphonyl group. Since the active species is also the triphosphate derivative, PMEA and PMEDAP need only two more phosphorylations to form the active compound. Other favorable properties of PMEA and PMEDAP are their known inhibi-



Scheme I



Scheme II

tory effects against other viruses that infect AIDS patients, the ability to sustain *in vivo* antiviral activity for several days after a single dose (20), and the demonstration of immunomodulating activity (21).

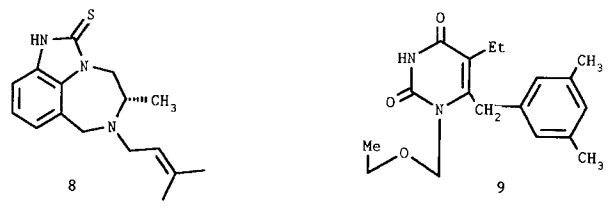
Nonnucleoside Derivatives

In the rational search for inhibitors of RT as singular agents for the treatment of AIDS, a crucial argument may be offered on the validity of this approach based on the known inability of the RT inhibition mechanism, as exemplified by the nucleosides, to cure AIDS in the clinic. A strong counterargument to this discussion would be the possibility of discovering RT inhibitors that interact with RT in a mode that is totally different to the anti-RT nucleosides. Indeed, many research groups actively searched their chemical inventories for nonnucleoside inhibitors of RT. These studies have revealed promising activity in many derivatives having diverse chemical structures and exhibiting potencies in nanomolar concentrations.

The tetrahydroimidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one and -thione (TIBO) derivatives were discovered after the evaluation of 600 classes of compounds. One compound in this series, R82150 (**8**) (Scheme III), is almost as potent as AZT (**1**) and has a therapeutic index that is five times higher than that of AZT (**1**). A unique property of the TIBO class of compounds is that they inhibit the replication of HIV-1 but are not active against HIV-2 or any other DNA or RNA viruses (22). It has been suggested that R82150 may bind to an allosteric site on RT (23).

Similarly, among the 1-[(2-hydroxyethoxy)methy]-6-(phenylthio)thymine (HEPT) derivatives (24,25), selective activity was shown against HIV-1 but no activity against HIV-2 or any other retroviruses. It is remarkable that among the RT inhibitors of this class (26), one derivative, **9** (Scheme III), has an *in vitro* therapeutic index of over 100,000 (27). Two pyridinone derivatives (**10** and **11**) (28) and a dipyridodiazepinone analogue, Nevirapine (**12**) (29), have also been shown to target RT (Scheme IV). It is intriguing that Nevirapine, TIBO, and the pyridinone derivatives share a common site of action on RT (28). Studies have revealed that Tyr-181 and Tyr-188 are located at the Nevirapine binding site (30) and that Nevirapine is active against an AZT-resistant strain of HIV-1 (31).

In a separate study, it has been demonstrated that derivatives from the TIBO and HEPT series bind to the same site on HIV-1 RT (32). It should be noted that *in vitro* HIV-1 resistance to pyridinone, TIBO, and Nevirapine have been reported. It remains to be determined whether this resistance will also manifest itself *in vivo* (33). The screening of 1500 compounds belonging to different classes has culminated in the discovery of derivative **13** (Scheme V), belonging to a class of bis(heteroaryl)piperazines (BHAPs). These agents show anti-HIV-1 potencies equal to AZT's in several laboratory and clinical isolates, possess activity in the HIV-1-infected SCID-hu mouse, and have no activity against HIV-2 (34). While all the above nonnucleoside derivatives are synthetic in nature, a recent report describes selective inhibition of HIV-1 RT by the natural product derivatives psychotrine (**14**) and its methyl ether (**15**) (35) (Scheme V).



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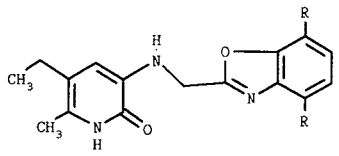
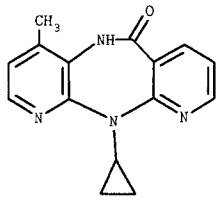
Scheme III



10, R = CH_3

11, R = Cl



12

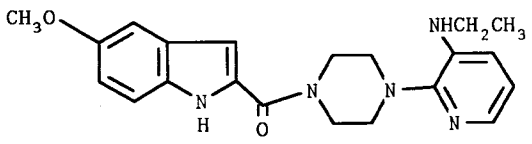
Scheme IV

PROTEASE INHIBITORS

The retroviral aspartyl protease was the first HIV-1 enzyme target for which the X-ray crystal structure was determined. The publication of these reports (36–38) generated wide interest in the design and synthesis of a broad variety of protease inhibitors. Three approaches have been documented. First, a large number of inhibitors were designed around one of the at least seven scissile bonds in the *gag* and *gag-pol* gene products that are the targets of the viral protease. In the inhibitors, this scissile bond is replaced by a nonhydrolyzable isostere having tetrahedral geometry in the transition state. These derivatives encompass the statine, hydroxyethylene, dihydroxyethylene, hydroxyethylamine, phosphinate, and reduced amide classes of compounds (39) and have produced several potent inhibitors (40–42).

The second approach to protease inhibitors has utilized the homodimeric structure of the enzyme and takes into account the symmetry of the protease active site. Based on this fact, C_2 symmetrical inhibitors such as **16** (Scheme VI) were rationally designed, synthesized, and shown to have potent anti-HIV-1 activity (43). In addition, the 2.8 Å crystal structure of the inhibitor–enzyme complex demonstrated that the inhibitor was bound to the enzyme in a highly symmetric mode (44). A third and more recent approach has been based on utilizing a structure-based computer-assisted search strategy using a computer program to create a negative image of the active-site cavity using the crystal structure of the HIV-1 protease. This image was matched with 10,000 molecules from a crystallographic data base and led to the discovery and testing of haloperidol, which can be considered a nonpeptide lead (45).

The early strategies of designing potent protease inhibitors around the scissile bond were borrowed from previously documented work on successful inhibitors of another aspartyl protease, renin. An important concern that faced the early design of protease inhibitors was the requirement for selective activity against the viral protease over the human asparty proteases, renin, pepsin, gastricsin, cathepsin D, and cathepsin E. Indeed, this has been achieved with inhibitor **17** (46) (Scheme VI). It has been reported that actin,



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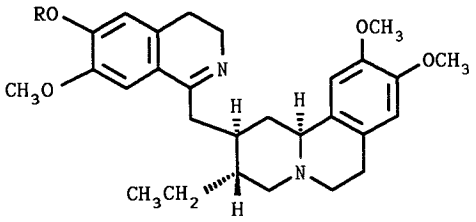


14, R = H

15, R = CH_3

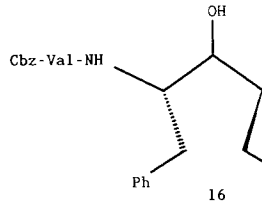
Scheme V

troponin C, Alzheimer amyloid protein, and pro-interleukin 1β can be substrates for the HIV-1 protease. This underscores the possibility of more serious damage to host cellular proteins if indeed it is possible for the protease to express activity outside the viral particle (47).

The most potent protease inhibitors have substantial peptide composition and will therefore be challenged with the problems of metabolism, distribution, bioavailability, and oral activity in the course of their clinical evaluation. However, the fact that the protease enzyme functions at a postintegration stage and a recent report on the possible need for the protease for the synthesis of proviral DNA (48) make further development of protease inhibitors attractive. Undoubtedly, the discovery of a potent nonpeptide inhibitor of the protease enzyme is highly desirable.

GLUCOSIDASE INHIBITORS

A critical step for maturation and infectivity of the virion involves the N-glycosylation of the envelope glycoprotein gp 120, followed by processing by a variety of enzymes. The processing also involves the trimming enzymes, α-glucosidases I and II, which sequentially remove one and two glucose units from the oligosaccharide chain. Castanospermine (18) and *N*-butyldeoxynojirimycin (*N*-butyl-DNJ) (19) are inhibitors of these enzymes (49) and the latter derivative is undergoing clinical evaluation (Scheme VII). Structurally, 18 and 19 are nitrogen analogues of glucose with the stereochemistry at C-6, C-7, C-8, and C-8a on the piperidine ring of castanospermine (18) resembling the C-2, C-3, C-4, and C-5 positions on the pyranose ring of glucose (50). This stereochemical disposition is also maintained at carbons 2, 3, 4, and 5 in *N*-butyl-DNJ (19), which can be considered a more flexible derivative of the rigid derivative, castanospermine (18,50). Many other similar analogues have also been synthesized and evaluated for anti-HIV-1 activity (51,52). A critical point in the development of these agents is that, apart from needing relatively higher doses to attain antiviral activity, they target an enzyme process that does not distinguish between viral and cellular glycosylation.



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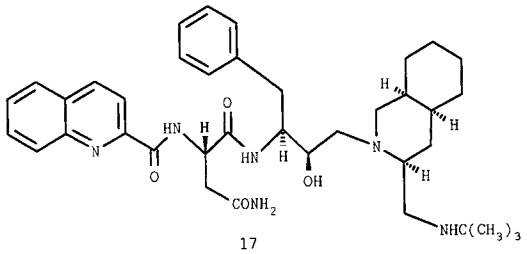


17

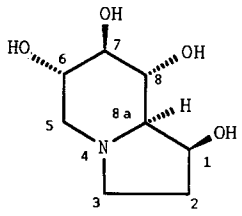
Scheme VI

INHIBITORS OF GENE EXPRESSION

Antisense Oligonucleotides

A pivotal argument in the design of drugs to fight AIDS has been the question of whether any agent will have the capability to cure the disease. The question is a highly pertinent one because it can be debated that once the proviral DNA is integrated into the host cell DNA (as in all AIDS patients), lifelong infection is guaranteed. Theoretically, a cure would be possible only if an agent excised the viral genome from all the infected host cells. Until this becomes a reality, it may be possible to design agents that would target and inhibit expression of certain viral genes. While this treatment may have to be indefinite, it is plausible to assume that this approach may delay or totally prevent the onset of AIDS. It is in this context that the antisense oligonucleotides are most provocative because theoretically they can be designed to have the potential to block gene expression.

In 1978, the first *in vitro* experiment that demonstrated



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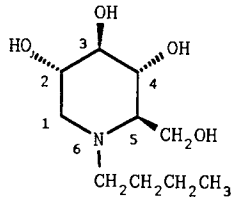

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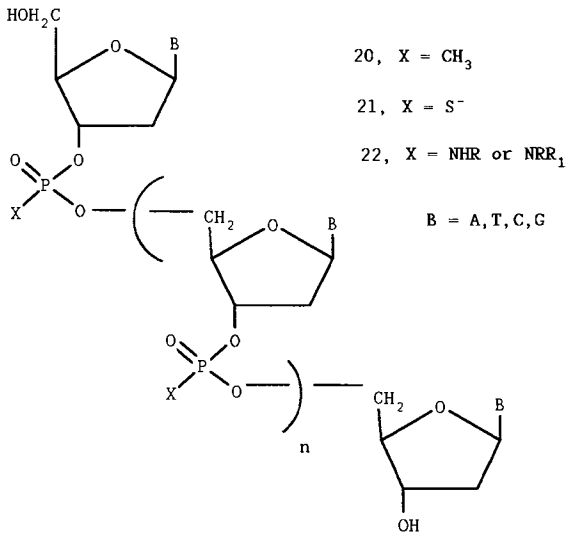
Scheme VII



Scheme VIII

the utility of the antisense approach was revealed (53). Since then, many analogues have been synthesized where the antisense oligonucleotide is designed to be complimentary to a specific segment of the genome or a definite sequence of mRNA. After hybridization with the target sequence, expression of the relevant segment is blocked. During the development of these agents, many issues need to be seriously considered. At the onset, it seemed that many of these agents would suffer from rapid *in vivo* metabolism by cellular exonucleases. This problem has been circumvented by preparing analogues that replaced the negatively charged oxygen on the phosphodiester linkages with methyl, sulfur, or amine groups, to produce methylphosphonates (**20**), phosphorothioates (**21**), or phosphoamidate (**22**) derivatives possessing anti-HIV-1 activity (54–56) (Scheme VIII). Another concern was the questionable cellular uptake of these agents. It has been suggested that oligonucleotides linked to poly-L-lysine (57) or cholesteryl (58) have better cellular penetration properties. Oligonucleotides have also been targeted to the *rev* gene (59). Antisense oligonucleotides also inhibit syncytia formation and RT and HIV-1 replication in chronically infected cells (60). Other matters for consideration include the control of chirality generation during the synthesis of some of these derivatives, the susceptibility of these agents to endonuclease activity, and the guarantee of hybridization *in vivo* (61).

INHIBITORS OF VIRAL BINDING

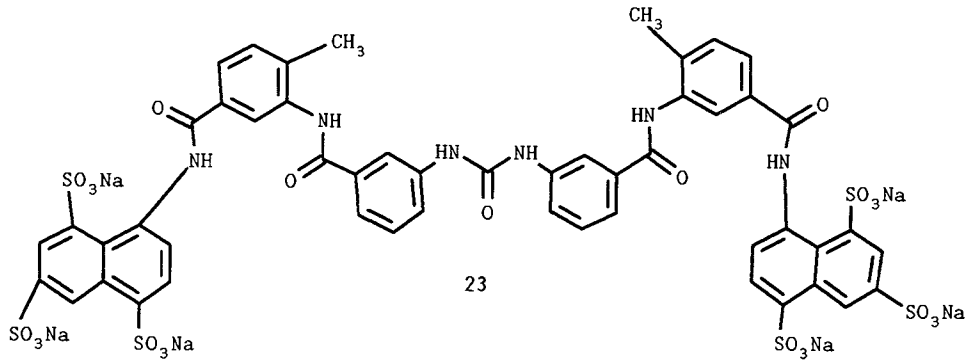
Soluble CD4 Derivatives

The high-affinity interaction between the T cell CD4 receptor and the virus envelope protein gp 120 is considered to be important for initiation of viral infectivity. Several *in vitro* studies have exhibited the antiviral activity of soluble CD4 derivatives (62–66). These agents have also shown to be able to prevent infection of macrophages (67). Soluble CD4 derivatives inhibit viral binding by competing with the T cell CD4 receptor for gp 120 attachment.

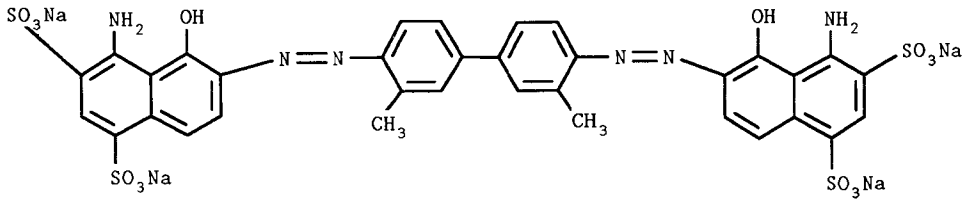
Some issues need to be resolved in connection with the development of soluble CD4 analogues. The short *in vivo* half-life of approximately 45 min (68) was elegantly resolved by producing hybrid molecules called immunoadhesins which contained the important gp 120 binding section of CD4 linked to the Fc portion of IgG. This modification increased the half-life of the hybrid 100-fold (69). In addition, an immunoadhesin has been shown to be able to cross the placenta of a primate, thereby implicating the potential to inhibit perinatally transmitted HIV infection (70). Studies have shown that HIV-2 is less sensitive to soluble CD4 than HIV-1 (71,72) and clinical isolates of HIV-1 require much higher doses of soluble CD4 than needed for inhibiting laboratory strains of HIV-1 (73). Further, the performance of soluble CD4 in the clinic has not been encouraging (74). Finally, the wide-scale therapeutic applicability of this mode of therapy requires further debate since the AIDS virus can also infect CD4-negative cells (75).

Anionic Compounds

The use of anionic compounds to arrest animal retroviral replication has been known for a long time. Therefore, it was not surprising that many anionic derivatives were tested for their ability to inhibit replication of the AIDS virus. In this context, it is worthy to note that the hexasulfonic acid derivative suramin (**23**) (Scheme IX) a known potent inhibitor of the RT of tumor viruses (76), was the first drug that was administered to AIDS patients (77), but it was no longer pursued because of its lack of clinical or immunological efficacy (78). Subsequently, several anionic derivatives were



Scheme IX



24

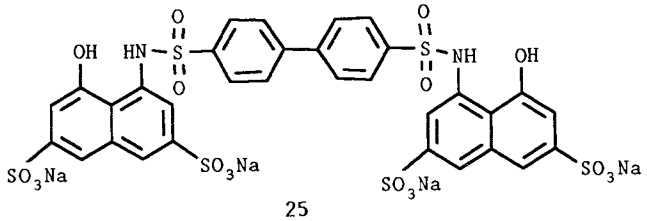
Scheme X

screened and demonstrated potent anti-HIV-1 activity. These derivatives may be broadly divided into the polymer and small molecule classes and generally contained the sulfate, sulfonic, carboxylic, and/or phenolic functionalities. The discovery of the *in vitro* anti-HIV-1 activity of suramin (79) was followed by the demonstration of anti-HIV-1 activity in sulfonic acid derivatives belonging to various dyes (80–82), stilbene (83), and natural product (84,85) classes. In addition, it has been shown that structural modification of suramin can lead to analogues that are more potent and less toxic than suramin (86).

The utility of anti-HIV-1 sulfonic acid azo dyes (87) as potential therapeutic agents is hampered by their known *in vivo* reduction to generate carcinogenic amine metabolites (88). One approach to remedy this problem could be to replace the spacer unit containing the carcinogenic fragment (as is present in the anti-HIV-1 dye Evans Blue, **24**) (Scheme X) with a relatively inert entity and to keep the naphthalenesulfonic acid unit intact. On the other hand, other naphthalenesulfonic acid units may also be employed with a suitable spacer. The use of naphthalenesulfonic acid as the anionic moiety is supported by their known nonmutagenicity (89).

Using the latter strategy, we have synthesized and evaluated a series of naphthalenesulfonic acid spacer derivatives utilizing a variety of HIV-1 and HIV-2 assays (90–92). Compound **25** (Scheme XI) emerged as the most potent compound in these studies (92). While activity was most pronounced in the bis derivatives, we were also able to demonstrate activity in a small naphthalenedisulfonic acid derivative, **26** (Scheme XI), against a clinical isolate of HIV-1 (93). In order to explain the activity of these agents against HIV-1 reverse transcriptase we employed molecular modeling analyses for a flexible bis-naphthalenesulfonic acid derivative, **27** (Scheme XI), and have shown that this agent can mimic the helical turn of B-DNA as demonstrated in Fig. 1 (94).

Antiretroviral activity has also been exhibited in sulfated derivatives, most notably in the sulfated polysaccharides. Among these, dextran sulfate (**28**) (Scheme XII) is a potent inhibitor of HIV-1 (95). Dextran sulfate, like the other anionic derivatives, pentosan polysulfate, heparin, aurintricarboxylic acid, suramin, and Evans Blue, specifically interacts with viral gp 120 (96). The optimum activity of dextran sulfate is clearly a function of its molecular weight and degree of sulfation (97). The activity of polymeric aurintricarboxylic acid has also been correlated with its molecular weight (98). It is interesting that dextran sulfate can mediate release of gp 120 from HIV-1. At first thought, it seems that this finding may be exploited as a novel strategy to inactivate the virus. However, in order to observe this effect *in vivo*, it will be necessary to administer higher doses of dextran sulfate than those that are presently being administered in the clinic (99). This may not be prudent due to the known effect of dextran sulfate on blood coagulation. Further, if this free-floating gp 120 is not eliminated by antibodies, the envelope protein would bind to T cells and initiate cell killing via an antibody-dependent complement-mediated pathway (100).



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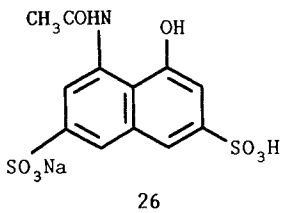

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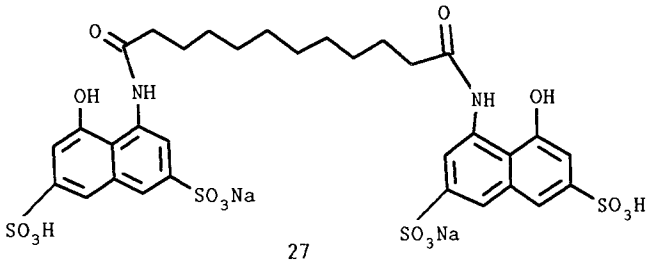

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Scheme XI

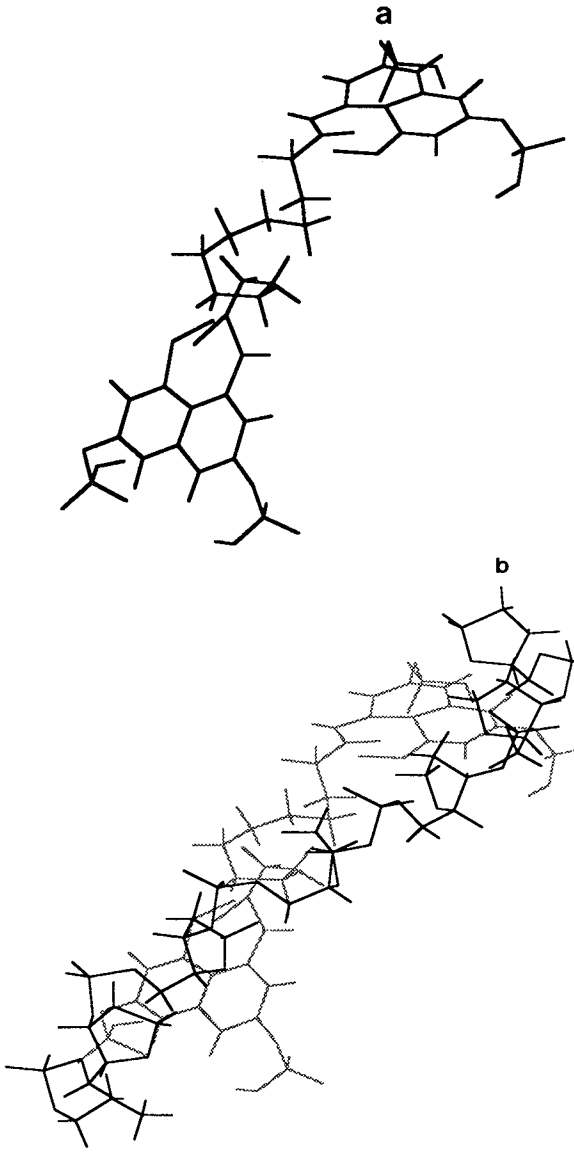
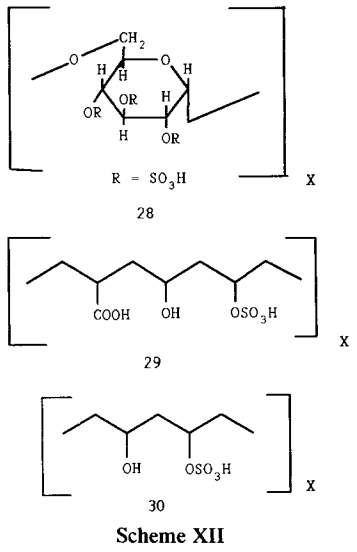


Fig. 1. (a) Global minimum energy helical conformation of 27. (b) Molecular superposition of 27 (dashed lines) on B-form DNA (solid lines).



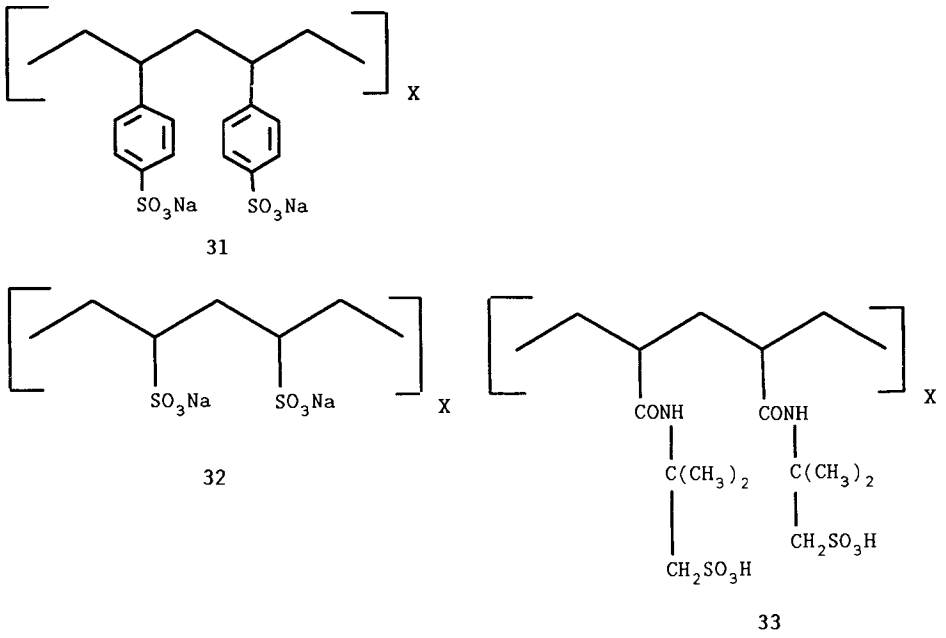
Scheme XII

An early and legitimate concern with the clinical use of sulfated polysaccharides, such as dextran sulfate, was its known anticoagulant activity. However, studies have shown that it is possible to dissociate anti-HIV-1 activity and anticoagulant activity in sulfated polysaccharides (101). An oral clinical study of dextran sulfate revealed no anti-HIV-1 activity (102). This should have been predicted due to the known polar nature of this derivative and its expected inability to cross cellular membranes. Although it was strongly advocated that parenteral administration may produce better results, another set of problems remained to be addressed. First, the i.v. administration of high molecular weight dextran sulfate is suggested to lead to allergic reactions and renal toxicity (103). Second, the glycosidic bonds in dextran sulfate should be predicted to suffer the same *in vivo* fate as other polysaccharides. Indeed, this has been experimentally confirmed, where it was shown that dextran sulfate is degraded into smaller molecular weight fragments that are known to be inactive (104).

On medicinal chemistry grounds, this problem of degradation is easily circumvented by eliminating the glycosidic bond and using an inert hydrocarbon backbone to carry the sulfate groups. This approach has been fruitful to produce the sulfated copolymer of acrylic acid and vinyl alcohol (PAVAS) (29) and the sulfated polymer of vinyl alcohol (PVAS) (30) (Scheme XII). Both PAVAS and PVAS demonstrate antiviral activity and are potent inhibitors of syncytia formation (105). While the *in vivo* stability of these agents can be expected to be better than dextran sulfate, they may still be prone to another catabolic step: the desulfation reactions catalyzed by sulfatase enzymes. Once again, structural modifications can be performed to avert this reaction.

Since dextran without the sulfate groups is inactive (106), any attempted structural manipulation should still contain a polyanionic functionality. In considering a suitable substitute anionic moiety it would be preferable if the group contained a similar presentation of the anionic charge as the sulfate group. This is pertinent because polymeric derivatives that separately employ carboxylic (107) or phenolic groups (108) to carry the anionic charge are inactive. It would also be desirable that the anionic group is not easily degraded during metabolism.

Being cognizant of these requirements, we tested several novel polymers containing the sulfonic acid group and discovered highly potent anti-HIV-1 activity in these derivatives. An important virtue of the sulfonic acid group as a moiety carrying the needed anionic charge is that it is relatively unreactive to metabolizing enzymes and is excreted largely unchanged with negligible desulfonation (109–111). All the sulfonic acid polymers exhibited activity at concentrations that were nontoxic to the host cells. However, in the HIV-1 RT assay, the aromatic polymers were the most potent, with poly(4-styrenesulfonic acid) (PSS) (31) (Scheme XIII) exhibiting a potency that was 161 times greater than suramin, a known potent inhibitor of RT. Among the active aliphatic polymers in the cytopathogenesis assay, poly(vinylsulfonic acid) (PVS) (32) (Scheme XIII) is an attractive



Scheme XIII

candidate since it has a molecular weight of 2000. In this assay, poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) (33) (Scheme XIII) is equally potent to dextran sulfate. All the sulfonic acid polymers inhibit binding of HIV-1 to the target cell (112).

Mechanistically, while all the sulfonic acid polymers inhibited binding of anti-gp 120 mAb (directed to the V3 fusion region) to HIV-1 gp 120, PSS (31) was one of two polymers that interfered with the binding of OKT4A/Leu3a mAb to the CD4 receptor (112). Based on all of the above observations, the sulfonic acid polymers are a promising class of new viral binding inhibitors. Prior to the clinical administration of these agents, it needs to be determined whether the anti-RT or the viral binding inhibitory property is the targeted activity. For the former case, prodrug design will be necessary. For the latter case, this modification may not be needed since the polar functionality may be required for interaction at the viral–target cell interface. It is worthy to note that inhibitors of viral adsorption have the potential to be used as chemoprophylactic agents against AIDS. For the future development of anionic viral binding inhibitors, it would be desirable to have a small molecule possessing a high potency, a low toxicity, and the ability to seek and bind selectively to a target site on the HIV-1 envelope which is crucial for the gp 120–CD4 interaction.

FUTURE PROSPECTS

Over the last decade, the human immunodeficiency virus has been the focal point of intense and sophisticated antiviral research. While the AIDS virus is the most complicated human pathogenic virus ever studied, it is also the most researched virus in medical history. In spite of the myriad of known inhibitors of HIV-1, the AIDS virus still successfully eludes all forms of curative therapy. Yet as further molecular biology and biochemistry of the virus continue to evolve, the medicinal chemist will be presented with a newer set of putative intervention targets. While these new targets will stimulate synthetic programs directed at inhibiting these sites, the search for novel anti-HIV-1 molecules should be an ongoing process. For example, the semirandom screening of synthetic derivatives should still be pursued. Already, this strategy of wide-scale screening has lead to the discovery of many highly potent anti-HIV-1 agents. In addition, the systemic evaluation of the vast reservoir of natural products needs to be undertaken, since it is well documented that the plant world has supplied diverse structures possessing palliative or curative properties (113).

Nonetheless, on the basis of mechanism-based discovery of new agents for AIDS, questions need to be raised constantly about the viability of certain targets for curing the disease. This is even more pertinent when agents with a known mechanism of action fail to cure the disease in the clinic. It is at this pivotal point that newer antiviral avenues must be sought and agents that attack novel targets need to be encouraged for extensive preclinical evaluation so that they may be quickly assessed for clinical efficacy. In this context, the regulatory genes, *tat* and *rev*, still remain as potential sites of drug intervention. The determination of the X-ray crystal structure (114) and solution secondary structure (115) of the ribonuclease H domain of RT will provide the impetus to design and synthesize inhibitors of these new targets.

Another plausible site of attack is the retroviral integrase enzyme. However, this approach remains questionable since in certain cell types viral replication continues despite the presence of a functioning integrase enzyme (116). A new putative target is the nuclear factor κB (NF-κB), the stimulation of which is related to the lowering of intracellular glutathione levels and the expression of genes controlled by the long terminal repeat of HIV-1. *N*-Acetyl-L-cysteine replenishes glutathione levels, thus inhibiting the activation of

NF-κB, which in turn is suggested to curb the replication and expression of HIV-1 (117,118). Finally, rigorous experimentation is required to ascertain whether inhibition of these novel targets will provide the necessary antiviral coup that will ultimately cure AIDS.

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REFERENCES

1. F. Barré-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, T. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. Isolation of a T cell lymphotropic virus from a patient at risk for the acquired immunodeficiency syndrome (AIDS). *Science* 220:868–871 (1983).
2. R. C. Gallo, S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleska, B. Safai, G. White, P. Foster, and P. D. Markham. Frequent detection and isolation of pathogenic retroviruses (HTLV-III) from patients with AIDS and at risk from AIDS. *Science* 224:500–503 (1984).
3. J. A. Levy, A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* 225:840–842 (1984).
4. J. P. Horowitz, J. Chua, and M. Novel. Nucleosides. V. The monomesylates of 1-(2-deoxy-β-D-lyxofuranosyl)thymine. *J. Org. Chem.* 29:2076–2078 (1964).
5. W. Ostertag, G. Roesler, C. J. Krieg, J. Kind, T. Cole, T. Crozier, G. Gaedicke, G. Steinheider, N. Kluge, and S. Dube. Induction of endogenous virus and of thymidine kinase by bromodeoxyuridine in cell cultures transformed by Friend virus. *Proc. Natl. Acad Sci. USA* 71:4980–4985 (1974).
6. H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder. 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 (1985).
7. R. Yarchoan, R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. N. Lehrman, R. M. Blum, D. W. Barry, G. M. Shearer, M. A. Fischl, H. Mitsuya, R. C. Gallo, J. M. Collins, D. P. Bolognesi, C. E. Myers, and S. Broder. Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication to patients with AIDS or AIDS-related complex. *Lancet* 1:575–580 (1986).
8. R. Yarchoan and S. Broder. Anti-retroviral therapy of AIDS and related disorders: general principles and specific development of dideoxynucleosides. *Pharmacol. Ther.* 40:329–348 (1989).
9. P. Van Roey, J. M. Salerno, C. K. Chu, and R. F. Schinazi. Correlation between preferred sugar ring conformation and activity of nucleoside analogues against human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 86:3929–3933 (1989).
10. A. Camerman, D. Mastropaolo, and N. Camerman. Azidothymidine: Crystal structure and possible functional role of the azido group. *Proc. Natl. Acad. Sci. USA* 84:8239–8242 (1987).
11. V. E. Marquez, C. K.-H. Tseng, H. Mitsuya, S. Aoki, J. Kelley, H. Ford Jr., J. S. Roth, S. Broder, D. G. Johns, and J. S. Driscoll. Acid-stable 2′-fluoro purine dideoxynucleosides as active agents against HIV. *J. Med. Chem.* 33:978–985 (1990).
12. T. Shirasaka, K. Murakami, H. Ford Jr., J. A. Kelley, H. Yoshioka, E. Kojima, S. Aoki, S. Broder, and H. Mitsuya. Lipophilic halogenated congeners of 2′,3′-dideoxypurine nucleosides active against human immunodeficiency virus in vitro. *Proc. Natl. Acad. Sci. USA* 87:9426–9430 (1990).
13. H. Mitsuya, R. Yarchoan, and S. Broder. Molecular targets for AIDS therapy. *Science* 249:1533–1544 (1990).
14. B. A. Larder, G. Darby, and D. D. Richman. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* 243:1731–1734 (1989).
15. B. A. Larder and S. D. Kemp. Multiple mutations in HIV-1 reverse transcriptase confer high level resistance to zidovudine (AZT). *Science* 246:1155–1158 (1989).
16. M. H. St. Clair, J. L. Martin, G. Tudor-Williams, M. C. Bach, C. L. Vavro, D. M. King, P. Kellam, S. D. Kemp, and B. A. Larder. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* 253:1557–1559 (1991).
17. E. De Clercq, A. Van Aerschot, P. Herdwijn, M. Baba, R. Pauwels, and J. Balzarini. Anti-HIV-1 activity of 2′,3′-dideoxynucleoside analogues: Structure activity relationship. *Nucleosides Nucleotides* 8:659–671 (1989).
18. J. Balzarini, L. Naesens, P. Herdewijn, I. Rosenberg, A. Holy, R. Pauwels, M. Baba, D. G. Johns, and E. De Clercq. 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 (1989).
19. L. Naesens, J. Balzarini, I. Rosenberg, A. Holy, and E. De Clercq. 9-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP): A novel agent with anti-human immunodeficiency virus activity in vitro and potent anti-Moloney murine sarcoma virus activity in vivo. *Eur. J. Clin. Microbiol. Infect. Dis.* 8: 1043–1047 (1989).
20. E. De Clercq. Basic approaches to anti-retroviral treatment. *J. Acquir. Immune Defic. Syndr.* 4:207–218 (1991).
21. V. D. Gobbo, A. Foli, J. Balzarini, E. De Clercq, E. Balestra, N. Villani, S. Marini, C. F. Perno, and R. Calio. Immunomodulatory activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA), a potent anti-HIV nucleotide analogue, on in vivo murine models. *Antiviral Res.* 16:65–75 (1991).
22. R. Pauwels, K. Andries, J. Desmyter, D. Schols, M. J. Kukla, H. J. Breslin, A. Raemaeckers, J. Van Gelder, R. Woestenborghs, J. Heykants, K. Schellekens, M. A. C. Janssen, E. De Clercq, and P. A. J. Janssen. Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. *Nature* 343:470–474 (1990).
23. K. B. Frank, G. J. Noll, E. V. Connell, and I. S. Sim. Kinetic interaction of human immunodeficiency virus type 1 reverse transcriptase with the antiviral tetrahydroimidazo[4,5,1-jk]-[1,4]-benzodiazepin-2(1H)-thione compound, R82150. *J. Biol. Chem.* 266:14232–14236 (1991).
24. H. Tanaka, M. Baba, H. Hayakawa, T. Sakamaki, T. Miyasaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, S. Shigeta, R. T. Walker, J. Balzarini, and E. De Clercq. A new class of HIV-1-specific 6-substituted acyclouridine derivatives: synthesis and anti-HIV-1 activity of 5- or 6-substituted analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine (HEPT). *J. Med. Chem.* 34:349–357 (1991).
25. M. Baba, E. De Clercq, S. Iida, H. Tanaka, I. Nitta, M. Ubasawa, H. Takashima, K. Sekiya, K. Umezu, H. Nakashima, S. Shigeta, R. T. Walker, and T. Miyasaka. Anti-human immunodeficiency virus type 1 activities and pharmacokinetics of novel 6-substituted acyclouridine derivatives. *Antimicrob. Agents Chemother.* 34:2358–2363 (1990).
26. M. Baba, E. De Clercq, H. Tanaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, K. Umezu, H. Nakashima, S. Mori, S. Shigeta, R. T. Walker, and T. Miyasaka. 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 (1991).

27. M. Baba, S. Shigeta, E. De Clercq, H. Tanaka, T. Miyasaka, M. Ubasawa, K. Umezu, and R. T. Walker. Highly potent and selective inhibition of HIV-1 replication by a novel series of 6-substituted acyclouridine derivatives. Abstracts Volume, 7th International Conference on AIDS, Florence, Italy, June 16–21, 1991, abstr TU.A.63.
28. M. E. Goldman, J. H. Nunberg, J. A. O'Brien, J. C. Quintero, W. A. Schleif, K. T. 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. Pyridinone derivatives: Specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. *Proc. Natl. Acad. Sci. USA* 88:6863–6867 (1991).
29. V. J. Merluzzi, K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C.-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosehthal, R. Faanes, R. J. Eckner, R. A. Koup, and J. L. Sullivan. Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. *Science* 250: 1411–1413 (1990).
30. K. A. Cohen, 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. 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 (1991).
31. D. Richman, A. S. Rosenthal, M. Skoog, R. J. Eckner, T.-C. Chou, J. P. Sabo, and V. J. Merluzzi. BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. *Antimicrob. Agents Chemother.* 35:305–308 (1991).
32. R. Pauwels, Z. Debyser, K. Andries, N. Yamamoto, M. Baba, D. Schols, M. Kukla, J. Desmyter, P. A. J. Janssen, and E. De Clercq. Interaction of TIBO and TIBO-like compounds with HIV-1 RT. Abstracts Volume, 7th International Conference on AIDS, Florence, Italy, June 16–21, 1991, abstr W.A.1007.
33. J. H. Nunberg, W. A. Schleif, E. J. Boots, J. A. O'Brien, J. C. Quintero, J. M. Hoffman, E. A. Emini, and M. E. Goldman. *J. Virol.* 65:4887–4892 (1991).
34. D. L. Romero, 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. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. *Proc. Natl. Acad. Sci. USA* 88:8806–8810 (1991).
35. G. T. Tan, A. D. Kinghorn, S. H. Hughes, and J. M. Pezzuto. Psychotrine and its methyl ether are selective inhibitors of human immunodeficiency virus-1 reverse transcriptase. *J. Biol. Chem.* 266:23529–23536 (1991).
36. M. A. Navia, 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. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature* 337:615–620 (1989).
37. A. Wlodawer, M. Miller, M. Jaskolski, B. K. Sathyanarayana, E. Baldwin, I. T. Weber, I. M. Selk, L. Clawson, J. Schneider, and S. B. H. Kent. Conserved folding in retroviral proteases: Crystal structure of a synthetic HIV-1 protease. *Science* 245:616–621 (1989).
38. R. Lapatto, T. Blundell, A. Hemmings, J. Overington, A. Wilderspin, S. Wood, J. R. Merson, P. J. Whittle, D. E. Danley, K. F. Geoghegan, S. J. Hawrylik, S. E. Lee, K. G. Scheld, and P. M. Hobart. X-ray analysis of HIV-1 proteinase at 2.7 A resolution confirms structural homology among retroviral enzymes. *Nature* 342:299–302 (1989).
39. J. R. Huff. HIV Protease: A novel chemotherapeutic target for AIDS. *J. Med. Chem.* 34:2305–2314 (1990).
40. G. B. Dreyer, B. W. Metcalf, T. A. Tomaszek, Jr., T. J. Carr, A. C. C. Chandler III, L. Hyland, S. A. Fakhoury, V. W. Magaard, M. L. Moore, J. E. Strickler, C. Debouck, and T. D. Meek. Inhibition of human immunodeficiency virus 1 protease *in vitro:* rational design of substrate analogue inhibitors. *Proc. Natl. Acad. Sci. USA* 86:9752–9756 (1989).
41. H. A. Overton, D. J. McMillan, S. J. Gridley, J. Brenner, S. Redshaw, and J. S. Mills. Effect of two novel inhibitors of the human immunodeficiency virus protease in the maturation of the HIV gag and gag-pol polyproteins. *Virology* 179:508–511 (1990).
42. T. J. McQuade, A. G. Tomasselli, L. Liu, V. Karacostas, B. Moss, T. K. Sawyer, R. L. Heinrikson, and W. G. Tarpley. A synthetic HIV-1 protease inhibitor with antiviral activity arrests HIV-like particle maturation. *Science* 247:454–456 (1990).
43. D. J. Kempf, 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. Structure-based C_2 symmetric inhibitors of HIV protease. *J. Med. Chem.* 33:2687–2689 (1990).
44. J. Erickson, 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. Kohlbrenner, R. Simmer, R. Helfrich, D. A. Paul, and M. Knigge. Design, activity, and 2.8 A crystal structure of a C_2 symmetric inhibitor complexed to HIV-1 protease. *Science* 249:527–533 (1990).
45. R. L. DesJarlais, G. L. Seibel, I. D. Kuntz, P. S. Furth, J. C. Alavarez, P. R. Ortiz De Montellano, D. L. DeCamp, L. M. Babe, and C. S. Craik. Structure-based design of nonpeptide inhibitors specific for the human immunodeficiency virus 1 protease. *Proc. Natl. Acad. Sci. USA* 87:6644–6648 (1990).
46. N. A. Roberts, J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay, A. Krohn, 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. Rational design of peptide-based HIV proteinase inhibitors. *Science* 248:358–361 (1990).
47. A. G. Tomasselli, J. O. Hui, L. Adams, J. Chosay, D. Lowery, B. Greenberg, A. Yem, M. R. Deibel, H. Zürcher-Neely, and R. L. Heinrikson. Actin, troponin C, alzheimer amyloid protein and pro-interleukin 1β as substrates of the protease from human immunodeficiency virus. *J. Biol. Chem.* 22:14548–14553 (1991).
48. C. Baboonian, A. Dalgleish, L. Bountiff, J. Gross, S. Oroszlan, G. Rickett, C. Smith-Burchnell, P. Troke, and J. Merson. HIV-1 proteinase is required for synthesis of proviral DNA. *Biochem. Biophys. Res. Commun.* 179:17–24.
49. G. W. J. Fleet, A. Karpas, R. A. Dwek, L. E. Fellows, A. S. Tyms, S. Petursson, S. K. Namgoong, N. G. Ramsden, P. W. Smith, J. C. Son, F. Wilson, D. R. Witty, G. S. Jacob, and T. W. Rademacher. Inhibition of HIV replication by amino-sugar derivatives. *FEBS Lett.* 237:128–132 (1988).
50. B. G. Winchester, I. Cenci Di Bello, A. C. Richardson, R. J. Nash, L. E. Fellows, N. G. Ramsden, and G. Fleet. The structural basis of the inhibition of human glycosidases by castanospermine analogues. *Biochem. J.* 269:227–231 (1990).
51. A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob, and T. W. Rademacher. Aminosugar derivatives as potential anti-human immunodeficiency virus agents. *Proc. Natl. Acad. Sci. USA* 85: 9229–9233 (1988).
52. R. A. Gruters, J. J. Neefjes, M. Tersmette, R. E. Y. De Goede, A. Tulp, H. G. Huisman, F. Miedema, and H. L. Ploegh. Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. *Nature* 330:74–77 (1987).
53. P. C. Zamecnik and M. L. Stephenson. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc. Natl. Acad. Sci. USA* 75:280–284 (1978).
54. C. A. Stein, M. Matsukura, C. Subasinghe, S. Broder, and J. S. Cohen. Phosphorothioate oligodeoxynucleotides are potent sequent nonspecific inhibitors of *de novo* infection by HIV. *AIDS Res. Hum. Retrovir.* 5:639–646 (1989).
55. D. C. Montefiori, R. W. Sobol, Jr., S. W. Li, N. L. Reichenbach, R. J. Suhadolnik, R. Charubala, W. Pfleiderer, A. Modliszewski, W. E. Robinson Jr., and W. M. Mitchell. Phosphorothioate and cordycepin analogues of 2′,5′-oligoadenylate: Inhibition of human immunodeficiency virus type 1 reverse transcriptase and infection *in vitro*. *Proc. Natl. Acad. Sci. USA* 86:7191–7194 (1989).

56. P. S. Sarin, S. Agrawal, M. P. Civeira, J. Goodchild, T. Ikeuchi, and P. Zamecnik. Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. *Proc. Natl. Acad. Sci. USA* 85:7448–7451 (1988).
57. M. LeMaitre, B. Bayard, and B. Lebleu. Specific antiviral activity of a poly(L-lysine)-conjugated oligodeoxyribonucleotide sequence complimentary to vesicular stomatitis virus N protein mRNA initiation site. *Proc. Natl. Acad. Sci. USA* 84:648–652 (1987).
58. R. L. Letsinger, G. Zhang, D. K. Sun, T. Ikeuchi, and P. S. Sarin. Cholesteryl-conjugated oligonucleotides: Synthesis, properties, and activity as inhibitors of replication of human immunodeficiency virus in cell culture. *Proc. Natl. Acad. Sci. USA* 86:6553–6556 (1989).
59. M. Matsukura, G. Zon, K. Shinozuka, M. Robert-Guroff, T. Shimada, C. A. Stein, H. Mitsuya, F. Wong-Staal, J. S.Cohen, and S. Broder. Regulation of viral expression of human immunodeficiency virus *in vitro* by an antisense phosphorothioate oligodeoxynucleotide against *rev(art/trs)* in chronically infected cells. *Proc. Natl. Acad. Sci. USA* 86:4244–4248 (1989).
60. S. Agrawal, T. Ikeuchi, D. Sun, P. S. Sarin, A. Konopka, J. Maizel, and P. C. Zamecnik. Inhibition of human immunodeficiency virus in early infected and chronically infected cells by antisense oligodeoxynucleotides and their phosphorothioate analogues. *Proc. Natl. Acad. Sci. USA* 86:7790–7794 (1989).
61. J. S. Cohen. Antisense oligodeoxynucleotides as antiviral agents. *Antiviral Res.* 16:121–133 (1991).
62. R. E. Hussey, N. E. Richardson, M. Kowalski, N. R. Brown, H.-C. Chang, R. F. Siliciano, T. Dorfman, B. Walker, J. Sodroski, and E. L. Reinherz. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. *Nature* 331:78–81 (1988).
63. D. H. Smith, R. A. Byrn, S. A. Marsters, T. Gregory, J. E. Groopman, and D. J. Capon. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. *Science* 238:1704–1707 (1987).
64. R. A. Fisher, J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell. HIV infection is blocked in vitro by recombinant soluble CD4. *Nature* 331:76–78 (1988).
65. K. C. Deen, J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature* 331:82–84 (1988).
66. A. Traunecker, W. Luke, and K. Karjalainen. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. *Nature* 331:84–86 (1988).
67. C. F. Perno, M. W. Baseler, S. Broder, and R. Yarchoan. Infection of monocytes by human immunodeficiency virus I blocked by inhibitors of CD4-gp 120 binding, even in the presence of enhancing antibodies. *J. Exp. Med.* 171:1043–1056 (1990).
68. S. Broder, H. Mitsuya, R. Yarchoan, and G. N. Pavlakis. Antiretroviral therapy in AIDS. *Ann. Intern. Med.* 113:604–618 (1990).
69. D. J. Capon, 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. Designing CD4 immunoadhesins for AIDS therapy. *Nature* 337:525–531 (1989).
70. R. A. Byrn, J. Mordenti, C. Lucas, D. Smith, S. A. Marsters, J. S. Johnson, P. Cossum, S. M. Chamow, F. M. Wurm, T. Gregory, J. E. Groopman, and D. J. Capon. Biological properties of a CD4 immunoadhesin. *Nature* 344:667–670 (1990).
71. P. R. Clapham, J. N. Weber, D. Whitby, K. McIntosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, and R. Weiss. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. *Nature* 337:368–370 (1989).
72. D. J. Looney, S. Hayashi, M. Nicklas, R. R. Redfield, S. Broder, F. Wong-Staal, and H. Mitsuya. Differences in the interaction of HIV-1 and HIV-2 with soluble CD4. *J. Acq. Immune Def. Syndr.* 3:649–657 (1990).
73. E. S. Daar, X. L. Li, T. Moudgil, and D. D. Ho. 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 (1990).
74. J. O. Kahn, J. D. Allan, T. L. Hodges, L. D. Kaplan, C. J. Arri, H. F. Fitch, A. E. Izu, J. Mordenti, S. A. Sherwin, J. E. Groopman, and P. A. Wolberding. The safety and pharmacokinetics of recombinant soluble CD4 (rCD4) in subjects with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. A phase I study. *Ann Intern. Med.* 112:254–261 (1990).
75. M. Tateno, F. Gonzalez-Scarano, and J. A. Levy. Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. *Proc. Natl. Acad. Sci. USA* 86:4287–4290 (1989).
76. E. De Clercq. Suramin: A potent inhibitor of the reverse transcriptase of RNA tumor viruses. *Cancer Lett.* 8:9–22 (1979).
77. S. Broder, R. Yarchoan, J. M. Collins, H. C. Lane, P. D. Markham, R. W. Klecker, R. R. Redfield, H. Mitsuya, D. F. Hoth, E. Gelman, J. E. Groopman, L. Resnick, R. C. Gallo, C. E. Myers, and A. S. Fauci. Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS-related complex: Clinical pharmacology and suppression of viral replication *in vivo*. *Lancet* 2:627–630 (1985).
78. L. D. Kaplan, P. R. Wolfe, P. A. Volberding, P. Feorino, J. A. Levy, D. I. Abrams, D. Kiprov, R. Wong, and L. Kaufman. Lack of response to suramin in patients with AIDS and AIDS-related complex. *Am. J. Med.* 82:615–620 (1987).
79. H. Mitsuya, M. Popovic, R. Yarchoan, S. Matsushita, R. C. Gallo, and S. Broder. Suramin protection of T cells in vitro against infectivity and cytopathic effect of HTLV-III. *Science* 226:172–174 (1984).
80. J. Balzarini, H. Mitsuya, E. De Clercq, and S. Broder. 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 (1986).
81. R. F. Schinazi, C. K. Chu, R. Babu, B. J. Oswald, V. Saalmann, D. L. Cannon, B. F. H. Eriksson, and M. Nasr. Anthraquinones as a new class of antiviral agents against human immunodeficiency virus. *Antiviral Res* 13:265–272 (1990).
82. M. Baba, D. Schols, R. Pauwels, J. Balzarini, and E. De Clercq. Fuchsin acid selectively inhibits human immunodeficiency virus (HIV) *in vitro*. *Biochem. Biophys. Res. Commun.* 155:1404–1411 (1988).
83. A. D. Cardin, P. L. Smith, L. Hyde, D. T. Blankenship, T. L. Bowlin, X. Schroder, K. A. Stauderman, D. L. Taylor, and A. S. Tyms. Stilbene disulfonic acids. CD4 anatagonists that block human immunodeficiency virus type-1 growth at multiple stages of the virus life cycle. *J. Biol. Chem.* 266:13355–13363 (1991).
84. H. Suzuki, T. S. Tochikura, K. Iiyama, S. Yamazaki, N. Yamamoto, and S. Toda. Lignosulfonate, a water soluble lignin from the waste liquor of the pulping process, inhibits the infectivity and cytopathic effects of human immunodeficiency virus in vitro. *Agr. Biol. Chem.* 53:3369–3380 (1989).
85. K. R. Gustafson, J. H. Cardellina II, R. W. Fuller, O. S. Weislow, R. F. Kiser, K. M. Snader, G. M. L. Patterson, and M. R. Boyd. AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). *J. Natl. Cancer. Inst.* 81:1254–1258 (1989).
86. K. D. Jentsch, G. Hunsmann, H. Hartmann, and P. Nickel. Inhibition of human immunodeficiency virus type 1 reverse transcriptase by suramin-related compounds. *J. Gen. Virol.* 68:2183–2192 (1987).
87. J. Balzarini, H. Mitsuya, E. De Clercq, and S. Broder. Comparative inhibitory effects of suramin and other selected compounds on the infectivity and replication of human T-cell lymphotropic virus (HTLV-III)/lymphadenopathy-associated virus (LAV). *Int. J. Cancer* 37:451–457 (1986).
88. M. C. Bowman, C. R. Nony, S. M. Billedeau, J. L. Martin, H. C. Thompson, Jr., and L. K. Lowry. Metabolism of nine benzidine-congener-based azo dyes in rats based on gas chro-

matography assays of the urine for potentially carcinogenic metabolites. *J. Anal. Toxicol.* 7:55–60 (1983).
89. H. S. Freeman, J. F. Esancy, M. K. Esancy, K. P. Mills, W. M. Whaley, and B. J. Dabney. An approach to the design od non-mutagenic azo dyes. 1. The identification of non-mutagenic precursors and potential metabolite. *Dyes Pigments* 8:417–430 (1987).
90. P. Mohan, R. Singh, J. Wepsiec, I. Gonzalez, D. K. Sun, and P. S. Sarin. Inhibition of HIV replication by naphthalene-monosulfonic acid derivatives and a bis naphthalenedisulfonic acid compound. *Life Sci.* 47:993–999 (1990).
91. P. Mohan, R. Singh, and M. Baba. Potential anti-AIDS agents. Synthesis and antiviral activity of naphthalenesulfonic acid derivatives against HIV-1 and HIV-2. *J. Med. Chem.* 34:212–217 (1991).
92. P. Mohan, R. Singh, and M. Baba. 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 (1991).
93. P. Mohan, R. Singh, and M. Baba. Novel naphthalenedisulfonic acid anti-HIV-1 agents. Synthesis and activity against reverse transcriptase, virus replication and syncytia formation. *Drug Design Discov.* 8:69–82 (1991).
94. P. Mohan, A. J. Hopfinger, and M. Baba. Naphthalenesulfonic acid derivatives as potential anti-HIV-1 agents. Chemistry, biology and molecular modeling of their inhibition of reverse transcriptase. *Antiviral Chem. Chemother.* 2:215–222 (1991).
95. M. Baba, R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, and E. De Clercq. Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc. Natl. Acad. Sci. USA* 85:6132–6136 (1988).
96. D. Schols, R. Pauwels, J. Desmyter, and E. De Clercq. Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T-cells persistently infected with HIV-1. *Virology* 175:556–561 (1990).
97. M. Baba, R. Snoeck, R. Pauwels, and E. De Clercq. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrob. Agents Chemother.* 32:1742–1745 (1988).
98. M. Cushman, P. Wang, S. H. Chang, C. Wild, E. De Clercq, D. Schols, M. E. Goldman, and J. A. Bowen. 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 (1991).
99. P. J. Bugelski, H. Ellens, T. K. Hart, and R. L. Kirsh. Soluble CD4 and dextran sulfate mediate release of gp 120 from HIV-1: Implications for clinical trials. *J. Acquir. Immune Def. Syndr.* 4:923–924 (1991).
100. W. A. Haseltine. Replication and pathogenesis of the AIDS virus. *J. Acquir. Immune Def. Syndr.* 1:217–240 (1988).
101. M. Baba, 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. Novel sulfated polysaccharides: Dissociation of anti-human immunodeficiency virus activity from antithrombin activity. *J. Infect. Dis.* 161:208–213 (1990).
102. K. J. Lorentsen, C. W. Hendrix, J. M. Collins, D. M. Kornhauser, B. G. Petty, R. W. Klecker, C. Flexner, R. H. Eckel, and P. S. Lietman. Dextran sulfate is poorly absorbed after oral administration. *Ann. Intern. Med.* 111:561–566 (1989).
103. H. Nakashima, O. Yoshida, M. Baba, E. De Clercq, and N. Yamamoto. Anti-HIV activity of dextran sulfate as determined under different experimental conditions. *Antiviral Res.* 11:233–246 (1989).
104. N. R. Hartman, D. G. Johns, and H. Mitsuya. Pharmacokinetic analysis of dextran sulfate in rats as pertains to its clinical usefulness for therapy of HIV infection. *AIDS Res Hum. Retrovir.* 6:805–812 (1990).
105. M. Baba, D. Schols, E. De Clercq, R. Pauwels, M. Nagy, J. Gyorgyi-Edelenyi, M. Low, and S. Gorog. Novel sulfated polymers as highly potent and selective inhibitors of human immunodeficiency virus replication and giant cell formation. *Antimicrob. Agents Chemother.* 34:134–138 (1990).
106. E. De Clercq. Potential drugs for the treatment of AIDS. *J. Antimicrob. Chemother.* 23(Suppl. A):35–46 (1989).
107. M. Baba, D. Schols, R. Pauwels, H. Nakashima, and E. De Clercq. Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: A new strategy towards AIDS chemotherapy. *J. Acq. Immune Defic. Syndr.* 3:493–499 (1990).
108. P. Mohan and M. Baba. Unpublished results.
109. R. T. Williams. Detoxication mechanisms. In *The Metabolism and Detoxication of Drugs, Toxic Substances and Other Inorganic Compounds*, Chapman and Hall, London, 1959, pp. 497–500.
110. P. L. Batten. Metabolism of 2-naphthylamine sulfonic acids. *Toxicol. Appl. Pharmacol.* 48:A171 (1979).
111. W. R. Michael. Metabolism of linear alkylate sulfonate and alkyl benzene sulfonate in albino rats. *Toxicol. Appl. Pharmacol.* 12:473–485 (1968).
112. P. Mohan, D. Schols, M. Baba, and E. De Clercq. Sulfonic acid polymers as a new class of human immunodeficiency virus inhibitors. *Antiviral Res.* (in press).
113. N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto, and Z.-G. Guo. Medicinal plants in therapy. *Bull. WHO* 63:965–981 (1985).
114. J. F. Davies II, Z. Hostomska, Z. Hostomsky, S. R. Jordan, and D. A. Matthews. Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. *Science* 252:88–95 (1991).
115. R. Powers, G. M. Clore, A. Bax, D. S. Garrett, S. J. Stahl, P. T. Wingfield, and A. M. Gronenborn. Secondary structure of the ribonuclease-H domain of the human immunodeficiency virus reverse transcriptase in solution using 3-dimensional double and triple resonance heteronuclear magnetic resonance spectroscopy. *J. Mol. Biol.* 221:1081–1090 (1991).
116. W. A. Haseltine. Development of antiviral drugs for the treatment of AIDS: Strategies and prospects. *J. Acq. Immune Defic. Syndr.* 2:311–334 (1989).
117. M. Roederer, F. J. T. Staal, P. A. Raju, S. W. Ela, L. A. Herzenberg, and L. A. Herzenberg. Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. *Proc. Natl. Acad. Sci. USA* 87:4884–4888 (1990).
118. F. J. T. Staal, M. Roederer, and L. A. Herzenberg. Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 87:9943–9947 (1990).