Reviews

Natural Product-Based Anti-HIV Drug Discovery and Development Facilitated by the NCI Developmental Therapeutics Program

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During the decade 1987−1996, the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) provided infrastructure support for both intramural and extramural anti-HIV (human immunodeficiency virus) drug discovery research and development. This retrospective review describes some of the anti-HIV lead discovery and development that took place under DTP auspices or which was substantially facilitated by resources made available through the DTP. Examples highlighted include leads identified through the initial screening of pure natural product derived compounds and those derived from bioassay-guided fractionation of crude natural product extracts, and these are classified according to the mechanism of action targeting the critical steps within the replication cycle of HIV.

Introduction

During the decade 1987−1996, the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) provided infrastructure support for both intramural and extramural anti-HIV (human immunodeficiency virus) drug discovery research and development. Resources available through the DTP have included the cell-based, *in vitro* anti-HIV primary and secondary screening assays,[1,2] *in vivo* assays employing the hollow fiber model,[3] and, for selected leads, a range of preclinical development activities including synthesis, pharmaceutical formulation, and toxicology in both AIDS and cancer.[4,5] The DTP also made available its natural products repository (NPR) to qualified intramural and extramural researchers and organizations. Currently, although the cell-based anti-HIV screening effort has been reduced, the overall antiviral program is being continued with a current emphasis on target-based assays.

The NPR comprises more than 150,000 diverse extracts of plant, marine, and microbial origin, derived from an NCI contracts-based, worldwide collection program. Details of the NPR and current access policies and procedures are available on the Internet (http://dtp.nci.nih.gov/). Reviews encompassing further details of the NPR and natural products collections programs are available,[4] as well as overviews of the origins of the DTP's anticancer[5] and anti-HIV screen and preclinical drug development programs.[6,7]

The present retrospective review describes some of the anti-HIV lead discovery and development that took place under DTP auspices or which was substantially facilitated by resources made available through the DTP. The examples to be highlighted include leads identified both through the initial screening of pure natural product derived compounds and those derived from bioassay-guided fractionation of crude natural product extracts showing activity in the NCI primary screen. Furthermore, the examples to be described are derived both from extramural and intramural investigator efforts and are not limited to isolates from the NPR.

Although the cell-based anti-HIV primary screen that has been provided by the DTP has not been specifically "mechanism-based", it has nonetheless facilitated the identification of natural product leads that block key points in the HIV life cycle. Some of these key points, as illustrated in Figure 1,[8] include (1) the point of viral attachment to the cell surface; (2) viral penetration of the cell membrane; (3) viral RNA decoding for reverse transcription of the proviral DNA; (4) integration of the proviral DNA into the host genome; (5) synthesis of viral proteins including the zinc fingers of the nucleocapsid protein; (6) the appropriate processing of the viral polypeptide with the HIV protease and the assembly of the viral DNA and proteins into a viral particle to the final maturation and shedding of the mature infectious viral particles (Figure 1[8]). It should be mentioned that since the anti-HIV screen was a cell-based screen, interpretation of the mechanism of action has been limited. Active leads discovered at a particular phase of the viral replication cycle have been subjected to further analysis by detailed mechanism studies including antibody/receptor competitive assays (e.g., CD4, CXCR4, or CCR5, etc.), enzymatic assays (additional reverse transcriptase, DNA polymerase, and RNA polymerase), and nucleic acid binding gel shift assays (for further details, refer to ref 2).

Natural Products and/or Natural Product-Based Anti-HIV Surface Acting Agents. At the earliest infection step, HIV-1 attaches to the surface of a CD4+ lymphoid cell via an interaction between the viral gp120 glycoprotein and the CD4 receptor molecule of the cell (Figure 1).[8,9] Accessory co-receptors have since been identified as part of this process but have not been employed in the routine anti-HIV screen. This event is then followed by fusion of the viral envelope with the cell membrane, a process facilitated by gp 41,[10] a cleavage product, along with gp120, of the viral glycoprotein gp160,[11] involving also secondary host cell chemokine receptors. At this initial infection phase, two targets can be exploited for the

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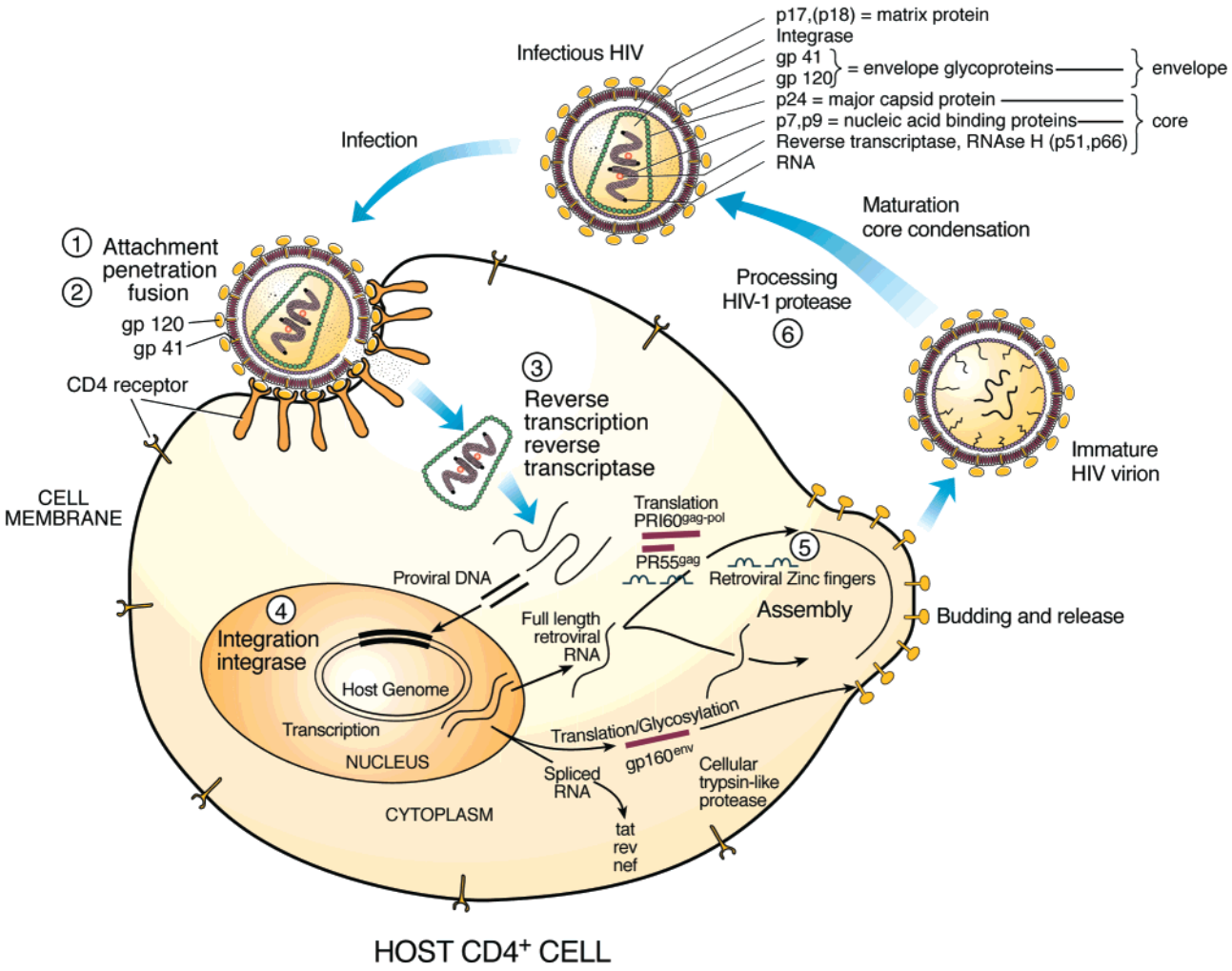


Figure 1. Targets within the different phases of HIV-1 viral replication cycle and infection of a T-cell, as used for anti-HIV cell-based assays.[1,2]

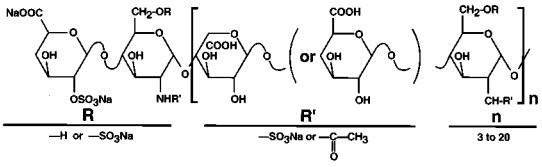
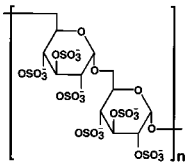
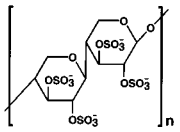
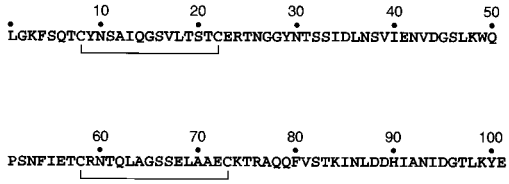
screening of antiviral agents. Specifically, the antiviral agents can act at the cell surface to inhibit the interaction of viral and cellular receptors, or they can prevent cell fusion by blocking the viral transmembrane gp 41. Blocking of cell fusion also blocks cell−cell transmission of HIV virus.

The DTP has identified a number of leads that appear to inactivate HIV, primarily by binding with the viral surface envelope glycoprotein gp120. They include the anionic sulfated polysaccharides, pentosan sulfate (NSC 626201), dextran sulfate (NSC 620255), and enoxaparin (NSC 648070), a phosphorothioate oligonucleotide (ISIS 5320; NSC 665353), and a microbial protein, cyanovirin-N (NSC 682999). When a cell surface acting agent was identified by the cell-based anti-HIV screening assay, a study of its mechanism of action was carried out using biochemical assays, such as the gp120-CD4 ELISA assay.[2]

Enoxaparin is a low molecular weight sulfated glycosaminoglycan with an average molecular weight of 4500 Da (Table 1).[12,13] It was produced by controlled depolymerization of a benzyl ester of unfractionated natural porcine heparin digest and is used clinically in Europe, Canada, and the United States as an antithrombolytic agent, especially in elective surgery to prevent thromboembolism.[12] Enoxaparin was submitted to the NCI by Rhone Poulenc for testing against HIV. In the primary antiviral screen, as well as in subsequent detailed mechanistic studies, enoxaparin was found to protect human T cells, both CEM-SS and MT-4, as well as peripheral blood leukocytes, against the cytopathogenic effect of a number of HIV-1 variants. It showed a range of inhibitory activities against HIV-1 wild-type IIIb and RF strains, HIV-2, and a pyridinone-resistant strain, A-17. In tissue culture, it also inhibited Rauscher murine leukemia virus infection of murine cells. Enoxaparin exhibited an EC_{50} value in the range of 0.2−1.6 μM against HIV-1 and exhibited little or no apparent toxicity. The cell inhibitory concentration at 50% of growth (IC_{50}) for exnoxaparin ranged from 56 to180 μM when tested in CEM cells, MT-4 lymphocytes, or peripheral blood lymphocytes (PBLs). This gave an averaged therapeutic index (TI_{50}) value of 280. Mechanistically, enoxaparin interacts with viral gp120 and competes with the binding of gp120 to the CD_4 recognition site on the T-lymphocyte cell surface, as suggested by the stoichiometric relationship observed between the number of CD_4−gp120 interactions and the concentration of enoxaparin required to prevent these interactions. This was observed with all the other sulfated polyanionic agents discussed below, where inhibitory action is based on binding with HIV gp120, a phenomenon well analyzed in experiments using anti-gp120 mAb and radioactive labeling.[13,14] Furthermore, enoxaparin exhibited no detectable effect on virus-specific enzymatic processes (NCI unpublished data).

A potential disadvantage associated with enoxaparin and some other sulfated polysaccharides is that it is an anticoagulant. The critical issue is whether an effective inhibitory plasma concentration level against HIV can be achieved

Table 1. Surface Acting Anti-HIV Compounds Discovered and/or Developed in the DTP/NCI

		EC_{50} μM	TI_{50}
Compounds targeting attachment			
Enoxaparin m.w. 4,500		0.20	280
Dextran sulfate m.w. 8,000		0.003	>582
Pentosan sulfate m.w. 8,000		0.014	>1,888
Compounds targeting attachment/fusion			
ISIS 5320 m.w. 2,582	T2G4T2	0.073	>172
Cyanovirin-N m.w. 10,957		0.500	2,200

Protein with 101 amino acids with two internal disulfide bonds isolated from *Nostoc ellipsosporum*

in patients without producing significant anticoagulant activity, thereby predisposing patients to the risk of bleeding. The difficulty of separating the anticoaggulating factor from the batch preparation of enoxaparin for anti-HIV clinical purposes became a handicap to its further advancement.

Dextran sulfate is essentially a natural product-based semisynthetic sulfated polyanion composed of α-(1→6) linked D-glucose residues in polymeric form (Table 1). Dextran was first identified as a high molecular weight polysaccharide produced by microbes (e.g., *Leuconostoc mesenteroides* and *L. dextranicum*) from sucrose. In a manner similar to enoxaparin, dextran sulfate inhibits HIV infection of T lymphocytes by competing with HIV-1 binding to the CD4+ recognition site on the cell surface. The mechanism of action of dextran sulfate has been well studied and defined. As early as 1988, Mitsuya et al.[15] reported that dextran sulfate was observed to be most effective in inactivating HIV when added to the cells during the virus adsorption. This was confirmed later by Baba et al.[16] In a time course study, the effectiveness of a cell surface acting agent is restricted to its presentation prior to or at the time of viral infection and attachment; synthesis of proviral DNA would then be blocked completely. If the surface acting agent is introduced during or after this time, thus allowing viral attachment to occur, proviral DNA synthesis and subsequent virus replication can occur. Likewise, in conditions where the T-cells are exposed chronically to HIV infection, as in an HIV-infected host, complete or total protection of T-cells by such surface acting agents becomes almost impossible.

Similarly, pentosan sulfate is a semisynthetic sulfated polyanion polymer composed of β-D-xylopyranose residues in polymeric form (Table 1). It is also a surface acting agent, inhibiting HIV infectivity by binding to the viral gp120 as discussed above. Since such inactivation is reversible, it is, therefore, not virucidal. Pentosan sulfate progressed to Phase I clinical trial in 1991 at the NCI with HIV-1-infected patients and with patients suffering from Kaposi's sarcoma. A separate Phase I trial was also conducted, focusing primarily on evaluating the toxicity and feasibility of administering pentosan sulfate by continuous intravenous infusion at anticoagulant doses up to eight weeks. Both trials were closed by the end of 1991, when adverse results attributed to dextran sulfate were observed elsewhere.

The phosphorothioate oligonucleotide ISIS 5320 (NSC 665353), $T_2G_4T_2$, was submitted to the NCI for antiviral screening against HIV by ISIS Pharmaceuticals. This oligonucleotide-based synthetic compound is an 8-mer oligonucleotide with a phosphorothioate backbone that forms a positively charged tetramer of a G quartet, in the shape of a barrel in solution.[17] This compound displayed an EC_{50} of 0.07−0.3 μM in the primary screen and was

found to be toxic at 125 μM. ISIS 5320 became inactive upon heat denaturation, but activity was restored upon renaturation, indicating that the stable tertiary structure is essential for antiviral activity.[17] Its range of anti HIV-1 action included strains such as wild-type IIIB and RF, the pyridinone-resistant A-17, the AZT-resistant and the nevirapine-resistant strains, and also the HIV-2 ROD strain. Although ISIS 5320 was reported to inhibit HIV-1 reverse transcriptase,[17] its other inhibitory mechanism was also consistent with the capability of the tetrameric configuration of ISIS 5320 to bind with the V3 loop[17] of the viral gp120, and therefore, effectively block viral infection. A distinction between ISIS 5320 and other surface acting agents is its ability to inhibit cell fusion (Figure 1) following viral attachment. This was well documented in a study[17] conducted with HeLa/CD4/LTR/ cells cocultivated with HIV-infected cells, in which ISIS 5320 inhibited cell fusion and, thus, subsequent β-gal expression at an EC_{50} of approximately 0.3 μM. Cocultivation of chronically HIV-infected CEM-SS cells with uninfected CEM-SS cells in the presence of ISIS 5320 confirmed this result. Preliminary toxicology studies suggested that high levels (relative to the EC_{50}) of this compound are retained preferentially in the lymph nodes and several other organs for 3−4 months, suggesting that it is a fairly stable compound. ISIS 5320 (NSC 665353) is undergoing clinical trials by ISIS Pharmaceuticals.

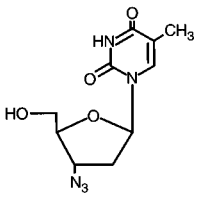
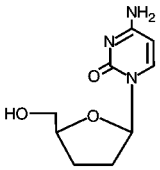
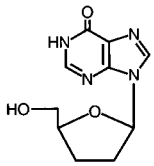
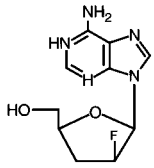
A novel anti-HIV protein, cyanovirin-N (CV-N; NSC 682999), was isolated from the cellular extract of a cyanobacterium, *Nostoc ellipsosporum*, by Boyd et al.[18] (Table 1). CV-N is a polypeptide consisting of 101 amino acids with four cysteine residues enabling two intrachain disulfide linkages. The intact disulfide linkage is essential for the remarkable anti-HIV activity of CV-N; disruption of the disulfide bond resulted in a loss of the anti-HIV activity.[19] CV-N inhibited a spectrum of T-tropic HIV-1 strains, HIV-2 and simian immunodeficiency virus (SIV), as well as macrophage tropic (M-) and also dual tropic (T- and M-) clinical isolates of HIV-1.[19] CV-N inhibition of HIV was mediated through interactions with the viral surface envelope glycoprotein, gp120. It was also reported to block *in vitro* cell fusion, thus blocking transmission of HIV-1 between infected and uninfected cells. Most importantly, its inhibitory effect, distinct from other cell surface acting agents, is irreversible, and therefore, it is virucidal. Currently, a recombinant form (NSC 683000) has been produced in large quantity in *Escherichia coli*. This recombinant polypeptide shows equipotent anti-HIV activity when compared to the original.[18]

Natural Product-Based Anti-HIV Reverse Transcriptase Inhibitors. The rationale behind the inclusion of deoxynucleosides as natural product-based antiviral drugs originated in a series of publications dating back as early as 1950, in which Bergmann et al.[20−22] described the discovery and isolation of thymine pentofuranoside from the air-dried sponges (*Cryptotethia crypta)* of the Bahamas. Bergman and Feeney[20,21] concluded that the compound was a xylofuranoside and named it spongothymidine to indicate its origin and its relationship to thymidine.[20−22] Until the discovery of biologically active nucleosides that did not contain ribose or deoxyribose, chemists had widely modified the bases but not the sugars in search of better antibiotics.[23] With the recognition that the sugar moiety could be modified while still maintaining biological activity, a significant number of nucleosides were made with regular bases but modified sugars, or both acyclic and cyclic derivatives, including AZT[24] and ultimately acyclovir.[25] Thus, extending this logic to other sugar derivatives such as dideoxy compounds, one may argue that the principle of sugar modification with maintenance of biological activity came from the identification of the sponge-derived products as non-ribose/deoxyribose nucleosides. This does not mean that the dideoxy compounds are natural products, but rather that the underlying chemical and biological principles came from natural product discoveries. Indeed, the discoveries of spongothymidine and spongouridine aided significantly in the research interests and synthetic modification of nucleoside compounds. This led to the generation of nucleoside antivirals and other antitumor agents by synthetic modification, such as cytosine arabinoside a number of years later[26] or adenine arabinoside more than 15 years later.[23,25−29] Subsequent second- and third-generation nucleosides led to the development of dideoxynucleosides such as dideoxycytidine and 3′-azido-2′,3′-dideoxythymidine, commonly known as AZT.[25,28,29]

Dideoxynucleoside Analogues. Reverse transcription of the viral RNA for the synthesis of proviral DNA (Figure 1) takes place within 2−4 h following initial viral infection. A logical approach to inhibit the reverse transcription process is to block the elongation of the nascent oligonucleotide chain during proviral DNA synthesis. In the early period (1985−1988) of the emergence of the human acquired immunodeficiency syndrome, a number of dideoxynucleoside derivatives and analogues were tested against HIV. NCI scientists working in collaboration with pharmaceutical companies developed a number of natural product-based anti-HIV 2′,3′-dideoxynucleosides (ddN's). Targeting the polynucleotide chain elongation during the proviral DNA synthesis, ddN analogues such as AZT, ddC, ddI, F-ddA, d4T, and 3TC (Table 2), developed fully or in part by the NCI, represent a class of synthetic agents based on modification of the natural nucleosides. These ddN's, upon intracellular phosphorylation, become competitive inhibitors with respect to the deoxynucleoside triphosphate substrate, thus blocking the viral reverse transcription process by acting as chain terminators[30,31] of the growing nascent DNA chain. As presented in Table 2, the average EC_{50} values of the ddN's are generally at nanomolar concentration levels. They are extremely effective when applied during the early phase from the time prior to viral attachment to 2 h post HIV infection, which is the time required for proviral DNA synthesis. The range of inhibitory activity of dideoxynucleoside analogues includes HIV-1, HIV-2 and the simian immunodeficiency viruses (SIV), and all the non-nucleoside inhibitor resistant HIV variants. AZT, 3′-azido-2′,3′-dideoxythymidine[31−35] (Table 2), commonly known as zidovudine or Retrovir, which has an azido substitutuent at the 3′ position of dideoxyribose, was the first ddN tested by the NCI in collaboration with Burroughs Wellcome, for treatment of AIDS patients. It was initially tested *in vitro* for its efficacy in inhibiting the cytopathic effect of human T-lymphotropic virus type III, HTLV-III, in 1985.[31] AZT was then used successfully in patients with AIDS in 1986[32,33] and was the first antiviral agent against HIV approved by the FDA. It has proven to be a most effective agent against HIV in patients with AIDS and is the most widely used globally in combination or in sequential therapy with other anti-HIV agents.

As well documented in the clinical history of viral diseases, in the 1980s, long-term treatment of AIDS patients with monotherapy of AZT inevitably resulted in the emergence of drug-resistant HIV variants. The most frequently observed drug-resistant HIV variants in AZT-treated patients generally bear mutations at codons 67

Table 2. Natural Product-Based Dideoxynucleoside Reverse Transcriptase Inhibitors

Dideoxynucleoside Analog	Structure	Anti-HIV-1$_{RF}$ or IIIB EC_{50} μM	TI_{50}
AZT (3'-azido-2',3'-dideoxythymidine) m.w. 267		0.001	>1000
ddC, dideoxycytidine m.w. 211		0.010 - 0.001	200
ddI, dideoxyinosine m.w. 218		0.0056	1,760
β-FddA, β-fluorodideoxyadenosine m.w. 253		1.16	>17.0

(D67N) aspartic acid→asparagine, 70 (K70R) lysine→arginine, and 215 (T215F/Y) threonine→phenylalanine or tyrosine.[36] Later reports showed that resistance mutations associated with long-term AZT treatment resulted in changes at five amino acid residues of the HIV-I reverse transcriptase; they are at codons 41 (M41L) methionine→leucine and 219 (K219Q) lysine→glutamine,[37,38] in addition to D67N, K70R, and T215F/Y. In light of this resistance problem, it became absolutely essential to look for other novel inhibitors of HIV reverse transcriptase. Other dideoxynucleoside analogues synthesized and undergoing testing and development during that period included ddC, ddI, d4T, and 3TC, and later 5-fluoro-ddA, which was discovered at the NCI (Table 2). Despite the inevitable emergence of HIV-resistant strains to AZT, this resistance profile was apparently directed specifically at AZT. Cross-resistance to ddC and ddI was rarely observed and, therefore, was not a deterrent to the use of ddC or ddI in therapy with AZT.[39−41] Phase I studies of ddC as a single agent, or in combination or alternating with AZT, in patients with severe HIV infection were undertaken by the NCI in 1988[39,40] and also by other clinical trial groups sponsored by the National Institute of Allergy and Infectious Diseases.[41,42]

ddI, also known as Didanosine or Videx (Table 2), was evaluated with AZT in combination therapy, as compared to an alternating regimen by Yarchoan et al.[43] at the NCI. A Phase I drug safety and immunology trial with AZT, levamisole, and ddI was also conducted by the NCI.[42] Other clinical trials conducted by NCI or sponsored by NIAID involved ddI in combination therapy with Ritonavir, a peptide-based protease inhibitor, in comparison with AZT plus Ritonavir, or Ritonavir alone, in pediatric AIDS patients.[42] Furthermore, earlier pharmacokinetics evaluation and a safety trial involving a triple regimen of ddI, 3TC, and AZT was also conducted at the NCI and another site.[41] ddI was the second anti-retroviral agent approved by the FDA for use against HIV infection in 1991. On the basis of results in two different clinical trials sponsored by NIAID, the FDA recommended ddI as the first-line therapy against HIV in both adults and pediatric AIDS patients.[42]

An HIV variant demonstrating resistance to ddI was later identified as associated with a leucine→valine substitution at codon 74 (L74V) of the reverse transcriptase,[44] whereas resistance to ddC was associated with a threonine→aspartic acid substitution at codon 69 (T69D).[45]

Fluoro-ddA (Table 2) was synthesized and developed by Driscoll et al.[46,47] Although F-ddA was comparatively less potent, it was found to be less toxic and was well tolerated *in vivo*. In addition, as in the case of ddC, it was effective against many clinically drug-resistant isolates from patients treated with AZT or ddI, or a combination of AZT and ddI.[47] F-ddA, like AZT, was also effective when tested *in vitro* against five clades of HIV (A, B, C, D, and E).[47] It was submitted for a Phase I pharmacokinetics and safety trial at the NCI in April of 1996. Currently, further clinical development is in progress under a collaborative agreement with a pharmaceutical company.

Both 3TC (NSC 697911) [2(1*H*)-pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl-,(2*R-cis*)-lamivudine] and d4T (NSC 163661) [3′-deoxy-2′-thymidine] were also submitted for development at the DTP. Both deoxynucleoside analogues were active against wild-type HIV-1/IIIB and RF strains with EC_{50} values in the 0.06−0.10 μM range. The efficacy of 3TC against the 4xAZT-resistant HIV variant[48] was found to be reduced only slightly.[50] Interestingly, HIV variants isolated from patients undergo-

ing 3TC treatment in a Phase I/II clinical trial at the NCI were reported to be more sensitive to AZT inhibition.[49] Thorough preclinical characterizations as well as clinical studies with 3TC, especially against pediatric HIV infections, have been carried out by the NCI. In 1993, Phase I/II safety and efficacy trials on 3TC were performed by Pizzo et al. at the NCI and also at the Childrens Hospital of Los Angeles.[41] Other Phase I/II dose-ranging open-label phamacokinetics and safety trials with 3TC in combination with AZT and ddI in pediatric HIV-infected patients were also conducted at these sites.[41] Similar efficacy trials with 3TC were carried out with patients suffering from HIV disease progression while undergoing treatment with AZT, ddC, or ddI.[42] The resistance profile of HIV-1 variants against 3TC have been well characterized by Wainberg's laboratory in Canada.[51] 3TC has now been approved by the FDA for AIDS therapy.

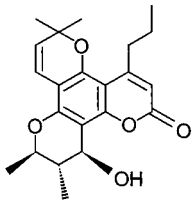
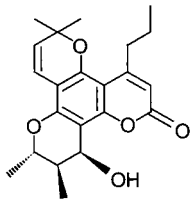
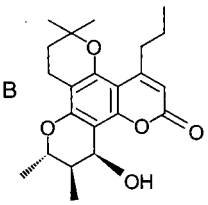
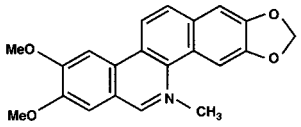
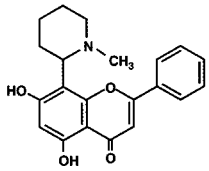
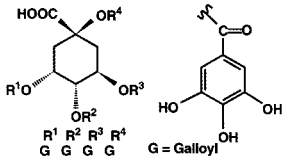
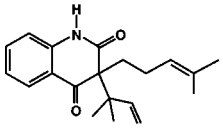
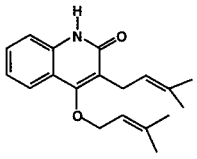
Natural Products and/or Natural Product-Based Non-nucleoside Reverse Transcriptase Inhibitors. The emergence of drug-resistant HIV variants in patients treated with the dideoxynucleoside analogues accelerated the search for other effective inhibitors of the HIV reverse transcriptase. A number of non-nucleoside agents have been discovered that inhibit the HIV reverse transcriptase by interacting at a site distinct from that of the catalytic site. The possibility of their use in combination therapy with AZT or other dideoxynucleoside analogues has led to some exciting approaches to drug selection as well as drug design and/or modification, based on drug-resistance analysis.[52,53] Since these agents basically represent pharmacophores distinctly different from that of the deoxynucleosides, they are called non-nucleoside reverse transcriptase inhibitors (NNRTI). The NNRTIs can be considered as a group of potent RT inhibitors having considerable structural diversity[54] but sharing certain common characteristics in their inhibitory profiles.[54] Specifically, they inhibit primarily HIV-1 RT, but fail to inhibit HIV-2 RT or SIV RT; this suggests that both HIV-2 RT and SIV RT differ structurally from HIV-1 RT to the extent that they cannot be recognized or are not accessible to the NNRTIs. Many NNRTIs target or bind with the enzyme at a non-nucleoside binding site (NNRB) in a reversible manner. This NNRTI binding site consists of 48 amino acids, residing within the p66 subunit of the RT.[55] Although the NNRB locates at a distance from the catalytic site, the NNRTI interacts allosterically with the RT, altering the structural conformation of the RT, resulting in a partial or total disruption of the catalytic complex of the RT, template-primer, and the substrate.[55,56] Since NNRTIs are allosteric inhibitors, certain NNRTIs were found to be synergistic with the dideoxynucleoside analogues, as will be discussed later. A number of the potent NNRTIs listed in Table 3 were isolated from plants collected through NCI contracts operating worldwide. Among the NNRTIs isolated by the NCI Laboratory of Drug Discovery Research and Development (LDDRD) (Table 3), one promising compound, (+)-calanolide A (NSC 660886), discovered[57] and isolated from a Malaysian (Sarawak) tropical rainforest tree, *Calophyllum lanigerum* Miq. var. *austrocoriaceum* (Clusiaceae), is discussed in detail.[58] Extraction of the fruit and twigs of *C. lanigerum*, followed by bioassay-guided fractionation and purification, yielded eight calanolide analogues.[59] Among these analogues, (+)-calanolide A and (−)-calanolide B (NSC 661122, costatolide), and its dihydro derivative, (−)-7,8-dihydrocalanolide B (NSC 661123, dihydrocostatolide), significantly inhibited the cytopathic effects of HIV-1 in T-cell lines, including both CEM-SS cells and MT-2 cells. It should be noted that the optical isomer of (+)-calanolide A, i.e., (−)-calanolide A (NSC 675449), exhibited no anti-HIV activity. The range of antiviral activity of these active calanolides has been well studied and reported.[58,60,61] The calanolides, however, were not active against HIV-2 or SIV, and they all shared inhibitory characteristics consistent with the NNRTI profile discussed above.[54] Detailed kinetic analysis of inhibition of HIV-1 reverse transcriptase by calanolide A using the template/primer systems with ribosomal RNA and the homopolymeric rA-dT 12-18 reported earlier indicated that two calanolide binding sites, one competitive and the other noncompetitive, exist in the RT, thus explaining the anti-RT inhibitory characteristic of this compound.[62] Other studies of the inhibition of NNRTI-resistant HIV-1 variants by the calanolides all suggested that they shared a common binding site for NNRTIs.[63]

(+)-Calanolide A also inhibited the AZT-resistant variant G9106 at an EC_{50} of $\leq 0.03\ \mu M$, and the variant A17 strain, developed by Merck, at an EC_{50} of $\leq 0.60\ \mu M$. The latter A17 pyridinone-resistant variant had two mutations at codon 181(Y181C), tyrosine→cysteine, and codon 131-(L103N), leucine→asparagine. This was considered unique because most NNRTIs were not able to inhibit the HIV-1 variant bearing the Y181C amino acid change.[64] This unique efficacy of calanolide A encouraged extensive, detailed analysis to fully characterize the antiviral spectrum of the calanolides. The most recent analysis includes the following findings and conclusions in a comparative study of the three calanolide isomers.[61]

All three calanolides inhibited the laboratory-adapted HIV-1 variants, the clinical viral isolates, inclusive of the diverse clades (A−F), syncytium-inducing and non-syncytium-inducing isolates, and T-tropic and monocyte-tropic isolates.[61] The calanolide isomers were also found to be effective against a wide spectrum of drug-resistant HIV strains isolated from patients' T-cells in tissue culture or HIV-infected T-cell cultures in the presence of the test drug. In most cases, their efficacy against the drug-resistant mutants remained the same with little increase in EC_{50}, when compared with their corresponding inhibition of the wild-type HIV-1. Exceptions were observed in assays against mutants with RT amino acid changes of V108I, L100I, and Y181C. Mutations at these amino acid residues V108, L100, and Y181 of the RT have been well documented as conferring a marked increase in drug resistance.[65] The V108I mutant is an isolate associated with thiazolobenzimidazole or TIBO treatment, and the calanolide isomers showed lessened effectiveness against this mutant.[61] The calanolide isomers also showed markedly lowered efficacy against the L100I mutant isolated from oxathiin carboxanilide (UC10; NSC 645129) treated, HIV-infected T-cell culture.[61,65] Other mutant variants against which the calanolide isomers showed reduced inhibitory efficacy include the K103N, the T139I, and the Y188H mutant.[61] The K103N and the T139I mutants were isolates derived from T-cell cultures treated with calanolide B alone or in combination with UC10. The Y188H mutant was isolated from T-cell culture treated with calanolide B alone.[61]

In the case of the Y181C-bearing mutant isolated from T-cell cultures exposed to either diphenyl sulfone, nevirapine, pyridinone, or UC38 (NSC 629243), the calanolide isomers were able to *sensitize* the mutant such that the inhibitory efficacy became manyfold more effective and exhibited much lower EC_{50} values.[61] The calanolide isomers also exhibited the characteristic of being able to synergize

Table 3. Non-nucleoside Reverse Transcriptase Inhibitory Leads Discovered by NCI LDDRD

Common Name	Structure	Taxonomy/Source	Anti-HIV-1_{RF} EC_{50} μM	TI_{50}
(+)-Calanolide A m.w. 370		*Calophyllum lanigerum* Sarawak, Malaysia	0.2	103
(−)-Calanolide B m.w. 370		*Calophyllum lanigerum* Sarawak, Malaysia	0.2	>20
(−)-7,8-Dihydrocalanolide B m.w. 370		*Calophyllum lanigerum* Sarawak, Malaysia	0.1	99.8
Nitidine m.w. 348		*Toddalia asiatica* India, China, Taiwan, Japan	14	3
O-demethyl-buchenavianine m.w. 351		*Buchenavia capitata* Dominican Republic	0.26	3
Galloylquinic acids		*Lepidobotrys staudtii* Cameroon	0.5	20
Buchapine m.w. 310		*Euodia roxburghiana* Thailand	0.94	31
Quinolone m.w. 297		*Euodia roxburghiana* Thailand	1.64	17

with other NNRTIs, such as nevirapine,[61] Uniroyal compound UC781 (NSC 675186),[61,66] the diarylsulfone (NSC 624231),[67,68] and the protease inhibitors, Ritonavir and Saquinavir,[61] in the inhibition of HIV. This characteristic was especially striking with (−)-calanolide B, which stood out among the three isomers. This property, in addition to the capability of the calanolide isomers to sensitize the Y181C-bearing drug-resistant HIV mutants, suggests that the calanolide isomers may be promising candidates for combination therapy with either nucleoside and/or protease inhibitors against HIV in patients.

The NCI has played an active and supportive role in the development of the calanolide class of compounds including aspects of preclinical development, such as the synthesis of analogues, *in vivo* animal assays,[69] serum-binding studies, formulation, pharmacology, and toxicology. A Phase I

clinical trial for assessing the safety and tolerability of (+)-calanolide A was conducted by Sarawak MediChem Research, Inc., a joint venture company formed by the Sarawak State Government and MediChem Research Inc., which has the exclusive license rights to the calanolides. Recently, multicenter Phase II clinical trials on patients with HIV infection were initiated in the Uniteds States and in Malaysia.

Other natural product-derived anti-HIV agents listed in Table 3 include the alkaloid nitidine, which was isolated from the roots of *Toddalia asiatica* (Rutaceae),[70] a plant native to India, the People's Republic of China, Taiwan, and Japan. Nitidine showed significant anti-HIV activity in the cell-based assay and was reported earlier to inhibit HIV reverse transcriptase.[71] An aporphine alkaloid, magnoflorine, isolated from the same plant showed insignificant anti-HIV activity.

The piperidine-flavone-related alkaloid, *O*-demethylbuchenavianine, isolated from *Buchenavia capitata* (Vahl) Eichl. (Combretaceae)[72] collected in the Dominican Republic (Table 3), showed activity in both the NCI anti-HIV and anti-cancer cell-based screens.

Triterpenes of the maprounic acid/aleuritolic acid class isolated from *Maprounea africana* Muell.-Arg. (Euphorbiaceae), collected in the Central African Republic, showed anti-HIV reverse transcriptase activity with IC_{50} values between 15 and 40 μM (Table 3).[73,74]

One of three galloylquinic acids, specifically 1,3,4,5-tetra-*O*-galloylquinic acid, isolated from the stem bark of the monotypic plant *Lepidobotrys staudtii* Engl. (Lepidobotryaceae),[75] obtained from Cameroon, demonstrated significant anti-HIV activity (Table 3). It protected CEM-SS cells from the cytopathic effects of HIV-1_{RF} at an EC_{50} value of 0.5 μM, but was much less effective against HIV-2_{ROD} with an EC_{50} of 10 mM in the cell-based assay. The same compound was also reported to inhibit recombinant reverse transcriptases of HIV-1 and HIV-2 at IC_{50} values of about 0.8 μM. Unfortunately, cellular DNA polymerases α and β were also affected.[75]

Several species of the genus *Euodia* have been used in folk medicine by indigenous peoples in Southeast Asia and Australia. Two known compounds, buchapine and 3-(3-methyl-2-butenyl)-4-[(3-methyl-2-butenyl)oxy]-2(1*H*)-quinolinone, extracted from *Euodia roxburghiana* Benth. (Rutaceae) collected in Thailand,[76] showed significant anti-HIV activity in the cell-based assay, with EC_{50} values of 0.94 and 1.64 μM, respectively (Table 3).[76] These quinolones also inhibited HIV-1 reverse transcriptase activity in a cell-free assay, with IC_{50} values of 10 and 8 μM, respectively.[76] These compounds may serve as useful leads for NNRTI drug design since others have also reported that similar compounds isolated from marine sponges exhibit anti-HIV RT activity.[77]

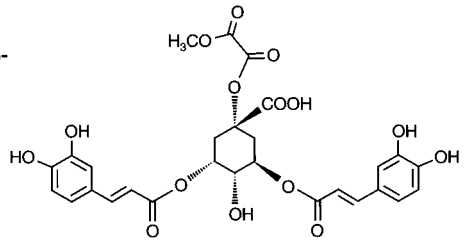
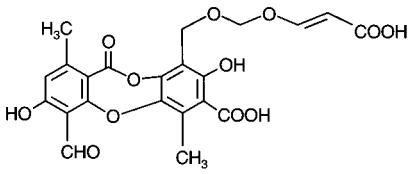
Integrase Inhibitors. The de novo viral DNA synthesized during reverse transcription is immediately integrated into the host cell DNA, via a step referred to as integration, to permit further transcription of viral RNA (Figure 1). HIV-1, like other retroviruses, depends on stable integration into the host genome so as to facilitate efficient replication of the viral RNA and maintenance of the infected state. There have been reports published on natural product HIV integrase inhibitors from the marine ascidian alkaloids, the lamellarins, and from terrestrial plants. Recently, lamellarin α 20-sulfate has been reported as a new HIV-1 integrase inhibiting alkaloid purified and characterized from the prosobranch mollusc *Lamellaria* sp.[79] Over 65 extracts of samples from the Asteraceae family of plants, *Baccharis genistelloides* (Asteraceae),[78] and two extracts of samples from the genus *Achyrocline satureioides* (Asteraceae) are available in the NCI Natural Products Repository. Out of the eight *Baccharis* species tested in the NCI program, six showed detectable anti-HIV activity. The most consistent anti-HIV activity was observed with extracts prepared from *B. dracunculifolia, B. latifolia, B. pedunculata,* and *B. punctulata*. Four major compounds derived from *Achyrocline satureioides* (Asteraceae), caffeic acid (NSC 57197), quinic acid (NSC 1115 and NSC 59258), and 5-caffeoylquinic acid (chlorogenic acid; NSC 70861 and NSC 407296), along with an acquired dicaffeoylquinic acid (cynarin; 1, 3-dicaffeoylquinic acid or 1,5-dicaffeoylquinic acid; NSC 91529), have been tested in the NCI anti-HIV screen using MT-2 cells infected with HIV-1_{RF}. It should be mentioned that cynarin was also extracted from the artichoke, *Cynara scolymus*, and is now commercially available. In agreement with the data reported by Robinson et al.,[78] little anti-HIV activity was observed with caffeic acid, quinic acid, 5-caffeoylquinic acid, and cynarin in our test results. An isomeric form of cynarin, the 3,5-dicaffeoylquinic acid (3,5-DCQA), showed significant anti-HIV_{Lai} activity. Its effective dosage determined at 50% protection against HIV-1-induced cytopathic effect (ED_{50}) was 1 μg/mL[78] in a cell-based assay. It also inhibited 50% of the enzymatic activity of integrase (IC_{50}) with a value of 0.66 μg/mL.[78] This gave a reasonable therapeutic index of 150 for 3,5-DCQA.[78] In the DTP cell-based assay, anti-HIV activity of cynarin (NSC 91529) was observed to vary between experiments. Unfortunately, the anti-HIV acitivity for cynarin was not comparable to the 3,5-DCQA isomer, and no further development was warranted. One other analogue of dicaffeoylquinic acid, the 1-methoxyoxalyl-3,5-dicaffeoylquinic acid (1-MO-3,5-DCQA) (Table 4), was also reported to show strong inhibitory action against HIV-1 integrase (Table 4).[78] It had an ED_{50} value of 4 μg/mL as well as a therapeutic index of 87 in the cell-based anti-HIV assay. It also inhibited the HIV integrase at a slightly better IC_{50} value of 0.57 μg/mL when measuring DNA disintegration. Altogether, the dicaffeoylquinic acid isomers provide lead compounds for improving anti-integrase drug research.

Depsides and depsidones, isolated from lichens, have also been reported to inhibit HIV integrase, as well as Gram-positive bacteria and mycobacteria, besides demonstrating antiviral, insecticidal, and nematocidal activity.[80] A depsidone (NSC 685588) exhibited significant anti-HIV activity in the cell-based assay (EC_{50} at 8.4 μM) and a correspondingly strong anti-integrase activity (IC_{50} = 4.9 μM for 3′-processing and 4.6 μM for strand transfer).[80] Likewise, the therapeutic index for this depsidone was also low (Table 4), and no further development was warranted.

The structure and function of HIV integrase, as well as the integration process itself which involves multistep enzymic reactions, have been intensely studied in different laboratories. A comprehensive review was published recently[81] and should aid in developing these natural product leads for rational design of improved anti-HIV integrase drugs.

Protease Inhibitors. In the late phase of HIV viral replication, the large precursor polyprotein (*gag-pol* precursor, Pr 160) must be appropriately cleaved by a viral protease, and the *Env* precursor, gp160, must be glycosylated and cleaved by a cellular protease to become the viral envelope gp120 and gp41 (Figure 1). Other cleavages involving the generation of the structural proteins, p17, p24, p7, and p6, and the generation of functional enzymes,

Table 4. Natural Product-Based Anti-HIV Compounds Targeting HIV Integrase

Compound	Structure	Anti-HIV-1		Anti-Integrase
		ED_{50}^{a} μg/mL	TI_{50}	IC_{50} μg/mL
1-methoxyoxalyl-3, 5-dicaffeoylquinic acid (1-MO-3, 5-DCQA) m.w. 602		4	87	0.57
		EC_{50}^{b} μM	TI_{50}	IC_{50} μM
Depsidone m.w. 474		8.4	>4.61	4.9

[a, b] In the anti-HIV cell-based assay, MT-2 cells were infected with HIV-1_{Lai} in [a], but with HIV-1_{RF} in [b]
ED_{50}: anti-HIV-1 activity was measured as 50% protection against HIV-1 induced cytopathic effect 72 h after infection
IC_{50}: anti-HIV-1 integrase activity at 50% inhibition

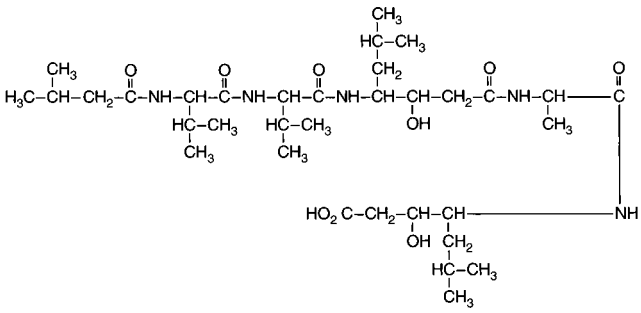
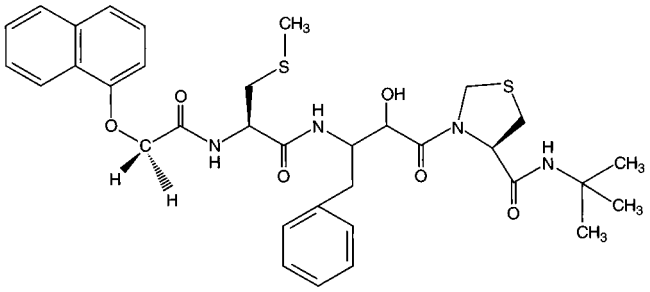
such as reverse transcriptase, integrase, and protease, also take place before the eventual packaging into a mature and infectious viral particle. The cleavage of the *gag* precursor protein of HIV (p55) is critical for the maturation and infectivity of the viral particle. Without the appropriate cleavages of the precursor polyproteins or if mutations affect the HIV protease coding region, noninfectious viral particles are generally produced. The role of HIV protease in the production of functionally infectious viral particles has been described as a critical process for retroviruses as well as HIV-1 replication.[82,83] The HIV protease, thus, lends itself as an appropriate therapeutic target, facilitating the search for anti-HIV inhibitors specifically targeting the viral protease. In this respect, inhibitors of HIV-1 protease are effective regulators of viral replication and can be applied even at the late stage of the HIV replication cycle.[83]

Between 1987 and 1990, the NCI invested considerable effort in evaluating the inhibitory activity of HIV-1 by pepstatin A, a known aspartyl protease inhibitor, whose anti-HIV activity varied over a wide concentration range. An averaged EC_{50} value of 51.8 μM together with a TI_{50} value of >1.4 were estimated for pepstatin A (Table 5). In recent confirmatory retests, peptatin A demonstrated an EC_{50} value of 69 and 150 μM when tested against CEM cells infected with the HIV-1_{RF} strain and with the Rojo clinical strain of HIV, respectively. This suggests that pepstatin A is able to penetrate the cell membrane, albeit at high concentrations. Pepstatin A, a metabolite of *Streptomyces testaceus* and *Streptomyces argentolus* var. *toyonakensis*, was shown to inhibit HIV-1 protease activity at a K_i value of 1.1 μM,[83] using as a substrate the fragment of HIV-1 protease Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val produced in a recombinant clone of *E. coli*. Structurally, pepstatin A (Table 5) is a small pentapeptide with a unique hydroxyamino acid, statine, that sterically blocks the active site of HIV-1 protease. The information from these initial studies, the elucidation of partial sequence homology between HIV-1 protease and that of aspartic protease, coupled with a large volume of data from the design of renin inhibitors, led to the testing of renin inhibitory agents against HIV-1 protease and the further derivation of peptidomimetics yielding nanomolar activity against the HIV-1 protease (for detail, refer to refs 84−86). The key strategy used in the development of the current HIV protease inhibitors was to substitute the scissile P1−P1′ amide bond by a nonhydrolyzable isostere with tetrahedral geometry; the designs were guided by assays and based on substrate specificity.[87] It eventually led to the optimization of a peptidomimetic lead structure and the development of a novel class of protease inhibitors including the current clinically available Crixivan, indinavir sulfate.[86−88]

In collaboration with Nikko Kyodo, Inc., KNI-272 (Table 5), a protease inhibitor of microbial origin, was advanced by NCI through preclinical testing to clinical trials. KNI-272, as well as its analogue KNI 227, was found to be highly potent against a spectrum of HIV-1 strains and HIV-2 at EC_{50} concentrations of 0.02−0.1 μM when tested *in vitro*[89] (Table 5). KNI-272 inhibition was also highly synergistic with AZT or with other NNRTIs in HIV-1-infected CEM-2 cells. Moreover, resistant HIV variants emerged in HIV-1-infected tissue culture exposed continuously to KNI-272 9 months later, whereas treatment with the currently available Ritonavir generally resulted in resistant mutants in as short a period as 1 month. In another study with KNI-227, no drug-resistant variants emerged in the HIV-1-infected cell cultures exposed to the drug until after 55 passages conducted over a period exceeding 1 year.[90] KNI-272 was entered into clinical trials in both NCI and Japan. Results of a 12-week Phase I trial on 37 patients with AIDS or symptomatic HIV infection treated with an escalating dose of KNI-272 via oral administration were reported recently.[91] Treated patients tolerated the treatment well and showed a continuous decrease in plasma HIV RNA copies throughout the 7−8 weeks of treatment, warranting further clinical study of KNI-272 to optimize its use in combination therapy.[91]

Other Anti-HIV Active Natural Products. A number of natural product-derived compounds have been identified as possessing potent cell-based anti-HIV activity, but their mechanisms of action have, as yet, not been fully resolved

Table 5. Natural Product-Based Protease Inhibitors Discovered and/or Developed at NCI with Collaboration

Common Name	Structure	Anti-HIV-1_{RF} EC_{50} μM	TI_{50}
Pepstatin m.w. 686		51.8	>1.4
KNI-272 m.w. 668		0.02	>4.6

(Table 6). Organic extracts of the stems, twigs, leaves, and flowers of the Western Australian plant *Conospermum incurvum* Lindley (Proteaceae) showed potent anti-HIV activity, and bioassay-guided fractionation and purification led to the isolation of a novel trimeric naphthoquinone, conocurvone, as the active agent.[92,93] It was shown not to act by any of the known mechanisms, and it was surmised that its inhibitory action occurs in the late phase of the viral replication cycle, since a time course study showed that conocurvone, if added 48 h post infection, could still protect T-cells from the cytopathogenic effect of HIV-1. Structure-activity studies showed that the trimeric central core of the compound is critical for antiviral activity; the compound appears to be well tolerated by the test T-cell line, CEM-SS. This compound is under development by the Australian company AMRAD, working in collaboration with Western Australian organizations.

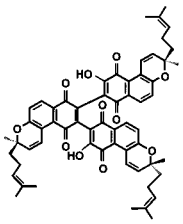
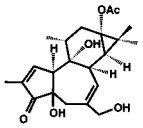
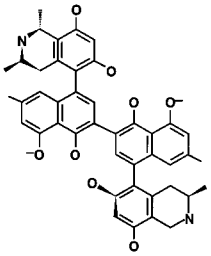
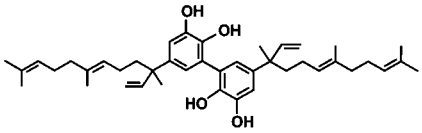
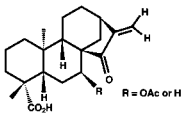
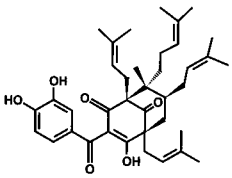
An organic extract prepared from the stem bark of *Homoalanthus nutans* (Forster) Pax (Euphorbiaceae) showed strong anti-HIV activity in the NCI cell-based screening assay. *H. nutans*, a native plant of Samoa, has been widely used by Samoan healers for diverse medicinal purposes. Upon fractionation, the phorbol ester, prostratin (Table 6), was isolated as the active agent.[94] Despite being a phorbol ester, it lacks tumor promoter effects and has actually been shown to compete against TPA (12-*O*-tetradecanoylphorbol 13-acetate, a potent tumor-promoting agent) when tested in tumor-promoting experiments on mouse skin treated with dimethylbenzanthracene. When added to T-cells or cells in tissue culture, prostratin exhibited typical cytostatic effects on the growth kinetics of the cells. Its range of anti-HIV activity spanned across certain HIV strains such as HIV-1_{RF}, HIV-1_{IIIB}, HIV-2, and certain drug-resistant HIV-1 variant strains in the appropriate host cells, CEM-SS cells and C8166 cells. Prostratin was able to provide complete cytoprotection even when added to HIV-infected cultures at as late as 24−30 h post-infection, but it failed to prevent viral replication. Its potency and extent of cytoprotection, however, are reported to depend on the HIV strain and the host cell type involved.

In two latently infected cell lines, prostratin was reported to activate viral expression;[95] in contrast, it failed to activate viral expression in cell lines chronically infected with HIV. It has been speculated[95] that HIV viral particles that stayed dormant in the host's tissue and, hence, escaped detection, might be responsible for the onset of eventual, sudden viremic crises. Compounds like prostratin may be able to stimulate the emergence of such dormant viral particles for exposure to effective anti-HIV chemotherapy, such as the highly active antiretroviral therapy (HAART).[95] The usual HAART regimen combines three or more different drugs such as two NNRTIs and a protease inhibitor, or other combination regimens recommended by leading HIV experts, to aggressively suppress viral replication as the viral particles emerge.

Prostratin's mechanism of action is not well understood. It was established that it did not inhibit the enzymatic reactions of HIV reverse transcriptase or protease, nor did it compete with the binding of gp120 to CD4. In its presence, cell-cell fusion of CEM-SS cells was prevented. Moreover, prostratin was observed to down-regulate CD4 expression in both CEM-SS and MT-2 cells. It was surmised that the anti-HIV mechanism of prostratin may be mediated via a protein kinase C enzyme pathway.[95]

The anti-HIV alkaloid michellamine B, NSC 649324, is a novel naphthalene tetrahydroisoquinoline alkaloid isolated from the leaves of *Ancistrocladus korupensis* (Ancistrocladaceae),[96,97] a plant native to the Korup National Park in Cameroon's Southwest Province. The leaves of this plant are the only known source of michellamine B. Michellamine B inhibits HIV-1 during the early phase of viral infection of T-lymphocytes. It was also noted that it inhibited HIV-2 in MT-2 cells equally well. Although other mechanisms of action remain viable for michellamine B, collective consideration of its inhibitory characteristics, range of activity, and the difficulty in cell membrane

Table 6. Other Natural Product-Based Anti-HIV Compounds

Compound	Structure	Taxonomy/Source	Anti-HIV-1 EC_{50} μM	Anti-HIV-1 TI_{50}
Conocurvone m.w. 602		*Conospermum proteaceae* Western Australia	0.02	250
Prostratin m.w. 390		*Homoalanthus nutans* Samoa	≤0.132	250
Michellamine B m.w. 756		*Ancistrocladus korupensis* Cameroon	1.0	40
Peltatol A m.w. 626		*Pothomorphe peltata* Dominican Republic	8.0	4
Xylopinic acid m.w. 370		*Xylopia* Peru	≤0.9	18
Guttiferone A m.w. 602		*Symphonia globulifera* Tanzania	8.0	10

penetration indicates that it most probably acts at the cell surface. The clinical development of michellamine B was not pursued due to the observation of unacceptable neural toxicity in preclinical studies with animal models.

Extracts prepared from a native plant of the Dominican Republic, *Pothomorphe peltata* (Piperaceae), demonstrated strong anti-HIV activity, and bioassay-guided fractionation yielded several prenylated catechol dimers, the peltatols, as the active agents.[98] An in-depth analysis of the anti-HIV activity of the peltatols has not been performed as yet.

An aqueous extract of fruits of a *Xylopia* sp. (Annonaceae), collected from Peru, demonstrated anti-HIV activity in the cell-based assay, and a new diterpene was isolated as the active agent[99] (Table 6). It protected CEM-SS cells from the cytopathic effects of HIV-1 infection at an EC_{50} of 0.9 μM. This new diterpene, xylopinic acid, could serve as a lead for anti-HIV drug development.

Guttiferone A was isolated from *Symphonia globulifera* (Clusiaceae), a native plant of Tanzania[98] and a member of the Clusiaceae (Guttiferae) family. Altogether, four different members of the Clusiaceae, *S. globulifera, Clusia rosea, Garcinia livingstonei,* and *G. ovalifolia* have yielded extracts that showed anti-HIV activity in the cell-based screening assay.[100] Guttiferone A provided cytoprotection of CEM-SS cells from HIV-1 infection at EC_{50} values of 1−10 μM. The family Clusiaceae includes the genus *Calophyllum,* which yielded the very potent NNRTIs, the calanolides, as described earlier.

Anti-HIV Compounds Isolated, Characterized, and Listed on the LDDRD Web Page. A comprehensive catalog of 90 anti-HIV compounds isolated and characterized by the Laboratory for Drug Discovery Research and Development (LDDRD)[101] chemists from natural product sources is open to the public. Almost all of the active anti-HIV compounds have been discussed in this paper, but a few that showed activity were not discussed herein because of redundancy or other limitations. Nigericin, isolated from an unknown actinomycete, showed anti-HIV activity with an EC_{50} value of 0.006 μM and a cytotoxicity index value, at 50% cell killing, (IC_{50}) of 0.14 μM. It is, however, an ionophore similar to the monensins, a class of compounds found to be difficult for anti-HIV drug development. An extremely active compound isolated from *Excoecaria agallocha* (Thymelaeaceae), having an EC_{50} value of 0.006 μM with an IC_{50} value of 2 μM in anti-HIV assay, was found to be a phorbol ester.[102] A number of sulfolipids[103] exhibit-

ing moderate *in vitro* anti-HIV activity were isolated from cyanobacteria, early in the antiviral screening program (late 1980s), but lacked significant *in vivo* activity.

Recently, a number of small macrocyclic peptides, the cycloviolins, have been successfully isolated from the tropical plant *Leonia cymosa* (Violaceae). The cycloviolins are composed of 28−31 amino acid residues and showed some anti-HIV activity.[104] The circulins, isolated from *Chassalia parvifolia* (Rubiaceae), constitute a more potent group of cyclic peptides; they are composed of 30 amino acid residues. They exhibited anti-HIV activity at an EC_{50} value of 0.01 μM and an IC_{50} value of 0.5 μM.[105] These cyclic peptides might offer opportunities for optimization or innovative therapeutic approaches.

Conclusion

Over the past 15 years, the NCI, through its Natural Products Program, has collected, cataloged, extracted, and screened terrestrial plants, marine plants and invertebrates, and microbial samples for both anti-HIV and anticancer activity. Despite this extensive program, the current natural product resource of the National Cancer Institute represents only a small portion of the immense global biodiversity available for drug discovery and development. It should be noted that many of the anticancer and anti-HIV drugs currently used in chemotherapy either are derived directly from natural products or are synthetics based on natural product models. Natural product-derived active compounds serve as important lead molecules, as demonstrated in the case of the development of anti-HIV reverse transcriptase and protease inhibitors discussed earlier. The rapid evolution of new HIV clades and drug-resistant variants in AIDS patients, as well as the resurgence of other infectious diseases, such as drug-resistant tuberculosis (TB) and hepatitis (A, B, and C viral infections), requires the development of innovative, but reproducible technology targeting the critical elements for drug discovery and development. Through its commitment to collaboration with the global scientific community, NCI is providing access to its natural product extract and open pure compound repositories, as well as information and technical support, with the goal of promoting improved health through drug discovery and development.

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