RECENT ADVANCES IN THE CHEMOTHERAPY OF AIDS

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Abstract : Acquired immunodeficiency syndrome (AIDS) continues to grow unabated and in pandemic proportions around the world. The complexity and high mutational ability of the etiologic agent, human immunodeficiency virus, has posed an unprecedented threat toward the chemotherapy of AIDS. Use of contemporary virology to unfold the mystery of HIV-1 and the discovery of key molecular targets for chemotherapeutic intervention has accelerated the quest for safe and effective anti-HIV agents. The vital stages in viral replication amenable to inhibition by novel chemical entities and representative examples from diverse chemical classes as potential anti-AIDS agents are presented in this review.

Key words : AIDS   HIV-1   dextran sulfate   PVAS   bicyclams

reverse transcriptase inhibitors   antisense oligonucleotides

protease inhibitors   glucosidase inhibitors   anti-HIV vaccine

Acquired immunodeficiency syndrome (AIDS) is a life threatening and debilitating disease state caused by retrovirus infection, and the etiologic agent is now widely known as the human immunodeficiency virus type 1 (HIV-1). A retroviral cause for AIDS was first established by French researchers, led by Luc Montagnier (1), who isolated a virus from a patient suffering from lymphadenopathy. They named the virus as lymphadenopathy associated virus (LAV), which was later renamed as HIV. Since the discovery of AIDS in 1981 in the United States, the disease has grown into pandemic proportions around the globe. According to present estimates, there are about 14 million people infected with HIV worldwide. Although the number of new infections per year are beginning to plateau in the western hemisphere, Asia is poised to become the next epicenter of the AIDS epidemic (2). Beside HIV-1, a closely related retrovirus HIV-2 has also been implicated in causing AIDS. But, so far, the spread of HIV-2 is mostly limited to the African continent.

It is now well established that the propagation of HIV-1 infection can take place by sexual contacts, administration of contaminated blood and blood related products and from an infected mother to her neonate. The severity of the problem has launched a vigorous search for chemotherapeutic agents, which would be able to arrest the replication of the virus, and a search for a safe and efficacious vaccine is also underway. So far, AZT (3'-azido-2',3'-dideoxythymidine, Zidovudine, Retrovir), DDI (2',3'-dideoxyinosine, Didanosine, Videx) and DDC (2',3'-dideoxycytidine, Zalcitabine, HIVID), drugs belonging to the nucleoside class of compounds, are licensed in the United States for the wide scale treatment of AIDS and AIDS-related complex (ARC). These drugs share a common mechanism of action i.e. inhibition of the important viral enzyme reverse transcriptase (3). However, drug-related toxicities, emergence of resistant viral strains and lack of total eradication of virus has necessitated the search for newer, potent and more efficacious anti-

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HIV agents, possibly with different mechanisms of action than the existing agents (4).

In this context, understanding of the molecular biology and life cycle of the AIDS virus is extremely important for designing new chemical entities for successful chemotherapeutic intervention. In the subsequent section, a brief overview of the biology and viable molecular targets for halting HIV-1 replication will be presented.

LIFE CYCLE OF HIV-1

Human immunodeficiency virus belongs to the *lentiviridae* family of pathogenic human retroviruses, which rely on RNA to encode their genetic message. HIV-1 is an enveloped virus and its surface consists of two major glycosylated proteins, gp 41 and gp 120. Inside the nucleocapsid are two copies of single stranded viral RNA and an enzyme, reverse transcriptase (RT). The virus shows great affinity for the helper T-cells, an important armament in the arsenal of cellular immune response (5a,b). The profound immune incompetence observed in HIV-1 infected individuals could be partly explained on the basis of the selective destruction of the helper T-cells by the virus. It has been shown that the viral surface glycoprotein gp 120 binds to the CD4 receptors on the T cells with great affinity (6a,b,c). Beside T-cells, other cells expressing CD4 on their surface may also harbor HIV-1 and thereby act as a reservoir for the virus, thus extending the latency period associated with the infection. These include macrophages, monocytes and lymphoid cells (7). CD4- cells could also harbor HIV and get infected; it was recently demonstrated that galactosyl ceramide and sulfatide receptors on colon and neural cells could also act as potential binding sites for HIV (8a,b). After the attachment to the target cell, the virus is internalized via pH independent cell fusion and then it uncoats (5b).

After the uncoating event, RT catalyzes the process of reverse transcription using viral RNA as the template. HIV-1 RT has been characterized as a heterodimer consisting of three functional domains, namely: RNA-dependent DNA polymerase; RNase H and double-stranded RNA-dependent RNase. Owing to the vital role of RT in the replicative cycle, the search for potent inhibitors of this enzyme still remains the most fertile area of anti-AIDS drug design. After reverse transcription, through a series of events, newly synthesized viral DNA is integrated into the host cell genome by the help of the enzyme integrase. Then, using the replication machinery of the host cell, other important enzymes and proteins, which are necessary for the formation, maturation and packaging of new virions, are synthesized. The budding virions are packaged into coat proteins which are cleaved off from a large peptide precursor by another important enzyme, HIV protease. Eventually, these virions are pinched off the cell membrane and seek to infect other healthy cells completing the viral life cycle (9).

The replicative cycle of HIV-1 presents several viable targets which could be aimed for the intervention of pathogenesis. Ideally, an antiretroviral agent should arrest the virulence and further infection of healthy cells without displaying toxicity toward normal cellular physiology. Availability of a wide rage of functional bioassays measuring antiviral activity has definitely accelerated the search for new chemical entitites as potential anti-AIDS agents. Theoretically, an anti-HIV compound may exert its activity by inhibiting a variety of important steps in the viral life cycle. However, medicinal chemists have predominantly focused on the following stages (Scheme 1):

a) Viral binding to target cells,

b) Viral uncoating event,

c) HIV reverse transcriptase,

d) HIV integrase,

e) Viral gene expression,

f) HIV protease, and

g) HIV glucosidase.

SCHEME 1
A simplified diagram depicting potential molecular targets in HIV-1 life-cycle amenable to chemotherapeutic intervention
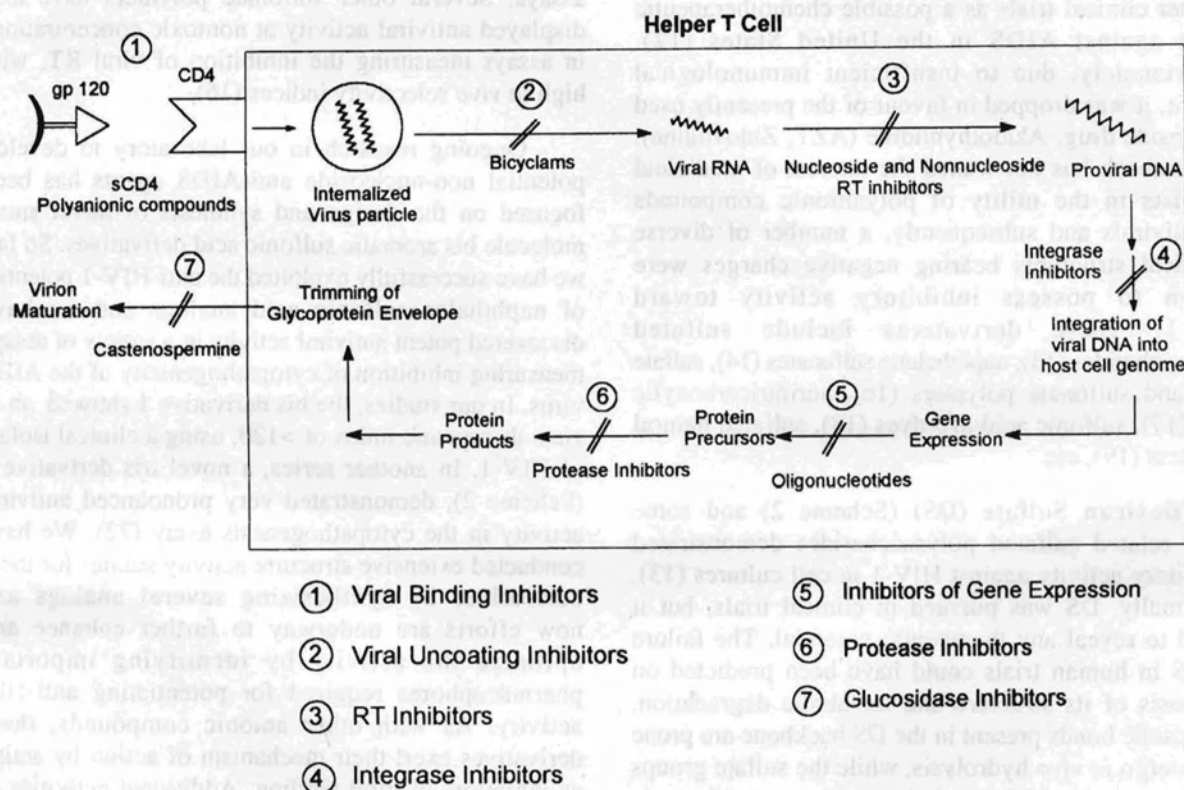

① Viral Binding Inhibitors
② Viral Uncoating Inhibitors
③ RT Inhibitors
④ Integrase Inhibitors
⑤ Inhibitors of Gene Expression
⑥ Protease Inhibitors
⑦ Glucosidase Inhibitors

Fig. 1.

INTERVENTION STRATEGIES AND INHIBITORS

a) VIRAL BINDING INHIBITORS

Soluble CD4 derivatives

As stated earlier, binding of gp 120 to the CD4 receptor acts as the first event of HIV infection. Using this crucial information, it was demonstrated that truncated soluble CD4 (sCD4) molecule was capable of inhibiting viral binding and replication in cell cultures (10). This approach provides an obvious promise regarding the neutralization of diverse viral isolates,

because the sCD4 molecule consists of the required binding region of the intact CD4 receptor. However, further clinical studies with the administration of sCD4 were disappointing. The reasons were attributed to the insensitivity of HIV-1 isolates for sCD4 and the difficulty in attaining sufficient therapeutic plasma levels due to short half-life. The problem arising from the short half-life was resolved using hybrid molecules of sCD4 and IgG (Immunoglobulin G), which increaed the plasma half-life of sCD4 more than 100-fold (11). Even after rigorous research devoted to this novel agent, sCD4 has failed to show clinical efficacy and also the long

term clinical utility is questionable, as many CD4- cells may also get infected and harbor HIV-1 during its latency period.

Polyanionic Compounds

Suramin, a hexasulfonate naphthylurea derivative and a potent RT inhibitor, was the first compound to enter clinical trials as a possible chemotherapeutic agent against AIDS in the United States (12). Unfortunately, due to insufficient immunological benefit, it was dropped in favour of the presently used nucleoside drug, Azidothymidine (AZT, Zidovudine). This setback has not waned the interest of medicinal chemists in the utility of polyanionic compounds as antivirals and subsequently, a number of diverse chemical structures bearing negative charges were shown to possess inhibitory activity toward HIV-1. These derivatives include sulfated polysaccharides (13), naphthalene sulfonates (14), sulfate (15) and sulfonate polymers (16), aurintricarboxylic acid (17), sulfonic acid azo dyes (18), sulfated natural products (19), etc.

Dextran Sulfate (DS) (Scheme 2) and some other related sulfated polysaccharides demonstrated inhibitory activity against HIV-1 in cell cultures (13). Eventually, DS was pursued in clinical trials, but it failed to reveal any therapeutic potential. The failure of DS in human trials could have been predicted on the basis of its structure and metabolic degradation. Glycosidic bonds present in the DS backbone are prone to undergo *in vivo* hydrolysis, while the sulfate groups present on carbohydrate skeleton are amenable to *in vivo* desulfation by non-specific sulfatases. It was indeed demonstrated that small and neutral fragments of DS were devoid of anit-HIV activity (20). However, the problem of hydrolytic cleavage of glycosidic bond was circumvented by employing a polymethylene hydrocarbon backbone, while keeping the sulfate groups intact to provide the essential anionic moiety. This approach resulted in the antiviral evaluation of a sulfated copolymer of acrylic acid and vinyl alcohol (**PAVAS**) and sulfated polymer of vinyl alcohol (**PVAS**) (Scheme 2) (21).

We envisioned that the important drawback of *in vivo* desulfation could be obviated by the incorporation of a metabolically stable anionic moiety. We chose the sulfonic acid group to replace the sulfate group, as the sulfonic acid group displays stability toward metabolism. This elegant design strategy resulted in the preclinical evaluation of an aliphatic (**PVS**) and an aromatic (**PSS**) sulfonic acid polymer (Scheme 2), which showed extremely potent inhibitory response in anti-HIV-1 assays. Several other sulfonate polymers have also displayed antiviral activity at nontoxic concentrations in assays measuring the inhibition of viral RT, with high *in vivo* selectivity indices (16).

Ongoing research in our laboratory to develop potential non-nucleoside anti-AIDS agents has been focused on the design and synthesis of novel small molecule bis aromatic sulfonic acid derivatives. So far, we have successfully exploited the anti-HIV-1 potential of naphthalenesulfonic acid analogs and we have discovered potent antiviral activity in a variety of assays measuring inhibition of cytopathogenicity of the AIDS virus. In our studies, the bis derivative 1 showed an *in vitro* therapeutic index of >120, using a clinical isolate of HIV-1. In another series, a novel tris derivative 2 (Scheme 2), demonstrated very pronounced antiviral activity in the cytopathogenesis assay (22). We have conducted extensive structure activity studies for these derivatives by synthesizing several analogs and now efforts are underway to further enhance and optimize the activity by identifying important pharmacophores required for potentiating anti-HIV activity. As with other anionic compounds, these derivatives exert their mechanism of action by acting as inhibitors of viral binding. Additional activities of these compounds in different assays will be discussed in subsequent sections.

In general, clinical utility of charged compounds could be debated on the basis of their polar nature, which will preclude their entry into the cells. However, by systematic modification of functional groups, prodrugs could be prepared to facilitate the cellular entry and uptake. On the basis of their mechanism of action, we believe that polar compounds are well suited to be used as chemoprophylactic agents as they interfere with the CD4/gp 120 interaction to inhibit the transmission of HIV-1 from the infected cell to the healthy cell.
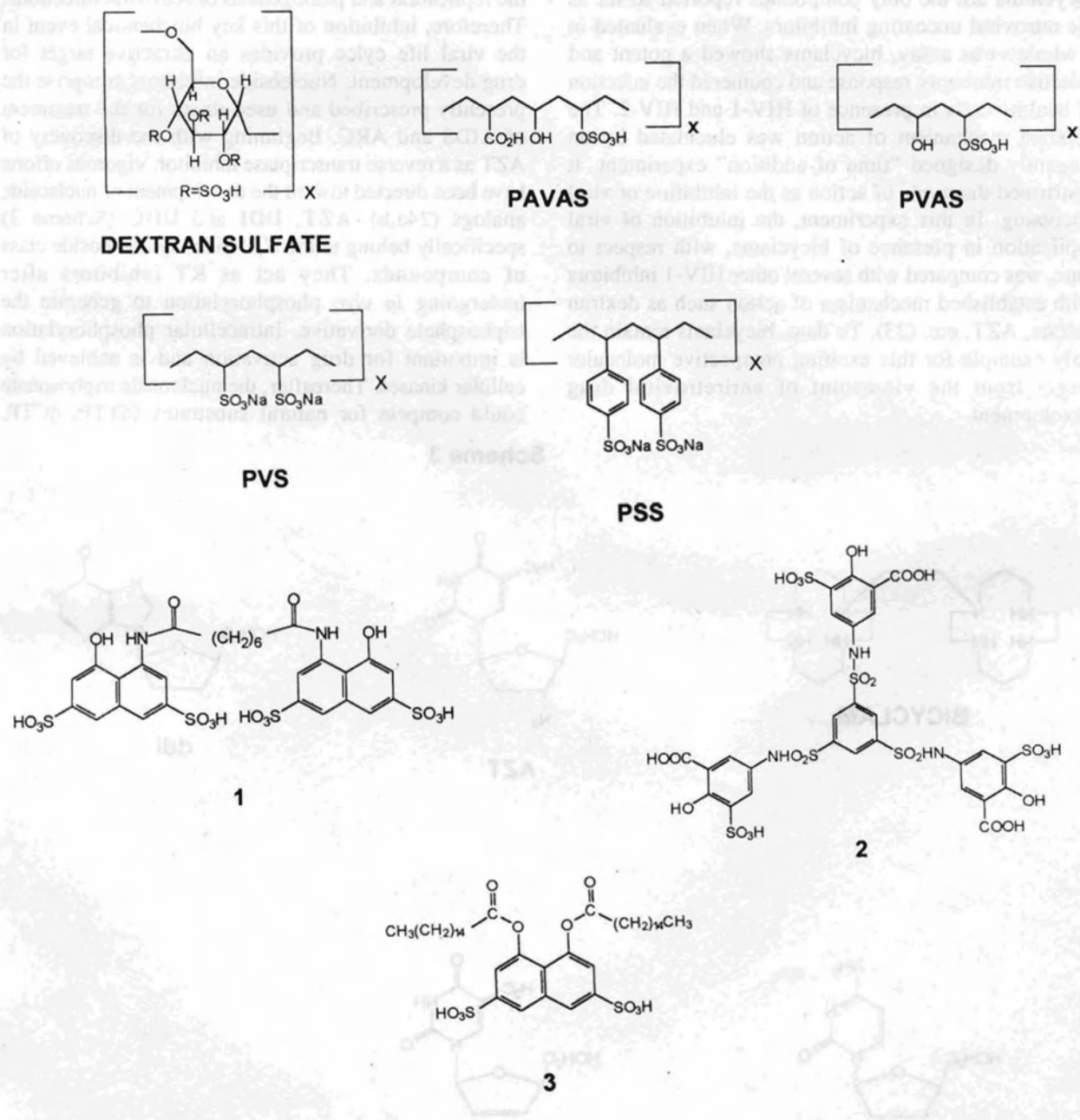
SCHEME 2

DEXTRAN SULFATE

PAVAS

PVAS

PVS

PSS

1

2

3

Fig. 2.

b) VIRAL UNCOATING INHIBITORS

Bicyclams

As exemplified by their structure (Scheme 3), **bicyclams** are the only compounds reported so far as the retroviral uncoating inhibitors. When evaluated in a whole virus assay, bicyclams showed a potent and selective inhibitory response and countered the infection of healthy cells in presence of HIV-1 and HIV-2. The detailed mechanism of action was elucidated by an elegantly designed "time of addition" experiment. It confirmed the mode of action as the inhibition of viral uncoating. In this experiment, the inhibition of viral replication in presence of bicyclams, with respect to time, was compared with several other HIV-1 inhibitors with established mechanism of action such as dextran sulfate, AZT, etc. (23). To date, bicyclams remain the only example for this exciting prospective molecular target from the viewpoint of antiretroviral drug development.

c) REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside RT Inhibitors

The process of reverse transcription is central to the replication and pathogenesis of retroviral infections. Therefore, inhibition of this key biochemical event in the viral life cylce provides an attractive target for drug development. Nucleoside inhibitors comprise the presently prescribed and used drugs for the treatment of AIDS and ARC. Beginning with the discovery of AZT as a reverse transcriptase inhibitor, vigorous efforts have been directed toward the development of nucleoside analogs (24a,b). **AZT, DDI** and **DDC** (Scheme 3) specifically belong to the 2',3'-dideoxy nucleoside class of compounds. They act as RT inhibitors after undergoing *in vivo* phosphorylation to generate the triphosphate derivative. Intracellular phosphorylation is important for drug activation and is achieved by cellular kinases. Thereafter, the nucleoside triphosphate could compete for natural substrates (dTTP, dCTP,

Scheme 3

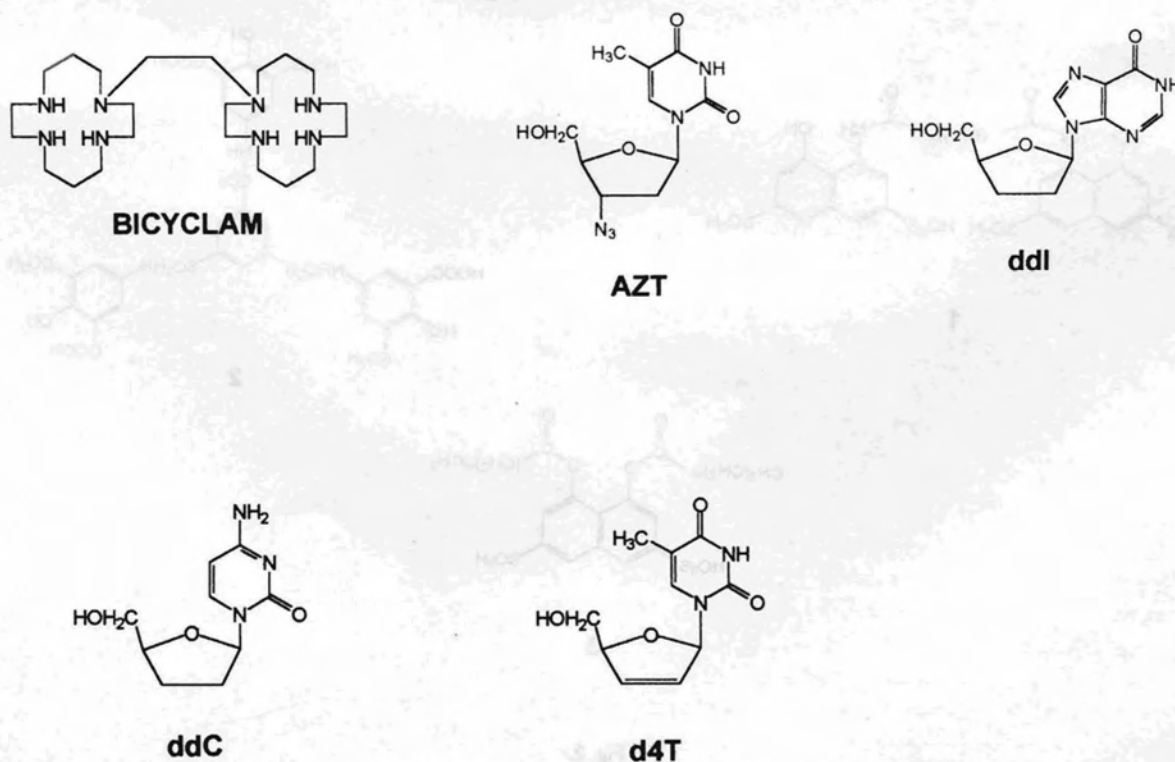
BICYCLAM

AZT

ddl

ddC

d4T

Fig. 3.

dATP, dGTP) and cause chain termination, thereby inhibiting RT. The success of AZT and other nucleosides has sparked keen interest in further modification of the basic structural features to generate novel analogs in order to attain better clinical efficacy. On this premise, several classes of nucleoside derivatives possessing antiretroviral activity have been reported. Extensive structure activity relationship studies have divulged important requirements at the sugar residue of nucleosides. It is now established that at the 3' position in the 2',3'-dideoxy skeleton, a substitution of azido, fluoro, or hydrogen is allowed, only when coupled with an appropriate purine or pyrimidine base (25). Also, a 2',3'-double bond is allowed, as in **D4T**, a nucleoside derivative presently undergoing clinical trials.

Drug related toxicities and emergence of viral resistance has limited the continuous long-term therapeutic potential of nucleoside drugs. Severe side effects of AZT treatment had been observed in patients which include macrocytic anemia, leukopenia and myopathy. In addition, some incidences of edema, meningoencephalitis, ulceration and nail pigmentation have been reported (26a,b). Similarly, prolonged use of DDI causes painful peripheral neuropathy, pancreatitis and hepatic failure (27). Cardiomyopathy, pancreatitis and ulceration are the result of dose-dependent toxicity of DDC (28).

The most serious concern regarding the therapeutic efficacy stems from the diminished susceptibility and sensitivity of AIDS patients toward prolonged nucleoside drug therapy. Viral resistance, which is caused by multiple mutations in the viral genome, has been implicated for the reduced sensitivity toward the drugs. Although the precise underlying mechanism for resistance is unclear at the present time, specific amino acid mutations in the enzyme reverse transcriptase have been found and studied. Mutations at amino acid residues 67, 70, 215 and 219 are implicated for AZT resistance (29). A single Leu → Val mutation at position 74 causes DDI resistance (30), while a single Thr → Asp mutation at position 69 confers DDC resistance (31). Along with the drug-related toxicities and viral resistance, and given the fact that AZT and other related nucleosides are unable to completely eradicate HIV-1 infection, the search for better drug candidates is of utmost importance.

Non-nucleoside RT inhibitors

To overcome the inherent problems associated with the development of nucleoside drugs, concerted efforts on the part of medicinal chemists has resulted in the discovery of several potent non-nucleoside RT (NNRT) inhibitors. Although acting at the same molecular target, these novel compound classes have displayed superior potency compared to nucleoside agents. A random screening of compounds, coupled with astute design, has revealed non-competitive RT inhibitory activities of **TIBO** (32), **HEPT** (33), **BHAP** (34), **pyridinone** (35) and **dipyridodiazepinone** (36) (Scheme 4) analogs. Mechanistically, all of these agents act on reverse transcriptase at sites distinct to that of nucleoside inhibitors. It is also interesting to note that all NNRT inhibitors mentioned above are selective for HIV-1 over HIV-2 and other retroviruses.

Our continuing endeavors in the area of naphthalenesulfonic acids as anti-AIDS agents has also revealed the potential of *in vitro* RT inhibition by these compounds. Derivative 3 (Scheme 2) demonstrated more potent inhibition of both HIV-1 and HIV-2 reverse transcriptases in our assays when compared to Suramin, a well known RT inhibitor (37). We have also shown that sulfonic acid polymers display excellent inhibitory activity against both of the viral RT's (16). These observations suggest the need of prodrug modification to mask the polarity of sulfonic acid groups to facilitate cellular penetration.

Most disturbingly, drug resistant viral strains have been observed against the NNRT inhibitors, after several continuous passages of the virus in cell cultures, in the presence of the drug (38a,b). This obstacle has invoked the researchers to establish the viability of multidrug regimen to initiate combination therapy. Indeed, the approach of "convergent combination therapy" using a three-drug regimen comprising of AZT, DDI and pyridinone, has shown success in cell cultures by completely attenuating HIV-1 replication, without any further observable resistance and mutations (39). This novel approach of putting limitation to the natural selection processes holds immense promise, but it remains to be seen if the multi-drug approach could be extrapolated into practical use in human clinical trials. Theoretically, this form of therapy could be extended

to a number of combinations of anti-HIV drugs acting at different sites of viral life cycle after confirming the synergistic ability of one (or more) agent(s) to act in presence of another.

Another breakthrough which may lead to the rational design of RT inhibitors, is the determination of crystal structures (40a,b) of the intact enzyme complexed with an inhibitor and its RNaseH domain (41). The X-ray crystal data will be valuable in the *de novo* design of chemical entities possessing anti-HIV activity using computational methods (42).

c) INTEGRASE INHIBITORS

Human immunodeficiency virus integrase has two important biochemical steps comprising overall enzyme function: nucleolytic cleavage and strand transfer (integration) reaction. Incorporation of viral DNA into the host cell genome could be translated as the basis of life-long infection. Therefore, this biochemical event, catalyzed by integrase, is a pivotal step in viral life cycle and worthy of further attention. At this time, there has been only one report pertaining to the evaluation of a wide array of compounds as integrase inhibitors. Although a definite structure activity pattern has not emerged from the study, it does provide important leads for inhibitors of this key molecular target. An evaluation of over thirty compounds was conducted belonging to diverse structural and pharmacological classes, including DNA topoisomerase inhibitors, mono and bifunctional intercalators and antimalarials. Out of all the compounds evaluated, Doxorubicin, a potent topoisomerase inhibitor and antitumor agent, displayed the most potent inhibitory activity (43). The discovery of integrase inhibitors represents an important lead, which should be further optimized in order to develop agents displaying synergism with other available anti-HIV agents.

d) GENE EXPRESSION INHIBITORS

Antisense Oligonucleotides

The integration of viral genes into the host cell genome assures chronic infection, as the replicative machinery of the host cell will continue producing the viral gene products. The only plausible therapeutic agents with potential to inhibit the gene expression are antisense oligonucleotides. Selective design strategy could be employed to synthesize oligonucleotides complimentary to a segment of genome or mRNA, which will interfere with the gene expression. Bioisosteric replacements of phosphodiester linkages with sulfur and amino groups to produce phosphorothioates and phosphoramidates (44a,b) have been made to obviate the problem of exonuclease mediated hydrolytic cleavage of oligonucleotides. Antisense oligonucleotides have shown inhibitory activities against HIV-1 replication in chronically infected cells, syncytia formation and reverse transcriptase (45). Some pertinent problems related to the cellular transport, site specific delivery and hybridization at a desired location will have to be addressed in order to fully realize the therapeutic potential of antisense oligonucleotides.

e) PROTEASE INHIBITORS

C_2 Symmetric inhibitors

HIV protease is a viral encoded, homodimeric aspartyl protease which is essential for the degradation of large polypeptide precursors into smaller, functional protein fragments required for the packaging and infectivity of budding virions. A catalytic triad of Asp-Thr-Gly contributed by each monomer comprises the active site of HIV protease. The discovery of C_2 symmetry by crystallographic analysis (46a,b) has unveiled an entirely new approach for antiretroviral drug design (47). A plethora of peptide based protease inhibitors (e.g. A77003, Scheme 4) (48) as well as inhibitors with isosteric replacements (49) are described in literature. Still, the realization of clinical efficacy has been marred due to limited water solubility of these derivatives, thus affecting the overall distribution and pharmacokinetics. Recently, the search for non-peptide protease inhibitors was extended to sulfonic acid azo dyes which showed inhibition at nontoxic doses (50). Presently, we too are successfully pursuing the design of aromatic sulfonates as protease inhibitors to obviate the inherent toxicological problems associated with azo dyes.

Availability of the crystal structure of protease initiated a computer based search for compounds having structural complimentarity to the enzyme active site which successfully identified Haloperidol as a potent inhibitor of the protease, at toxic concentrations (51). This approach will be useful for the indentification of

Scheme 4

TIBO

HEPT

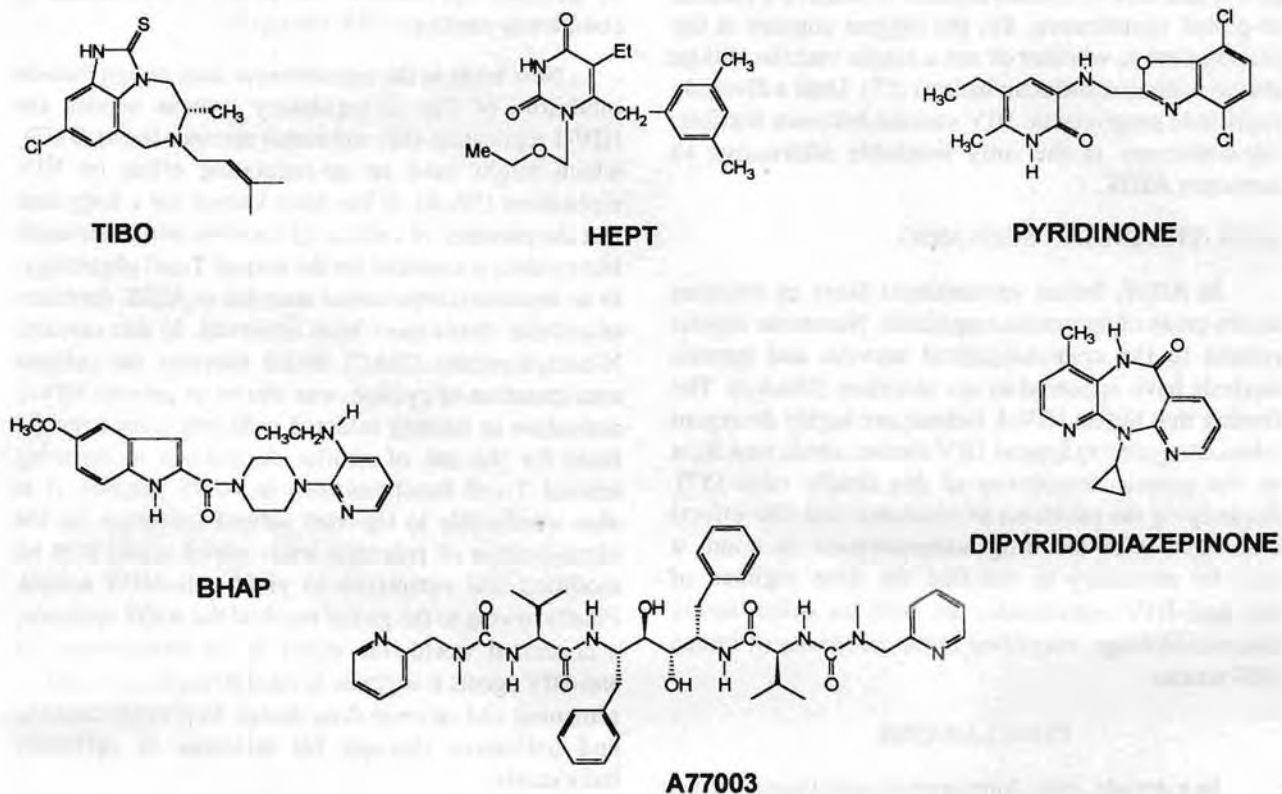**PYRIDINONE**

BHAP

DIPYRIDODIAZEPINONE

A77003

Fig. 4.

newer chemical entities and their molecular modifications, in order to discover potential inhibitors of HIV protease. Further, the synergism of protease inhibitors with other presently prescribed anti-HIV agents is being established for the usage of multi-agent regimen for the long term treatment and prevention of AIDS (52).

f) GLUCOSIDASE INHIBITORS

Polyhydroxylated Compounds

The final step in the HIV life cycle, before the budding of virion, involves the processing of the surface glycoproteins by the enzyme HIV glucosidase. This is another key step in the viral replicative cycle which is gaining attention for the chemotherapy of AIDS (53).

This enzyme cleaves off glucose units from the oligosaccharide chain, thus contributing to the maturation of infectious viral progency. Polyhydroxylated compounds such as castenospermine and N-butyldeoxynojirimycin have demonstrated inhibitory potential in preclinical evaluation (54). However, the selectivity of these compounds and their ability to distinguish between cellular and viral glycosylation has to be confirmed before their wide scale use.

ANTI-HIV VACCINE DESIGN

Beside chemotherapeutic approaches, rigorous research efforts are also being devoted for the development of safe and efficacious vaccine candidates against HIV infection. In order to bolster immunity in immune compromised individuals, equal contributions

from the humoral and the cellular immune response is usually required. The successful vaccine strategy will have to overcome the problem of genetic diversity of HIV-1 and HIV-2 isolates in order to achieve a vaccine of global significance. So, the biggest concern at the present time is whether or not a single vaccine will be able to neutralize different isolates (55). Until a diversely applicable prophylactic HIV vaccine becomes feasible, chemotherapy is the only available alternative to surmount AIDS.

AIDS: THE INDIAN SCENARIO

In AIDS, Indian subcontinent faces an ominous health crisis of enormous magnitude. Numerous reports related to the epidemiological surveys and genetic analysis have appeared in the literature (56a,b,c). The finding that Indian HIV-1 isolates are highly divergent when compared to typical HIV strains, sheds new light on the genetic complexity of this deadly virus (57). So, keeping the problems of resistance and side-effects associated with the drug administration in mind, it may be necessary to redefine the dose regimen of the anti-HIV nucleosides as well as other newly discovered drugs, according to the sensitivity of Indian HIV strains.

CONCLUSIONS

In a decade, since human immunodeficiency virus was first isolated, modern biology has successfully unraveled most of the intricate pathways regulating the viral replication. However, as we continue to get fresh insights into the complex life cycle of the virus, new molecular targets for chemotherapeutic intervention will continue to emerge. Until now, much emphasis and efforts have been directed toward the inhibiton of conventional targets like reverse transcriptase and protease, despite the availability of a multitude of putative inhibition sites in the viral life cycle. Also, it is now becoming increasingly clear that a combination of different approaches will probably be needed to completely eradicate HIV infection.

New leads in the antiretroviral drug design include inhibitors of Tat, a regulatory protein needed for HIV-1 replication (58) and tumor necrosis factor (TNF), which might have an up-regulating effect on HIV replication (59a,b). It has been known for a long time that the presence of cellular glutathione and other thiols like cystine, is essential for the normal T-cell physiology. In an immune compromised state due to AIDS, depletion of cellular thiols have been observed. In this context, N-acetylcysteine (NAC) which elevates the cellular concentration of cystine, was shown to prevent HIV-1 activation in latently infected cells and it presents the basis for the use of similar derivatives in restoring normal T-cell functions (60) in AIDS patients. It is also worthwhile to tap vast natural resources for the identification of potential leads which could then be modified and optimized to yield anti-AIDS agents. Finally, owing to the global reach of the AIDS epidemic, a concerted worldwide effort in the development of anti-HIV agents is urgently needed through semi-random screening and rational drug design to provide curative and palliative therapy for millions of suffering individuals.

ACKNOWLEDGMENTS

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