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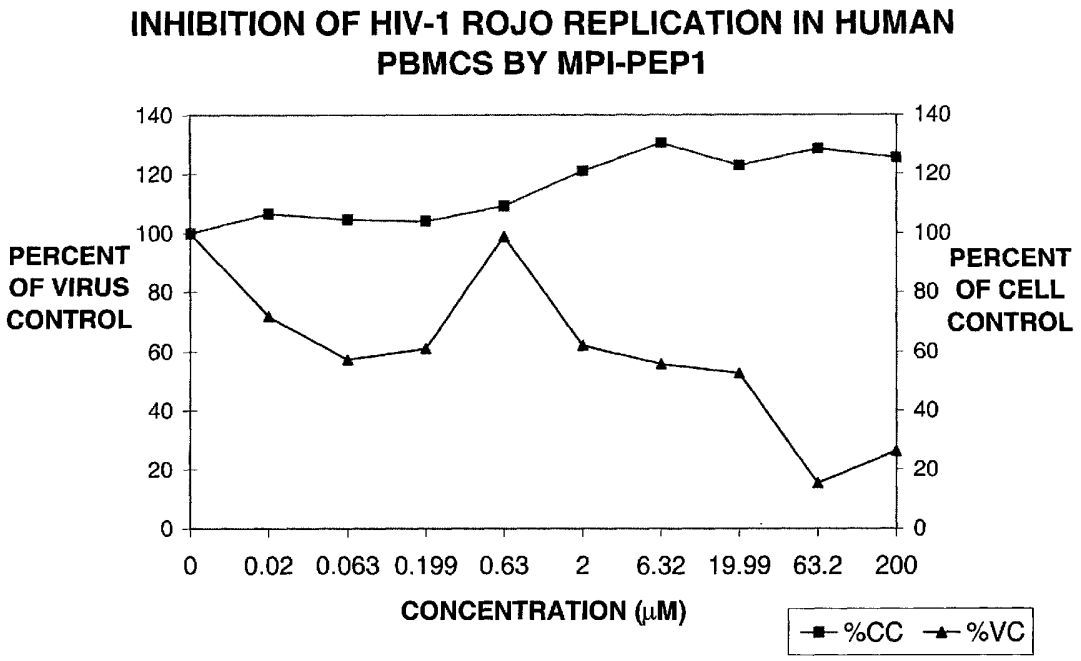
(54) Title: COMPOSITION AND METHOD FOR TREATING HIV INFECTION



(57) Abstract: Methods for inhibiting HIV propagation and treating HIV infection are provided which include administering to cells infected with HIV a compound capable of inhibiting viral budding from the infected host cells. The methods are especially useful in treating HIV infection and in treating and preventing AIDS.

WO 03/015708 A2

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COMPOSITION AND METHOD FOR TREATING HIV INFECTION

Technical Field of the Invention

The present invention generally relates to pharmaceuticals and methods of treating diseases, particularly to methods and pharmaceutical compositions for treating HIV infection and AIDS.

Technical Background of the Invention

Human immunodeficiency virus (HIV) infection causes the acquired immunodeficiency syndrome (commonly known as AIDS). HIV is a retrovirus that primarily infects T cells expressing the CD4 glycoprotein, i.e., $CD4^+$ T-cells, which are also known as helper T-cells. HIV virus multiplies in helper T-cells and quickly destroys the host helper T-cells, resulting in cellular immunity depression and leaving the infected patient susceptible to opportunistic infections, malignancies and various other pathological conditions. Ultimately, HIV infection can cause depletion of helper T-cells and collapse of a patient's immune defenses. Not surprisingly, HIV-infected individuals and AIDS patients typically develop AIDS-related conditions such as AIDS-related complex (ARC), progressive generalized lymphadenopathy (PGL), dementia, tropical paraparesis, Kaposi's sarcoma, thrombocytopenia purpurea, herpes infection, cytomegalovirus infection, Epstein-Barr virus related lymphomas among others. In any case, the HIV viruses in an infected individual are infectious and can be transmitted to other people through blood transfusion or sexual contacts.

There has been a great deal of effort in the past fifteen years or so in developing pharmaceutical compounds for treating HIV infection and AIDS. The therapeutic

approaches have been mostly focused on a limited number of drug targets, namely HIV reverse transcriptase, HIV protease, and HIV integrase. A number of reverse transcriptase inhibitors and protease inhibitors have been developed or marketed. Examples of nucleoside reverse transcriptase inhibitors include Zidovudine, Stavudine, Lamivudine, and ddI. Examples of non-nucleoside reverse transcriptase inhibitors include Efavirenz, Delavirdine, and Abacavir. In addition, a number of HIV protease inhibitors are commercially available including Ritonavir, Nelfinavir, Indinavir and Saquinavir.

However, HIV typically undergoes active mutations as it multiplies. In addition, there are extensive genetic variations in HIV partly due to high mutation rate. Therefore, mutations in HIV reverse transcriptase and protease arise frequently in infected individuals and render the virus resistant to the inhibitor administered to the individuals. Combination therapy, generally referred to as HAART (highly active anti-retroviral therapy), has been developed in which a combination of different anti-HIV inhibitors is administered to a patient. However, viral resistance to combination therapies still frequently develops.

In addition, many of the anti-HIV compounds known in the art have other serious drawbacks. For example, the reverse transcriptase inhibitors such as AZT and ddI are fairly toxic and cause serious side effects in patients treated with such compounds. Therefore, although limited success for controlling HIV infection and AIDS has been achieved with previously developed anti-HIV compounds, there is a need for alternative therapeutic approaches that overcome the shortcomings of currently available drugs.

Summary of the Invention

The present invention provides a method for inhibiting HIV budding from HIV-infected cells and thus inhibiting HIV propagation in the cells. The method comprises administering to cells a composition comprising a peptide that has a contiguous amino acid sequence of an HIV GAG protein. The contiguous amino acid sequence encompasses the late domain motif of said GAG protein. In addition, the peptide is capable of binding the UEV domain of Tsg101.

In preferred embodiments, the peptide in the composition is associated with a transporter capable of increasing the uptake of the peptide by the cells. Also preferably, the peptide consists of a contiguous amino acid sequence of 8 to 50 residues, more preferably 9 to 20 residues of an HIV GAG protein.

The method of inhibiting HIV budding in accordance with the present invention is useful in treating HIV infection and preventing AIDS.

Accordingly, the present invention provides a method for treating HIV infection, which comprises administering to a patient in need of such treatment a composition comprising a peptide associated with a transporter capable of increasing the uptake of the peptide by the cells. The peptide includes a contiguous amino acid sequence of an HIV GAG protein, encompassing the late domain motif of said GAG protein. Particularly, the peptide is capable of binding the UEV domain of Tsg101.

In a preferred embodiment of the treatment method, the peptide is covalently linked to the transporter. Advantageously, the transporter is selected from the group consisting of penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D-arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histidine oligomers, D-histidine oligomers, L-ornithine oligomers, D-ornithine oligomers, fibroblast growth factor and fragments thereof, Galparan and fragments thereof, and HSV-1 structural protein VP22 and fragments thereof, and peptoid analogs thereof. Alternatively, the transporter can be non-peptidic molecules or structures such as liposomes, dendrimers, and siderophores.

In another preferred embodiment of the treatment method, the peptide consists of from about 9 to about 50, more preferably from 9 to 20 amino acid residues. Examples of preferred peptides include, but are not limited to those consisting of a sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

When the transporter used in the method of the present invention is a peptide, a hybrid polypeptide or fusion polypeptide is provided. The hybrid polypeptide includes (a) a first portion capable of binding the UEV domain of Tsg101 and having a contiguous

amino acid sequence of an HIV GAG protein encompassing the late domain motif of the GAG protein, and (b) a second portion which is a peptidic transporter capable of increasing the uptake of the first portion by human cells. Preferably, the first portion consists of from 8 to 50, more preferably 9 to 20 amino acid residues. The hybrid polypeptide can be chemically synthesized or produced by recombinant expression. Thus, the present invention also provides isolated nucleic acids encoding the hybrid polypeptides, and host cells recombinantly expressing the hybrid polypeptides.

The peptide of the present invention can be administered to a patient in the presence or absence of a transporter. The peptide with or without a transporter can be administered directly to a patient in a pharmaceutical composition. Alternatively, the peptide or hybrid polypeptide according to the present invention can be introduced into a patient indirectly by administering to the patient a nucleic acid encoding the peptide or hybrid polypeptide.

Various modifications may be made to improve the stability and solubility of the peptides or hybrid polypeptides, and/or optimize its binding affinity to the UEV domain of Tsg101. In particular, various protection groups can be incorporated into the amino acid residues of the peptides or hybrid polypeptides. In addition, the compounds according to the present invention can also be in various pharmaceutically acceptable salt forms.

In another aspect of the present invention, methods of combination therapy for treating or preventing HIV and/or AIDs are provided. In such methods, both a compound of the present invention (in the presence or absence of a transporter) and one or more other anti-HIV compounds are administered to a patient in need of treatment. Such other anti-HIV compounds should be pharmaceutically compatible with the compound of the present invention. Compounds suitable for use in combination therapies with the Tsg101-binding compounds according to the present invention include, but are not limited to, HIV protease inhibitors, nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV integrase inhibitors, HIV fusion inhibitors, immunomodulators, and vaccines.

In accordance with yet another aspect of the present invention, an isolated peptide is provided having a contiguous amino acid sequence of 8 to 50 residues, more preferably

9 to 20 residues of an HIV GAG protein. The contiguous amino acid sequence encompasses the late domain motif of said GAG protein. In addition, the peptide is capable of binding the UEV domain of Tsg101. In preferred embodiments, the isolated peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

The foregoing and other advantages and features of the invention, and the manner in which the same are accomplished, will become more readily apparent upon consideration of the following detailed description of the invention taken in conjunction with the accompanying examples, which illustrate preferred and exemplary embodiments.

Brief Description of the Drawings

Figure 1 is a competitive inhibition curve showing that the p(1-14) peptide having the first 14 amino acid residues is capable of inhibiting protein-protein interaction between GST-p6 and myc-Tsg101(1-207);

Figure 2 is a Dixon plot showing p6(1-14) inhibition of the interaction between GST-p6 and myc-Tsg101(1-207);

Figure 3 is another Dixon plot showing p6(1-14) inhibition of the interaction between GST-p6 and myc-Tsg101(1-207);

Figure 4 is the graphical test results showing the effect of the compound MPI-PEP1 at various concentrations on HIV viral propagation in cell culture and on cell viability in the cell culture;

Figure 5 is the graphical test results of the compound MPI-PEP2;

Figure 6 is the graphical test results of the compound MPI-PEP3; and

Figure 7 is the graphical test results of AZT as a positive control compound.

Detailed Description of the Invention

As used herein, the term "HIV infection" generally encompasses infection of a host animal, particularly a human host, by the human immunodeficiency virus (HIV)

family of retroviruses including, but not limited to, HIV I, HIV II, HIV III (a.k.a. HTLV-III, LAV-1, LAV-2), and the like. "HIV" can be used herein to refer to any strains, forms, subtypes, clades and variations in the HIV family. Thus, treating HIV infection will encompass the treatment of a person who is a carrier of any of the HIV family of retroviruses or a person who is diagnosed of active AIDS, as well as the treatment or prophylaxis of the AIDS-related conditions in such persons. A carrier of HIV may be identified by any methods known in the art. For example, a person can be identified as HIV carrier on the basis that the person is anti-HIV antibody positive, or is HIV-positive, or has symptoms of AIDS. That is, "treating HIV infection" should be understood as treating a patient who is at any one of the several stages of HIV infection progression, which, for example, include acute primary infection syndrome (which can be asymptomatic or associated with an influenza-like illness with fevers, malaise, diarrhea and neurologic symptoms such as headache), asymptomatic infection (which is the long latent period with a gradual decline in the number of circulating CD^{4+} T cells), and AIDS (which is defined by more serious AIDS-defining illnesses and/or a decline in the circulating CD4 cell count to below a level that is compatible with effective immune function). In addition, "treating or preventing HIV infection" will also encompass treating suspected infection by HIV after suspected past exposure to HIV by e.g., contact with HIV-contaminated blood, blood transfusion, exchange of body fluids, "unsafe" sex with an infected person, accidental needle stick, receiving a tattoo or acupuncture with contaminated instruments, or transmission of the virus from a mother to a baby during pregnancy, delivery or shortly thereafter. The term "treating HIV infection" may also encompass treating a person who has not been diagnosed as having HIV infection but is believed to be at risk of infection by HIV.

The term "treating AIDS" means treating a patient who exhibits more serious AIDS-defining illnesses and/or a decline in the circulating CD4 cell count to below a level that is compatible with effective immune function. The term "treating AIDS" also encompasses treating AIDS-related conditions, which means disorders and diseases incidental to or associated with AIDS or HIV infection such as AIDS-related complex (ARC), progressive generalized lymphadenopathy (PGL), anti-HIV antibody positive conditions, and HIV-positive conditions, AIDS-related neurological conditions (such as

dementia or tropical paraparesis), Kaposi's sarcoma, thrombocytopenia purpurea and associated opportunistic infections such as Pneumocystis carinii pneumonia, *Mycobacterial tuberculosis*, esophageal candidiasis, toxoplasmosis of the brain, CMV retinitis, HIV-related encephalopathy, HIV-related wasting syndrome, etc.

Thus, the term "preventing AIDS" as used herein means preventing in a patient who has HIV infection or is suspected to have HIV infection or is at risk of HIV infection from developing AIDS (which is characterized by more serious AIDS-defining illnesses and/or a decline in the circulating CD4 cell count to below a level that is compatible with effective immune function) and/or AIDS-related conditions.

The terms "polypeptide," "protein," and "peptide" are used herein interchangeably to refer to amino acid chains in which the amino acid residues are linked by peptide bonds or modified peptide bonds. The amino acid chains can be of any length of greater than two amino acids. Unless otherwise specified, the terms "polypeptide," "protein," and "peptide" also encompass various modified forms thereof. Such modified forms may be naturally occurring modified forms or chemically modified forms. Examples of modified forms include, but are not limited to, glycosylated forms, phosphorylated forms, myristoylated forms, palmitoylated forms, ribosylated forms, acetylated forms, etc. Modified forms also encompass pharmaceutically acceptable salt forms. In addition, modifications also include intra-molecular crosslinking and covalent attachment to various moieties such as lipids, flavin, biotin, polyethylene glycol or derivatives thereof, etc. In addition, modifications may also include cyclization, and branching. Further, amino acids other than the conventional twenty amino acids encoded by genes may also be included in a polypeptide.

As used herein, the term "Tsg101" means human Tsg101 protein, unless otherwise specified.

In accordance with a first aspect of the present invention, a method is provided for inhibiting lentivirus budding from lentivirus-infected cells and thus inhibiting lentivirus propagation in the cells. The method includes administering to the cells a compound comprising an amino acid sequence motif of PX_1X_2P and capable of binding the UEV domain of Tsg101, wherein X_1 is any amino acid and X_2 is an amino acid other than arginine (R). The compounds can be administered to cells in vitro or cells in vivo in a

human or animal body. In the case of in vivo applications of the method, lentivirus infection can be treated and alleviated by using the compound to inhibit lentivirus propagation.

As is known in the art, lentiviruses are a group of retroviruses capable of long-term latent infection of vertebrate cells. They replicate in host cells only when activated. Lentiviruses typically have enveloped virions. Non-primate lentiviruses include bovine lentiviruses (e.g. bovine immunodeficiency virus (BIV), Jembrana disease virus), feline lentiviruses (e.g. feline immunodeficiency virus (FIV) which causes immunodeficiency, wasting, and encephalitis in cats), ovine/caprine lentivirus (e.g. caprine arthritis-encephalitis virus (CAEV) which causes anemia and wasting in goats, ovine lentivirus, Visna virus which causes pneumonia, wasting, encephalitis and arthritis), and Equine lentiviruses (e.g. Equine infectious anemia virus (EIAV), which infects horses causing arthritis and encephalitis). Examples of primate lentiviruses include human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), human immunodeficiency virus type 3 (HIV-3) (all of which cause AIDS), and various simian immunodeficiency viruses that infect hosts such as chimpanzee, mangabey, African Green monkey, mandrill, L'Hoest, Sykes' monkey, or Guereza Colobus monkey.

In one embodiment, the method is used for inhibiting HIV viral budding from HIV-infected cells and for inhibiting HIV propagation in the cells. By inhibiting HIV propagation in cells in a patient, the HIV viral load in the patient body can be prevented from increasing and can even be decreased. Accordingly, the method of the present invention can also be used in treating HIV infection as well as AIDS. In addition, when applied early before a patient develops AIDS, the method can be used to prevent AIDS by inhibiting HIV propagation and decreasing the viral load in the patient.

The compound which comprises the amino acid sequence motif PX_1X_2P and is capable of binding the UEV domain of Tsg101 can be of any type of chemical compounds so long as the compound is capable of binding the UEV domain of human Tsg101 and/or Tsg101 orthologs in animals such as cattles, feline, monkey, sheeps, goats, horses, and other lentivirus hosts. For example, the compound can be a peptide, a modified peptide, an oligonucleotide-peptide hybrid (e.g., PNA), etc. In a preferred

embodiment, the compound administered is capable of binding the UEV domain of human Tsg101.

In one embodiment, in the compound comprising an amino acid sequence motif PX_1X_2P and capable of binding the UEV domain of Tsg101, X_1 is selected from the group consisting of threonine (T), serine (S), and isoleucine (I), and X_2 is not R. In another embodiment, the X_2 in the motif is alanine (A) or threonine (T). In a more preferred embodiment, the compound administered has the amino acid sequence motif of PX_1X_2P, wherein X_1 is selected from the group consisting of T, S, and I, and X_2 is A or T.

Thus, the compound can be a tetrapeptide having an amino acid sequence of PX_1X_2P, wherein X_2 is an amino acid other than arginine. In one embodiment, the tetrapeptide has an amino acid sequence of P(T/S/I)(A/T)P (SEQ ID NOs:1-6). In a preferred embodiment, the tetrapeptide has the sequence of PTAP (SEQ ID NO:1). In another preferred embodiment, the tetrapeptide has the sequence of PSAP (SEQ ID NO. 2).

The compound can also include a longer peptide comprising the amino acid sequence motif of PX_1X_2P and capable of binding the UEV domain of Tsg101. For example, the compound may include a peptide of 5, 6, 7, 8 or 9 amino acids, preferably 10, 11, 12, 13, 14, 15 or more amino acids.

In a preferred embodiment, the compound includes a peptide that contains a contiguous amino acid sequence of an HIV GAG protein and is capable of binding the UEV domain of Tsg101. The contiguous amino acid sequence encompasses the late domain motif of the GAG protein, which can be the P(T/S/I)(A/T)P motif or a variant thereof.

In specific embodiments, the compound includes an amino acid sequence selected from the group of EPTAP (SEQ ID NO:7), EPSAP (SEQ ID NO:8), PTAPP (SEQ ID NO:9), PSAPP (SEQ ID NO:10), EPTAPP (SEQ ID NO:11), EPSAPP (SEQ ID NO:12), PEPTAP(SEQ ID NO:13), PEPSAP (SEQ ID NO:14), RPEPTAP (SEQ ID NO:15), RPEPSAP (SEQ ID NO:16), PEPTAPP (SEQ ID NO:17), PEPSAPP (SEQ ID NO:18), EPTAPPEE (SEQ ID NO:19), EPSAPPEE (SEQ ID NO:20), EPTAPPAE (SEQ ID NO:21), PEPTAPPEE (SEQ ID NO:22), PEPTAPPAE (SEQ ID NO:23), PEPSAPPEE

(SEQ ID NO:24), PGPTAPPEE (SEQ ID NO:25), PGPTAPPAE (SEQ ID NO:26), PGPSAPPEE (SEQ ID NO:27), RPEPTAPPEE (SEQ ID NO:28), RPEPSAPPEE (SEQ ID NO:29), RPEPTAPPAE (SEQ ID NO:30), RPEPSAPPAE (SEQ ID NO:31), RPGPTAPPEE (SEQ ID NO:32), RPGPSAPPEE (SEQ ID NO:33), RPGPTAPPAE (SEQ ID NO:34), RPGPSAPPAE (SEQ ID NO:35) LQSRPEPTAPPEE (SEQ ID NO:36), LQSRPEPSAPPEE (SEQ ID NO:37).

Advantageously, the compound is a peptide that contains a contiguous amino acid sequence of less than about 400, 375, 350, 325, 300, 275, 250, 225 or 200 residues of an HIV GAG protein, which encompasses the late domain motif of the GAG protein, and is capable of binding the UEV domain of Tsg101. Preferably, the peptide contains a contiguous amino acid sequence of less than about 175, 150, 125, 115, 100, 95, 90, 85, 80, 75, 70, 65, 60 or 55 residues of an HIV GAG protein, which encompasses the late domain motif of the GAG protein, and is capable of binding the UEV domain of Tsg101. More preferably, the peptide contains a contiguous amino acid sequence of less than about 50, 48, 45, 42, 40, 38, 35, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21 or 20 residues of an HIV GAG protein, which encompasses the late domain motif of the GAG protein, and is capable of binding the UEV domain of Tsg101. In preferred embodiments, the peptide contains a contiguous amino acid sequence of from about 4 to about 50, preferably from about 6 to about 50, from about 8 to about 50, more preferably from about 9 to about 50, from about 9 to 45, 9 to 40, 9 to 37, 9 to 35, 9 to 30, 9 to 25 residues of an HIV GAG protein, which encompasses the late domain motif of the GAG protein, and is capable of binding the UEV domain of Tsg101. More advantageously, the peptide contains a contiguous amino acid sequence of from 9 to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 residues of an HIV GAG protein, even more advantageously, from 10 to about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 residues of an HIV GAG protein, which encompasses the late domain motif of the GAG protein, and is capable of binding the UEV domain of Tsg101. Preferably, the late domain motif in the contiguous span is the P(T/S)AP motif.

In another embodiment, the PX_1X_2P motif in the compound according to the present invention is within an amino acid sequence that is at least 70 percent, preferably at least 80 percent or 85 percent, more preferably at least 90 percent or 95 percent

identical to a contiguous span of at least 5, 6, 7, 8 or 9 amino acids, preferably 10, 11, 12, 13, 14, 15 or more amino acids of a naturally occurring HIV Gag sequence that spans the HIV late domain motif. In this respect, the percentage identity is determined by the algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA*, 90:5873-77 (1993), which is incorporated into the various BLAST programs. Specifically, the percentage identity is determined by the "BLAST 2 Sequences" tool, which is available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. *See* Tatusova and Madden, *FEMS Microbiol. Lett.*, 174(2):247-50 (1999). For pairwise protein-protein sequence comparison, the BLASTP 2.1.2 program is employed using default parameters (Matrix: BLOSUM62; gap open: 11; gap extension: 1; x_dropoff: 15; expect: 10.0; and wordsize: 3, with filter). It should be understood that such homologue peptides should retain the ability to bind the UEV domain of Tsg101. Preferably, in this embodiment of the present invention, X_1 in the PX_1X_2P motif is selected from the group consisting of T, S, and I, and X_2 is not R. More preferably, X_1 is selected from the group consisting of T, S, and I, and X_2 is A or T. Most preferably, X_1 is T or S, and X_2 is A.

The homologues can be made by site-directed mutagenesis based on a late domain motif-containing Gag polyprotein sequence of HIV or other lentiviruses. The site-directed mutagenesis can be designed to generate amino acid substitutions, insertions, or deletions. Methods for conducting such mutagenesis should be apparent to skilled artisans in the field of molecular biology. The resultant homologues can be tested for their binding affinity to the UEV domain of Tsg101.

The peptide portion in the compounds according to the present invention can also be in a modified form. Various modifications may be made to improve the stability and solubility of the compound, and/or optimize its binding affinity to the UEV domain of Tsg101. Examples of modified forms include, but are not limited to, glycosylated forms, phosphorylated forms, myristoylated forms, palmitoylated forms, ribosylated forms, acetylated forms, etc. Modifications also include intra-molecular crosslinking and covalent attachment to various moieties such as lipids, flavin, biotin, polyethylene glycol or derivatives thereof, etc. In addition, modifications may also include cyclization, and branching. Amino acids other than the conventional twenty amino acids encoded by genes may also be included in a polypeptide sequence in the compound of the present

invention. For example, the compounds may include D-amino acids in place of L-amino acids.

To increase the stability of the compounds according to the present invention, various protection groups can also be incorporated into the amino acid residues of the compounds. In particular, terminal residues are preferably protected. Carboxyl groups may be protected by esters (e.g., methyl, ethyl, benzyl, p-nitrobenzyl, t-butyl or t-amyl esters, etc.), lower alkoxyl groups (e.g., methoxy, ethoxy, propoxy, butoxy, etc.), aralkyloxy groups (e.g., benzyloxy, etc.), amino groups, lower alkylamino or di(lower alkyl)amino groups. The term "lower alkoxy" is intended to mean an alkoxy group having a straight, branched or cyclic hydrocarbon moiety of up to six carbon atoms. Protection groups for amino groups may include lower alkyl, benzyloxycarbonyl, t-butoxycarbonyl, and sobornyloxycarbonyl. "Lower alkoxy" is intended to mean an alkyl group having a straight, branched or cyclic hydrocarbon moiety of up to six carbon atoms. In one example, a 5-oxo-L-prolyl residue may be used in place of a prolyl residue. A 5-oxo-L-prolyl residue is especially desirable at the N-terminus of a peptide compound. In another example, when a proline residue is at the C-terminus of a peptide compound, a N-ethyl-L-prolinamide residue may be desirable in place of the proline residue. Various other protection groups known in the art useful in increasing the stability of peptide compounds can also be employed.

In addition, the compounds according to the present invention can also be in various pharmaceutically acceptable salt forms. "Pharmaceutically acceptable salts" refers to the relatively non-toxic, organic or inorganic salts of the compounds of the present invention, including inorganic or organic acid addition salts of the compound. Examples of such salts include, but are not limited to, hydrochloride salts, hydrobromide salts, sulfate salts, bisulfate salts, nitrate salts, acetate salts, phosphate salts, nitrate salts, oxalate salts, valerate salts, oleate salts, borate salts, benzoate salts, laurate saltes, stearate salts, palmitate salts, lactate salts, tosylate salts, citrate salts, maleate, salts, succinate salts, tartrate salts, naththylate salts, fumarate salts, mesylate salts, laurylsuphonate salts, glucoheptonate salts, and the like. *See, e.g.,* Berge, *et al. J. Pharm. Sci.*, 66:1-19 (1977).

Suitable pharmaceutically acceptable salts also include, but are not limited to, alkali metal salts, alkaline earth salts, and ammonium salts. Thus, suitable salts may be

salts of aluminum, calcium, lithium, magnesium, potassium, sodium and zinc. In addition, organic salts may also be used including, e.g., salts of lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), procaine and tris. In addition, metal complex forms (e.g. copper complex compounds, zinc complex compounds, etc.) of the compounds of the present invention may also exhibit improved stability.

Additionally, as will be apparent to skilled artisans apprised of the present disclosure, peptide mimetics can be designed based on the above-described compounds according to the present invention. However, it is noted that the mimetics must be capable of binding the UEV domain of Tsg101. For example, peptoid analogs of the P(T/S)(A/T)P motif can be prepared using known methods. Peptoids are oligomeric N-substituted glycines. Typically, various side chain groups can be included when forming an N-substituted glycine (peptoid monomer) that mimics a particular amino acid. Peptoid monomers can be linked together to form an oligomeric N-substituted glycines - peptoid. Peptoids are easy to synthesize in large amounts. In contrast to peptides, the backbone linkage of peptoids are resistant to hydrolytic enzymes. In addition, since a variety of functional groups can be presented as side chains off of the oligomeric backbone, peptoid analogs corresponding to any peptides can be produced with improved characterics. *See* Simon *et al., Proc. Natl. Acad. Sci. USA,* 89:9367-9371 (1992); Figliozzi *et al., Methods Enzymol.,* 267:437-447 (1996); Horwell, *Trends Biotechnol.,* 13:132-134 (1995); and Horwell, *Drug Des. Discov.,* 12:63-75 (1994), all of which are incorporated herein by reference.

Thus, peptoid analogs of the above-described compounds of the present invention can be made using methods known in the art. The thus prepared peptoid analogs can be tested for their binding affinity to Tsg101. They can also be tested in anti-viral assays for their ability to inhibit lentivirus budding from infected host cells and ability to inhibit lentivirus propagation. In particular, they can be tested for ability to suppress HIV budding from infected human cells and inhibit HIV propagation.

Mimetics of the compounds of the present invention can also be selected by rational drug design and/or virtual screening. Methods known in the art for rational drug design can be used in the present invention. *See, e.g.,* Hodgson *et al., Bio/Technology,*

9:19-21 (1991); U.S. Patent Nos. 5,800,998 and 5,891,628, all of which are incorporated herein by reference. An example of rational drug design is the development of HIV protease inhibitors. *See* Erickson *et al.*, *Science,* 249:527-533 (1990). Structural information on the UEV domain of Tsg101 and/or the binding complex formed by the Tsg101 UEV domain and the HIV Gag p6 PTAP motif are obtained. The interacting complex can be studied using various biophysics techniques including, e.g., X-ray crystallography, NMR, computer modeling, mass spectrometry, and the like. Likewise, structural information can also be obtained from protein complexes formed by the Tsg101 UEV domain and a variation of the PTAP motif.

Computer programs are employed to select compounds based on structural models of the binding complex formed by the Tsg101 UEV domain and the HIV Gag p6 PTAP motif. In addition, once an effective compound is identified, structural analogs or mimetics thereof can be produced based on rational drug design with the aim of improving drug efficacy and stability, and reducing side effects.

In addition, understanding of the interaction between the Tsg101 UEV domain and compounds of the present invention can also be derived from mutagenesis analysis using yeast two-hybrid system or other methods for detection protein-protein interaction. In this respect, various mutations can be introduced into the interacting proteins and the effect of the mutations on protein-protein interaction is examined by a suitable method such as in vitro binding assay or the yeast two-hybrid system.

Various mutations including amino acid substitutions, deletions and insertions can be introduced into the protein sequence of the Tsg101 UEV domain and/or a compound of the present invention using conventional recombinant DNA technologies. Generally, it is particularly desirable to decipher the protein binding sites. Thus, it is important that the mutations introduced only affect protein-protein interaction and cause minimal structural disturbances. Mutations are preferably designed based on knowledge of the three-dimensional structure of the interacting proteins. Preferably, mutations are introduced to alter charged amino acids or hydrophobic amino acids exposed on the surface of the proteins, since ionic interactions and hydrophobic interactions are often involved in protein-protein interactions. Alternatively, the "alanine scanning mutagenesis" technique is used. *See* Wells, *et al., Methods Enzymol.*, 202:301-306

(1991); Bass *et al., Proc. Natl. Acad. Sci. USA*, 88:4498-4502 (1991); Bennet *et al., J. Biol. Chem.*, 266:5191-5201 (1991); Diamond *et al., J. Virol.*, 68:863-876 (1994). Using this technique, charged or hydrophobic amino acid residues of the interacting proteins are replaced by alanine, and the effect on the interaction between the proteins is analyzed using e.g., an in vitro binding assay. In this manner, the domains or residues of the proteins important to compound-target interaction can be identified.

Based on the structural information obtained, structural relationships between the Tsg101 UEV domain and a compound of the present invention are elucidated. The moieties and the three-dimensional structures critical to the interaction are revealed. Medicinal chemists can then design analog compounds having similar moieties and structures.

The residues or domains critical to the modulating effect of the identified compound constitute the active region of the compound known as its "pharmacophore." Once the pharmacophore has been elucidated, a structural model can be established by a modeling process that may incorporate data from NMR analysis, X-ray diffraction data, alanine scanning, spectroscopic techniques and the like. Various techniques including computational analysis, similarity mapping and the like can all be used in this modeling process. *See e.g.,* Perry *et al., in OSAR: Quantitative Structure-Activity Relationships in Drug Design*, pp.189-193, Alan R. Liss, Inc., 1989; Rotivinen *et al., Acta Pharmaceutical Fennica,* 97:159-166 (1988); Lewis *et al., Proc. R. Soc. Lond.*, 236:125-140 (1989); McKinaly *et al., Annu. Rev. Pharmacol. Toxiciol.*, 29:111-122 (1989). Commercial molecular modeling systems available from Polygen Corporation, Waltham, MA, include the CHARMm program, which performs the energy minimization and molecular dynamics functions, and QUANTA program which performs the construction, graphic modeling and analysis of molecular structure. Such programs allow interactive construction, visualization and modification of molecules. Other computer modeling programs are also available from BioDesign, Inc. (Pasadena, CA.), Hypercube, Inc. (Cambridge, Ontario), and Allelix, Inc. (Mississauga, Ontario, Canada).

A template can be formed based on the established model. Various compounds can then be designed by linking various chemical groups or moieties to the template. Various moieties of the template can also be replaced. These rationally designed

compounds are further tested. In this manner, pharmacologically acceptable and stable compounds with improved efficacy and reduced side effect can be developed. The compounds identified in accordance with the present invention can be incorporated into a pharmaceutical formulation suitable for administration to an individual.

The mimetics including peptoid analogs can exhibit optimal binding affinity to the UEV domain of human Tsg101 or animal orthologs thereof. Various known methods can be utilized to test the Tsg101-binding characteristics of a mimetics. For example, the entire Tsg101 protein or a fragment thereof containing the UEV domain may be recombinantly expressed, purified, and contacted with the mimetics to be tested. Binding can be determined using a surface plasmon resonance biosensor. *See e.g.,* Panayotou *et al., Mol. Cell. Biol.*, 13:3567-3576 (1993). Other methods known in the art for estimating and determining binding constants in protein-protein interactions can also be employed. *See* Phizicky and Fields, *et al., Microbiol. Rev.*, 59:94-123 (1995). For example, protein affinity chromatography may be used. First, columns are prepared with different concentrations of an interacting member, which is covalently bound to the columns. Then a preparation of its interacting partner is run through the column and washed with buffer. The interacting partner bound to the interacting member linked to the column is then eluted. Binding constant is then estimated based on the concentrations of the bound protein and the eluted protein. Alternatively, the method of sedimentation through gradients monitors the rate of sedimentation of a mixture of proteins through gradients of glycerol or sucrose. At concentrations above the binding constant, the two interacting members sediment as a complex. Thus, binding constant can be calculated based on the concentrations. Other suitable methods known in the art for estimating binding constant include but are not limited to gel filtration column such as nonequilibrium "small-zone" gel filtration columns (*See e.g.,* Gill *et al., J. Mol. Biol.,* 220:307-324 (1991)), the Hummel-Dreyer method of equilibrium gel filtration (*See e.g.,* Hummel and Dreyer, *Biochim. Biophys. Acta,* 63:530-532 (1962)) and large-zone equilibrium gel filtration (*See e.g.,* Gilbert and Kellett, *J. Biol. Chem.,* 246:6079-6086 (1971)), sedimentation equilibrium (*See e.g.,* Rivas and Minton, *Trends Biochem.,* 18:284-287 (1993)), fluorescence methods such as fluorescence spectrum (*See e.g.,* Otto-Bruc *et al, Biochemistry,* 32:8632-8645 (1993)) and fluorescence polarization or

anisotropy with tagged molecules (*See e.g.,* Weiel and Hershey, *Biochemistry,* 20:5859-5865 (1981)), and solution equilibrium measured with immobilized binding protein (*See e.g.,* Nelson and Long, *Biochemistry,* 30:2384-2390 (1991)).

The compounds capable of binding Tsg101 UEV domain according the present invention can be delivered into cells by direct cell internalization, receptor mediated endocytosis, or via a "transporter." It is noted that the compound administered to cells in vitro or in vivo in the method of the present invention preferably is delivered into the cells in order to achieve optimal results. Thus, preferably, the compound to be delivered is associated with a transporter capable of increasing the uptake of the compound by an animal cell susceptible to infection by a lentivirus, particularly HIV. As used herein, the term "associated with" means a compound to be delivered is physically associated with a transporter. The compound and the transporter can be covalently linked together, or associated with each other as a result of physical affinities such as forces caused by electrical charge differences, hydrophobicity, hydrogen bonds, van der Waals force, ionic force, or a combination thereof. For example, the compound can be encapsulated within a transporter such as a liposome.

As used herein, the term "transporter" refers to an entity (e.g., a compound or a composition or a physical structure formed from multiple copies of a compound or multiple different compounds) that is capable of facilitating the uptake of a compound of the present invention by animal cells, particularly human cells. Typically, the cell uptake of a compound of the present invention in the presence of a "transporter" is at least 50% higher, preferably at least 60%, 75% or 90% higher, and more preferably at least 100% higher than the cell uptake of the compound in the absence of the "transporter." Methods of assaying cell uptake of a compound should be apparent to skilled artisans. For example, the compound to be delivered can be labeled with a radioactive isotope or another detectable marker (e.g., a fluorescence marker), and added to cultured cells in the presence or absence of a transporter, and incubated for a time period sufficient to allow maximal uptake. Cells can then be separated from the culture medium and the detectable signal (e.g., radioactivity) caused by the compound inside the cells can be measured. The result obtained in the presence of a transporter can be compared to that obtained in the absence of a transporter.

Many molecules and structures known in the art can be used as "transporter." In one embodiment, a penetratin is used as a transporter. For example, the homeodomain of Antennapedia, a *Drosophila* transcription factor, can be used as a transporter to deliver a compound of the present invention. Indeed, any suitable member of the penetratin class of peptides can be used to carry a compound of the present invention into cells. Penetratins are disclosed in, e.g., Derossi *et al., Trends Cell Biol.,* 8:84-87 (1998), which is incorporated herein by reference. Penetratins transport molecules attached thereto across cytoplasm membranes or nucleus membranes efficiently in a receptor-independent, energy-independent, and cell type-independent manner. Methods for using a penetratin as a carrier to deliver oligonucleotides and polypeptides are also disclosed in U.S. Patent No. 6,080,724; Pooga *et al., Nat. Biotech.,* 16:857 (1998); and Schutze *et al., J. Immunol.,* 157:650 (1996), all of which are incorporated herein by reference. U.S. Patent No. 6,080,724 defines the minimal requirements for a penetratin peptide as a peptide of 16 amino acids with 6 to 10 of which being hydrophobic. The amino acid at position 6 counting from either the N- or C-terminal is tryptophan, while the amino acids at positions 3 and 5 counting from either the N- or C-terminal are not both valine. Preferably, the helix 3 of the homeodomain of *Drosophila* Antennapedia is used as a transporter. More preferably, a peptide having a sequence of the amino acids 43-58 of the homeodomain Antp is employed as a transporter. In addition, other naturally occurring homologs of the helix 3 of the homeodomain of *Drosophila* Antennapedia can also be used. For example, homeodomains of Fushi-tarazu and Engrailed have been shown to be capable of transporting peptides into cells. *See* Han *et al., Mol. Cells,* 10:728-32 (2000). As used herein, the term "penetratin" also encompasses peptoid analogs of the penetratin peptides. Typically, the penetratin peptides and peptoid analogs thereof are covalently linked to a compound to be delivered into cells thus increasing the cellular uptake of the compound.

In another embodiment, the HIV-1 tat protein or a fragment or derivative thereof is used as a "transporter" covalently linked to a compound according to the present invention. The use of HIV-1 tat protein and derivatives thereof to deliver macromolecules into cells has been known in the art. *See* Green and Loewenstein, *Cell,* 55:1179 (1988); Frankel and Pabo, *Cell,* 55:1189 (1988); Vives *et al., J. Biol. Chem.,*

272:16010-16017 (1997); Schwarze *et al., Science*, 285:1569-1572 (1999). It is known that the sequence responsible for cellular uptake consists of the highly basic region, amino acid residues 49-57. *See e.g.,* Vives *et al., J. Biol. Chem.,* 272:16010-16017 (1997); Wender *et al., Proc. Nat'l Acad. Sci. USA,* 97:13003-13008 (2000). The basic domain is believed to target the lipid bilayer component of cell membranes. It causes a covalently linked protein or nucleic acid to cross cell membrane rapidly in a cell type-independent manner. Proteins ranging in size from 15 to 120 kD have been delivered with this technology into a variety of cell types both in vitro and in vivo. *See* Schwarze *et al., Science*, 285:1569-1572 (1999). Any HIV tat-derived peptides or peptoid analogs thereof capable of transporting macromolecules such as peptides can be used for purposes of the present invention. For example, any native tat peptides having the highly basic region, amino acid residues 49-57 can be used as a transporter by covalently linking it to the compound to be delivered. In addition, various analogs of the tat peptide of amino acid residues 49-57 can also be useful transporters for purposes of this invention. Examples of various such analogs are disclosed in Wender *et al., Proc. Nat'l Acad. Sci. USA,* 97:13003-13008 (2000) (which is incorporated herein by reference) including, e.g., *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57} (i.e., *l*-Tat_{57-49} and *d*-Tat_{57-49}), L-arginine oligomers, D- arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histidine oligomers, D-histidine oligomers, L-ornithine oligomers, D-ornithine oligomers, and various homologues, derivatives (e.g., modified forms with conjugates linked to the small peptides) and peptoid analogs thereof. As used herein, the term "oligomer" means a molecule that includes a covalently linked chain of amino acid residues of the same amino acids having a large enough number of such amino acid residues to confer transporter activities on the molecule. Typically, an oligomer contains at least 6, preferably at least 7, 8, or at least 9 such amino acid residues. In one embodiment, the transporter is a peptide that includes at least six contiguous amino acid residues, all of which are L-arginine, D-arginine, L-lysine, D-lysine, L-histidine, D-histidine, L-ornithine, D-ornithine, or a combination thereof.

Other useful transporters known in the art include, but are not limited to, short peptide sequences derived from fibroblast growth factor (*See* Lin *et al., J. Biol. Chem.,*

270:14255-14258 (1998)), Galparan (*See* Pooga *et al., FASEB J.* 12:67-77 (1998)), and HSV-1 structural protein VP22 (*See* Elliott and O'Hare, *Cell*, 88:223-233 (1997)).

As the above-described various transporters are generally peptides, fusion proteins can be conveniently made by recombinant expression to contain a transporter peptide covalently linked by a peptide bond to a peptide having the PX_1X_2P motif. Alternatively, conventional methods can be used to chemically synthesize a transporter peptide or a peptide of the present invention or both.

In addition to peptide-based transporters, various other types of transporters can also be used, including but not limited to cationic liposomes (*see* Rui *et al., J. Am. Chem. Soc.*, 120:11213-11218 (1998)), dendrimers (Kono *et al., Bioconjugate Chem.*, 10:1115-1121 (1999)), siderophores (Ghosh *et al., Chem. Biol.*, 3:1011-1019 (1996)), etc. In a specific embodiment, the compound according to the present invention is encapsulated into liposomes for delivery into cells.

Additionally, when a compound according to the present invention is a peptide, it can be administered to cells by a gene therapy method. That is, a nucleic acid encoding the peptide can be administered to in vitro cells or to cells in vivo in a human or animal body. Various gene therapy methods are well known in the art. Successes in gene therapy have been reported recently. *See e.g.,* Kay *et al., Nature Genet.*, 24:257-61 (2000); Cavazzana-Calvo *et al., Science*, 288:669 (2000); and Blaese *et al., Science*, 270: 475 (1995); Kantoff, *et al., J. Exp. Med.*, 166:219 (1987).

Any suitable gene therapy methods may be used for purposes of the present invention. Generally, an exogenous nucleic acid encoding a peptide compound of the present invention is incorporated into a suitable expression vector and is operably linked to a promoter in the vector. Suitable promoters include but are not limited to viral transcription promoters derived from adenovirus, simian virus 40 (SV40) (e.g., the early and late promoters of SV40), Rous sarcoma virus (RSV), and cytomegalovirus (CMV) (e.g., CMV immediate-early promoter), human immunodeficiency virus (HIV) (e.g., long terminal repeat (LTR)), vaccinia virus (e.g., 7.5K promoter), and herpes simplex virus (HSV) (e.g., thymidine kinase promoter). Where tissue-specific expression of the exogenous gene is desirable, tissue-specific promoters may be operably linked to the exogenous gene. In this respect, a CD^{4+} T cell-specific promoter will be most desirable.

In addition, selection markers may also be included in the vector for purposes of selecting, in vitro, those cells that contain the exogenous nucleic acid encoding the peptide compound of the present invention. Various selection markers known in the art may be used including, but not limited to, e.g., genes conferring resistance to neomycin, hygromycin, zeocin, and the like.

In one embodiment, the exogenous nucleic acid is incorporated into a plasmid DNA vector. Many commercially available expression vectors may be useful for the present invention, including, e.g., pCEP4, pcDNAI, pIND, pSecTag2, pVAX1, pcDNA3.1, and pBI-EGFP, and pDisplay.

Various viral vectors may also be used. Typically, in a viral vector, the viral genome is engineered to eliminate the disease-causing capability, e.g., the ability to replicate in the host cells. The exogenous nucleic acid to be introduced into a patient may be incorporated into the engineered viral genome, e.g., by inserting it into a viral gene that is non-essential to the viral infectivity. Viral vectors are convenient to use as they can be easily introduced into tissue cells by way of infection. Once in the host cell, the recombinant virus typically is integrated into the genome of the host cell. In rare instances, the recombinant virus may also replicate and remain as extrachromosomal elements.

A large number of retroviral vectors have been developed for gene therapy. These include vectors derived from oncoretroviruses (e.g., MLV), lentiviruses (e.g., HIV and SIV) and other retroviruses. For example, gene therapy vectors have been developed based on murine leukemia virus (*See*, Cepko, *et al.*, Cell, 37:1053-1062 (1984), Cone and Mulligan, *Proc. Natl. Acad. Sci. U.S.A.*, 81:6349-6353 (1984)), mouse mammary tumor virus (*See*, Salmons *et al.*, *Biochem. Biophys. Res. Commun.*,159:1191-1198 (1984)), gibbon ape leukemia virus (*See*, Miller *et al.*, *J. Virology*, 65:2220-2224 (1991)), HIV, (*See* Shimada *et al.*, *J. Clin. Invest.*, 88:1043-1047 (1991)), and avian retroviruses (*See* Cosset *et al.*, *J. Virology*, 64:1070-1078 (1990)). In addition, various retroviral vectors are also described in U.S. Patent Nos. 6,168,916; 6,140,111; 6,096,534; 5,985,655; 5,911,983; 4,980,286; and 4,868,116, all of which are incorporated herein by reference.

Adeno-associated virus (AAV) vectors have been successfully tested in clinical trials. *See e.g.*, Kay *et al.*, *Nature Genet.* 24:257-61 (2000). AAV is a naturally

occurring defective virus that requires other viruses such as adenoviruses or herpes viruses as helper viruses. *See* Muzyczka, *Curr. Top. Microbiol. Immun.*, 158:97 (1992). A recombinant AAV virus useful as a gene therapy vector is disclosed in U.S. Patent No. 6,153,436, which is incorporated herein by reference.

Adenoviral vectors can also be useful for purposes of gene therapy in accordance with the present invention. For example, U.S. Patent No. 6,001,816 discloses an adenoviral vector, which is used to deliver a leptin gene intravenously to a mammal to treat obesity. Other recombinant adenoviral vectors may also be used, which include those disclosed in U.S. Patent Nos. 6,171,855; 6,140,087; 6,063,622; 6,033,908; and 5,932,210, and Rosenfeld *et al.*, *Science*, 252:431-434 (1991); and Rosenfeld *et al.*, *Cell*, 68:143-155 (1992).

Other useful viral vectors include recombinant hepatitis viral vectors (*See, e.g.*, U.S. Patent No. 5,981,274), and recombinant entomopox vectors (*See, e.g.*, U.S. Patent Nos. 5,721,352 and 5,753,258).

Other non-traditional vectors may also be used for purposes of this invention. For example, International Publication No. WO 94/18834 discloses a method of delivering DNA into mammalian cells by conjugating the DNA to be delivered with a polyelectrolyte to form a complex. The complex may be microinjected into or taken up by cells.

The exogenous nucleic acid fragment or plasmid DNA vector containing the exogenous gene may also be introduced into cells by way of receptor-mediated endocytosis. *See e.g.*, U.S. Patent No. 6,090,619; Wu and Wu, *J. Biol. Chem.*, 263:14621 (1988); Curiel *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:8850 (1991). For example, U.S. Patent No. 6,083,741 discloses introducing an exogenous nucleic acid into mammalian cells by associating the nucleic acid to a polycation moiety (e.g., poly-L-lysine, having 3-100 lysine residues), which is itself coupled to an integrin receptor binding moiety (e.g., a cyclic peptide having the amino acid sequence RGD).

Alternatively, the exogenous nucleic acid or vectors containing it can also be delivered into cells via amphiphiles. *See e.g.*, U.S. Patent No. 6,071,890. Typically, the exogenous nucleic acid or a vector containing the nucleic acid forms a complex with the

cationic amphiphile. Mammalian cells contacted with the complex can readily absorb the complex.

The exogenous nucleic acid can be introduced into a patient for purposes of gene therapy by various methods known in the art. For example, the exogenous nucleic acid alone or in a conjugated or complex form described above, or incorporated into viral or DNA vectors, may be administered directly by injection into an appropriate tissue or organ of a patient. Alternatively, catheters or like devices may be used for delivery into a target organ or tissue. Suitable catheters are disclosed in, e.g., U.S. Patent Nos. 4,186,745; 5,397,307; 5,547,472; 5,674,192; and 6,129,705, all of which are incorporated herein by reference.

In addition, the exogenous nucleic acid encoding a peptide compound of the present invention or vectors containing the nucleic acid can be introduced into isolated cells using any known techniques such as calcium phosphate precipitation, microinjection, lipofection, electroporation, gene gun, receptor-mediated endocytosis, and the like. Cells expressing the exogenous gene may be selected and redelivered back to the patient by, e.g., injection or cell transplantation. The appropriate amount of cells delivered to a patient will vary with patient conditions, and desired effect, which can be determined by a skilled artisan. *See e.g.,* U.S. Patent Nos. 6,054,288; 6,048,524; and 6,048,729. Preferably, the cells used are autologous, i.e., obtained from the patient being treated.

When the transporter used in the method of the present invention is a peptide, a hybrid polypeptide or fusion polypeptide is provided. In preferred embodiments, the hybrid polypeptide includes (a) a first portion capable of binding the UEV domain of Tsg101 and having a contiguous amino acid sequence of an HIV GAG protein encompassing the late domain motif of the GAG protein, and (b) a second portion which is a peptidic transporter capable of increasing the uptake of the first portion by human cells. Preferably, the first portion consists of from 8 to 50, more preferably 9 to 20 amino acid residues. The hybrid polypeptide can be produced in a patient's body by administering to the patient a nucleic acid encoding the hybrid polypeptide by a gene therapy method as described above. Alternatively, the hybrid polypeptide can be chemically synthesized or produced by recombinantly expression.

Thus, the present invention also provides isolated nucleic acids encoding the hybrid polypeptides and host cells recombinantly expressing the hybrid polypeptides. Such a host cell can be prepared by introducing into a suitable cell an exogenous nucleic acid encoding one of the hybrid polypeptides by standard molecular cloning techniques as described above.

The compounds according to the present invention capable of binding Tsg101 are a novel class of anti-HIV compounds distinct from other commercially available compounds. While not wishing to be bound by any theory or hypothesis, it is believed that the compounds according to the present invention inhibit HIV through a mechanism distinct from those of the anti-HIV compounds known in the art, which typically are either protease inhibitors or reverse transcriptase inhibitors. Therefore, it may be desirable to employ combination therapies to administer to a patient both a compound according to the present invention, with or without a transporter, and another anti-HIV compound of a different class. However, it is to be understood that such other anti-HIV compounds should be pharmaceutically compatible with the compound of the present invention. By "pharmaceutically compatible" it is intended that the other anti-viral agent(s) will not interact or react with the above composition, directly or indirectly, in such a way as to adversely affect the effect of the treatment, or to cause any significant adverse side reaction in the patient. In this combination therapy approach, the two different pharmaceutically active compounds can be administered separately or in the same pharmaceutical composition. Compounds suitable for use in combination therapies with the Tsg101-binding compounds according to the present invention include, but are not limited to, HIV protease inhibitors, nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV integrase inhibitors, immunomodulators, and vaccines.

Examples of nucleoside HIV reverse transcriptase inhibitors include 3'-Azido-3'-deoxythymidine (Zidovudine, also known as AZT and RETROVIR®), 2',3'-Didehydro-3'-deoxythymidine (Stavudine, also known as 2',3'-dihydro-3'-deoxythymidine, d4T, and ZERIT®), (2R-cis)-4-Amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone (Lamivudine, also known as 3TC, and EPIVIR®), and 2', 3'-dideoxyinosine (ddI).

Examples of non-nucleoside HIV reverse transcriptase inhibitors include (-)-6-Chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one (efavirenz, also known as DMP-266 or SUSTIVA®) (*see* U.S. Pat. No. 5,519,021), 1-[3-[(1-methylethyl)aminol]-2-pyridinyl]-4-[[5-[(methylsulfonyl)amino]-1H -indol-2-yl]carbonyl]piperazine (Delavirdine, *see* PCT International Patent Application No. WO 91/09849), and (1S,4R)-cis-4-[2-amino-6-(cycloprpoylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (Abacavir).

Examples of protease inhibitors include [5S-(5R*,8R*, 10R*,11R*)]-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2, 4, 7, 12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester (Ritonavir, marketed by Abbott as NORVIR®), [3S-[2(2S*,3S*),3a,4ab,8ab]]-N-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinolinecarb oxamide monomethanesulfonate (Nelfinavir, marketed by Agouron as VIRACEPT®), N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4-(S)-hydroxy-5-(1-(4-(2-benzo[b]furanylmethyl)-2(S)-N'(t-butylcarboxamido)-piperazinyl))-pentaneamide (*See* U.S. Pat. No. 5,646,148), N-(2(R)-hydroxy-1(S)-indanyl)2(R)-phenylmethyl-4-(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide (Indinavir, marketed by Merck as CRIXIVAN®), 4-amino-N-((2 syn,3S)-2-hydroxy-4-phenyl-3-((S)-tetrahydrofuran-3-yloxycarbonylamino)-butyl)-N-isobutyl-benzenesulfonamide (amprenavir, *see* U.S. Pat. No. 5,585,397), and N-tert-butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide (Saquinavir, marketed by Roche Laboratories as INVIRASE®).

Examples of suitable HIV integrase inhibitors are disclosed in U.S. Patent Nos. 6,110,716; 6,124,327; and 6,245,806, which are incorporated herein by reference.

In addition, antifusogenic peptides disclosed in, e.g., U.S. Patent No. 6,017,536 can also be included in the combination therapies according to the present invention. Such peptides typically consist of a 16 to 39 amino acid region of a simian immunodeficiency virus (SIV) protein and are identified through computer algorithms capable of recognizing the ALLMOTI5, 107x178x4, or PLZIP amino acid motifs. *See* U.S. Patent No. 6,017,536, which is incorporated herein by reference.

Typically, a compound of the present invention is administered to a patient in a pharmaceutical composition, which typically includes one or more pharmaceutically acceptable carriers that are inherently nontoxic and non-therapeutic. That is, the compounds are used in the manufacture of medicaments for use in treating HIV infection and treating and/or preventing AIDS.

The pharmaceutical composition according to the present invention may be administered to a subject needing treatment or prevention through any appropriate routes such as parenteral, oral, or topical administration. The active compounds of this invention are administered at a therapeutically effective amount to achieve the desired therapeutic effect without causing any serious adverse effects in the patient treated. Generally, the toxicity profile and therapeutic efficacy of therapeutic agents can be determined by standard pharmaceutical procedures in suitable cell models or animal models or human clinical trials. As is known in the art, the LD_{50} represents the dose lethal to about 50% of a tested population. The ED_{50} is a parameter indicating the dose therapeutically effective in about 50% of a tested population. Both LD_{50} and ED_{50} can be determined in cell models and animal models. In addition, the IC_{50} may also be obtained in cell models and animal models, which stands for the circulating plasma concentration that is effective in achieving about 50% of the maximal inhibition of the symptoms of a disease or disorder. Such data may be used in designing a dosage range for clinical trials in humans. Typically, as will be apparent to skilled artisans, the dosage range for human use should be designed such that the range centers around the ED_{50} and/or IC_{50}, but significantly below the LD_{50} obtained from cell or animal models.

Typically, the compounds of the present invention can be effective at an amount of from about 0.01 microgram to about 5000 mg per day, preferably from about 1 microgram to about 2500 mg per day. However, the amount can vary with the body weight of the patient treated and the state of disease conditions. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at predetermined intervals of time. The suitable dosage unit for each administration of the compounds of the present invention can be, e.g., from about 0.01 microgram to about 2000 mg, preferably from about 1 microgram to about 1000 mg.

In the case of combination therapy, a therapeutically effective amount of another anti-HIV compound can be administered in a separate pharmaceutical composition, or alternatively included in the pharmaceutical composition that contains a compound according to the present invention. The pharmacology and toxicology of many of such other anti-HIV compounds are known in the art. *See e.g., Physicians Desk Reference*, Medical Economics, Montvale, NJ; and *The Merck Index*, Merck & Co., Rahway, NJ. The therapeutically effective amounts and suitable unit dosage ranges of such compounds used in art can be equally applicable in the present invention.

It should be understood that the dosage ranges set forth above are exemplary only and are not intended to limit the scope of this invention. The therapeutically effective amount for each active compound can vary with factors including but not limited to the activity of the compound used, stability of the active compound in the patient's body, the severity of the conditions to be alleviated, the total weight of the patient treated, the route of administration, the ease of absorption, distribution, and excretion of the active compound by the body, the age and sensitivity of the patient to be treated, and the like, as will be apparent to a skilled artisan. The amount of administration can also be adjusted as the various factors change over time.

The active compounds according to this invention can be administered to patients to be treated through any suitable routes of administration. Advantageously, the active compounds are delivered to the patient parenterally, i.e., by intravenous, intramuscular, intraperiotoneal, intracisternal, subcutaneous, or intraarticular injection or infusion.

For parenteral administration, the active compounds can be formulated into solutions or suspensions, or in lyophilized forms for conversion into solutions or suspensions before use. Lyophilized compositions may include pharmaceutically acceptable carriers such as gelatin, DL-lactic and glycolic acids copolymer, D-mannitol, etc. To convert the lyophilized forms into solutions or suspensions, diluent containing, e.g., carboxymethylcellulose sodium, D-mannitol, polysorbate 80, and water may be employed. Lyophilized forms may be stored in, e.g., a dual chamber syringe with one chamber containing the lyophilized composition and the other chamber containing the diluent. In addition, the active ingredient(s) can also be incorporated into sterile lyophilized microspheres for sustained release. Methods for making such microspheres

are generally known in the art. *See* U.S. Patent Nos. 4,652,441; 4,728,721; 4,849,228; 4,917,893; 4,954,298; 5,330,767; 5,476,663; 5,480,656; 5,575,987; 5,631,020; 5,631,021; 5,643,607; and 5,716,640.

In a solution or suspension form suitable for parenteral administration, the pharmaceutical composition can include, in addition to a therapeutically or prophylactically effective amount of a compound of the present invention, a buffering agent, an isotonicity adjusting agent, a preservative, and/or an anti-absorbent. Examples of suitable buffering agent include, but are not limited to, citrate, phosphate, tartrate, succinate, adipate, maleate, lactate and acetate buffers, sodium bicarbonate, and sodium carbonate, or a mixture thereof. Preferably, the buffering agent adjusts the pH of the solution to within the range of 5-8. Examples of suitable isotonicity adjusting agents include sodium chloride, glycerol, mannitol, and sorbitol, or a mixture thereof. A preservative (e.g., anti-microbial agent) may be desirable as it can inhibit microbial contamination or growth in the liquid forms of the pharmaceutical composition. Useful preservatives may include benzyl alcohol, a paraben and phenol or a mixture thereof. Materials such as human serum albumin, gelatin or a mixture thereof may be used as anti-absorbents. In addition, conventional solvents, surfactants, stabilizers, pH balancing buffers, and antioxidants can all be used in the parenteral formulations, including but not limited to dextrose, fixed oils, glycerine, polyethylene glycol, propylene glycol, ascorbic acid, sodium bisulfite, and the like. The parenteral formulation can be stored in any conventional containers such as vials, ampoules, and syringes.

The active compounds can also be delivered orally in enclosed gelatin capsules or compressed tablets. Capsules and tablets can be prepared in any conventional techniques. For example, the active compounds can be incorporated into a formulation which includes pharmaceutically acceptable carriers such as excipients (e.g., starch, lactose), binders (e.g., gelatin, cellulose, gum tragacanth), disintegrating agents (e.g., alginate, Primogel, and corn starch), lubricants (e.g., magnesium stearate, silicon dioxide), and sweetening or flavoring agents (e.g., glucose, sucrose, saccharin, methyl salicylate, and peppermint). Various coatings can also be prepared for the capsules and tablets to modify the flavors, tastes, colors, and shapes of the capsules and tablets. In addition, liquid carriers such as fatty oil can also be included in capsules.

Other forms of oral formulations such as chewing gum, suspension, syrup, wafer, elixir, and the like can also be prepared containing the active compounds used in this invention. Various modifying agents for flavors, tastes, colors, and shapes of the special forms can also be included. In addition, for convenient administration by enteral feeding tube in patients unable to swallow, the active compounds can be dissolved in an acceptable lipophilic vegetable oil vehicle such as olive oil, corn oil and safflower oil.

The active compounds can also be administered topically through rectal, vaginal, nasal, bucal, or mucosal applications. Topical formulations are generally known in the art including creams, gels, ointments, lotions, powders, pastes, suspensions, sprays, drops and aerosols. Typically, topical formulations include one or more thickening agents, humectants, and/or emollients including but not limited to xanthan gum, petrolatum, beeswax, or polyethylene glycol, sorbitol, mineral oil, lanolin, squalene, and the like.

A special form of topical administration is delivery by a transdermal patch. Methods for preparing transdermal patches are disclosed, e.g., in Brown, *et al., Annual Review of Medicine*, 39:221-229 (1988), which is incorporated herein by reference.

The active compounds can also be delivered by subcutaneous implantation for sustained release. This may be accomplished by using aseptic techniques to surgically implant the active compounds in any suitable formulation into the subcutaneous space of the anterior abdominal wall. *See, e.g.,* Wilson *et al., J. Clin. Psych.* 45:242-247 (1984). Sustained release can be achieved by incorporating the active ingredients into a special carrier such as a hydrogel. Typically, a hydrogel is a network of high molecular weight biocompatible polymers, which can swell in water to form a gel like material. Hydrogels are generally known in the art. For example, hydrogels made of polyethylene glycols, or collagen, or poly(glycolic-co-L-lactic acid) are suitable for this invention. *See, e.g.,* Phillips et al., *J. Pharmaceut. Sci.,* 73:1718-1720 (1984).

The active compounds can also be conjugated, i.e., covalently linked, to a water soluble non-immunogenic high molecular weight polymer to form a polymer conjugate. Preferably, such polymers do not undesirably interfere with the cellular uptake of the active compounds. Advantageously, such polymers, e.g., polyethylene glycol, can impart solubility, stability, and reduced immunogenicity to the active compounds. As a result, the active compound in the conjugate when administered to a patient, can have a longer

half-life in the body, and exhibit better efficacy. In one embodiment, the polymer is a peptide such as albumin or antibody fragment Fc. PEGylated proteins are currently being used in protein replacement therapies and for other therapeutic uses. For example, PEGylated adenosine deaminase (ADAGEN®) is being used to treat severe combined immunodeficiency disease (SCIDS). PEGylated L-asparaginase (ONCAPSPAR®) is being used to treat acute lymphoblastic leukemia (ALL). A general review of PEG-protein conjugates with clinical efficacy can be found in, e.g., Burnham, *Am. J. Hosp. Pharm.*, 15:210-218 (1994). Preferably, the covalent linkage between the polymer and the active compound is hydrolytically degradable and is susceptible to hydrolysis under physiological conditions. Such conjugates are known as "prodrugs" and the polymer in the conjugate can be readily cleaved off inside the body, releasing the free active compounds.

Alternatively, other forms controlled release or protection including microcapsules and nanocapsules generally known in the art, and hydrogels described above can all be utilized in oral, parenteral, topical, and subcutaneous administration of the active compounds.

Another preferable delivery form is using liposomes as carrier. Liposomes are micelles formed from various lipids such as cholesterol, phospholipids, fatty acids, and derivatives thereof. Active compounds can be enclosed within such micelles. Methods for preparing liposomal suspensions containing active ingredients therein are generally known in the art and are disclosed in, e.g., U.S. Pat. No. 4,522,811, and Prescott, Ed., *Methods in Cell Biology,* Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq., both of which are incorporated herein by reference. Several anticancer drugs delivered in the form of liposomes are known in the art and are commercially available from Liposome Inc. of Princeton, New Jersey, U.S.A. It has been shown that liposomes can reduce the toxicity of the active compounds, and increase their stability.

Example 1

Yeast two-hybrid assays were utilized to determine the effect of amino acid substitution mutations in the PTAP motif of HIV p6gag on the interaction between Tsg101 and p6gag. To prepare a yeast two-hybrid activation domain-Tsg101 construct, a

DNA fragment encompassing the full-length coding sequence for Tsg101 according to GenBank Accession No. U82130 was obtained by PCR from a human fetal brain cDNA library and cloned into the EcoRI/Pst1 sites of the activation domain parent plasmid GADpN2 (LEU2, CEN4, ARS1, ADH1p-SV40NLS-GAL4 (768-881)-MCS (multiple cloning site)-PGK1t, AmpR, ColE1_ori).

To prepare the yeast two-hybrid DNA binding domain-HIV1 p6gag construct, a DNA fragment corresponding to the HIV1 p6 peptide derived from the HIV1.NL43 strain GAG protein was obtained by PCR from the NL43 containing plasmid R9Δapa and was cloned into the EcoRI/Sal1 sites of the binding domain parent plasmid pGBT.Q. The sequence of the amplified insert is shown in SEQ ID NO:41. In addition, the amino acid sequence of the HIV-$1_{NYU/BR5}$ GAG is provided in GenBank under Accession No. AF324493 and is listed in SEQ ID NO:42.

The following amino acid substitution mutations were introduced by PCR into the HIV1 p6gag sequence in the yeast two-hybrid binding domain-HIV1 p6gag construct described above. The mutations were verified by DNA sequence analysis. Such mutations are summarized in Table 1 below.

Table 2. Tested Mutations in p6gag Protein

Mutant Construct	p6gag Peptide Sequence Surrounding the PTAP Motif														
p6(wt)	S	R	P	E	P	T	A	P	P	E	E	S	F	R	F
p6(E6G)				G											
p6(P7L)					L										
p6(A9R)							R								
p6(P10L)								L							

To test the effect of the mutations, yeast cells of the strain Y189 purchased from Clontech (ura3-52 his3*200 ade2-101 trp1-901 leu2-3,112 met gal4 gal80 URA3::GAL1p-lacZ) were co-transformed with the activation domain-Tsg101 construct and one of the binding domain-mutant p6gag constructs or the binding domain-wild type p6gag construct. Filter lift assays for β-Gal activity were conducted by lifting the transformed yeast colonies with filters, lysing the yeast cells by freezing and thawing, and contacting the lysed cells with X-Gal. Positive β-Gal activity indicates that the p6gag wild type or mutant protein interacts with Tsg101. All binding domain constructs were also tested for self-activation of β-Gal activity. The results are shown in Table 2.

Table 2. Interactions Between Tsg101 and p6gag

	p6(wt)	p6(E6G)	p6(P7L)	p6(A9R)	p6(P10L)
Tsg101	+	+	-	-	-
p6(wt)	-				
p6(E6G)		-			
p6(P7L)			-		
p6(A9R)				-	
p6(P10L)					-

Thus, as is clear from Table 2, the mutations in the PTAP motif of HIV p6gag abolished the interaction between Tsg101 and HIV p6gag, while the p6/E6G mutation outside the PTAP motif did not result in the elimination of the Tsg101-p6gag interaction.

The interactions between TSG101 and wild-type p6gag (WT) or the p6gag PTAP mutants were further quantitated by performing liquid culture β-galactosidase assays. Cultures were grown overnight in synthetic media (-Leu, -Trp, + glucose) in 96 well plates, normalized for optical density, and lysed by addition of 6X lysis/substrate solution in 6X Z-buffer (60mM KCl, 6mM $MgSO_4$, 360mM Na_2HPO_4, 240 mM NaH_2PO_4, 6mg/ml CPRG, 0.12U/ml lyticase, 0.075% NP-40). Cultures were incubated for 2 hr at 37°C, clarified by centrifugation, and the optical absorbance of each supernatant was measured (575 nm). Full length Tsg101 bound wild-type p6 in the two-hybrid liquid culture assay, resulting in high levels of β-galactosidase activity (>300-fold over background). Three different p6 point mutants were used to test whether the Tsg101 binding interaction required the PTAP late domain motif within HIV-1 p6, and all three (P6L, A9R and P10L) reduced β-galactosidase activity to background levels. Each of these point mutations also arrests HIV-1 budding at a late stage (Huang et al. 1995). These results are consistent with the hypothesis that the interaction between HIV p6gag and the human cellular protein TSG101 is essential for viral budding to occur.

Example 2

A fusion protein with a GST tag fused to the HIV-1 GAGp6 domain was recombinantly expressed and purified by chromatography. In addition, a GAGp6 peptide containing the first 14 amino acid residues ("p6(1-14)") was synthesized chemically by

standard peptide synthesis methods. The peptide was purified by conventional protein purification techniques, e.g., by chromatography.

Nunc/Nalgene Maxisorp plates were incubated overnight at 4°C or for 1-2 hrs at room temperature in 100 µl of a protein coupling solution containing purified GST-p6 and 50mM Carbonate, pH=9.6. This allowed the attachment of the GST-p6 fusion protein to the plates. Liquids in the plates were then emptied and wells filled with 400 µl/well of a blocking buffer (SuperBlock; Pierce-Endogen, Rockford, IL). After incubating for 1 hour at room temperature, 100 µl of a mixture containing Drosophila S2 cell lysate myc-tagged Tsg101 (residues 1-207) and a specific amount of the p6(1-14) peptide were applied to the wells of the plate. This mixture was allowed to react for 2 hours at room temperature to form p6:Tsg101 protein-protein complexes.

Plates were then washed 4 x 100µl with 1 x PBST solution (Invitrogen; Carlsbad, CA). After washing, 100µl of 1µg/ml solution of anti-myc monoclonal antibody (Clone 9E10; Roche Molecular Biochemicals; Indianapolis, IN) in 1 x PBST was added to the wells of the plate to detect the myc-epitope tag on the Tsg101 protein. Plates were then washed again with 4 x 100µl with 1 x PBST solution and 100µl of 1µg/ml solution of horseradish peroxidase (HRP) conjugated Goat anti-mouse IgG (Jackson Immunoresearch Labs; West Grove, Pennsylvania) in 1 x PBST was added to the wells of the plate to detect bound mouse anti-myc antibodies. Plates were then washed again with 4 x 100µl with 1 x PBST solution and 100 µl of fluorescent substrate (QuantaBlu; Pierce-Endogen, Rockford, IL) was added to all wells. After 30 minutes, 100 µl of stop solution was added to each well to inhibit the function of HRP. Plates were then read on a Packard Fusion instrument at an excitation wavelength of 325 nm and an emission wavelength of 420nm. The presence of fluorescent signals indicates binding of Tsg101 to the fixed GST-p6. In contrast, the absence of fluorescent signals indicates that the p6(1-14) peptide is capable of disrupting the interaction between Tsg101 and HIV p6.

Different concentrations of the p6(1-14) peptide were tested, and the relative intensities of the fluorescence signals obtained at different concentrations were plotted against the peptide concentrations. The competitive inhibition curve is shown in Figure 1. Two Dixon plots are shown in Figure 2 and Figure 3, respectively.

Example 3

1. Materials

For antiviral tests, the following peptidic compounds (in Table 3) were chemically synthesized and purified by conventional protein purification techniques:

Table 3

Compound	Formula	SEQ ID
MPI-PEP1	NH_2-$(R)_9$-PEPTAPEE-COOH	38
MPI-PEP2	NH_2-$(R)_9$-PEPTALEE-COOH	39
MPI-PEP3	NH_2-RPEPTAP-CO-NH_2	40

The compounds were solubilized in sterile RPMI 1640 tissue culture medium to yield 40 mM stock solutions. AZT was used as a positive control antiviral compound.

Fresh human blood was obtained commercially from Interstate Blood Bank, Inc. (Memphis, TN). The lymphotropic clinical isolate HIV-1 $_{ROJO}$ was obtained from a pediatric patient attending the AIDS Clinic at the University of Alabama at Birmingham. The laboratory-adapted HIV-1_{IIIB} strain was propagated and tittered in fresh human PBMCs; pre-titered aliquots of HIV-1_{ROJO} and Hiv-1_{IIIB} were removed from the freezer (-80° C) and thawed rapidly to room temperature in a biological safety cabinet immediately before use. Phytohemagglutinin (PHA-P) was obtained from Sigma (St. Louis, MO) and recombinant IL-2 was obtained from Amgen (San Francisco, CA).

2. Anti-HIV Efficacy Evaluation in Fresh Human PBMCs

Fresh human PBMCs were isolated from screened donors, seronegative for HIV and HBV. Leukophoresed blood was diluted 1:1 with Dulbecco's phosphate buffered saline (PBS), layered over 14 mL of Ficoll-Hypaque density gradient in a 50 mL centrifuge tube and then centrifuged for 30 minutes at 600 X g. Banded PBMCs were aspirated from the resulting interface and subsequently washed 2X with PBS by low speed centrifugation. After the final wash, cells were enumerated by trypan blue exclusion and re-suspended at 1x 10^7 cells /mL in RPMI 1640 supplemented with 15% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 4 μg/mL PHA-P. The cells were allowed to incubate for 48-72 hours at 37°C. After incubation, PBMCs were centrifuged and reset in RPMI 1640 with 15% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/mL

streptomycin, 10 μg/mL gentamycin, and 20 U/mL recombinant human IL-2. PBMCs were maintained in this medium at a concentration of 1-2 x 10^6 cells/mL with biweekly medium changes until used in the assay protocol.

For the standard PBMC assay, PHA-P stimulated cells from at least two normal donors were pooled, diluted in fresh medium to a final concentration of 1 x 10^6 cells/mL, and plated in the interior wells of 96 well round bottom microplate at 50 μL/well (5 x 10^4 cells/well). Test drug dilutions were prepared at a 2X concentration in microtiter tubes and 100 μL of each concentration was placed in appropriate wells in a standard format. 50 μL of a predetermined dilution of virus stock was placed in each test well (final MOI ≈ 0.1). Wells with cells and virus alone were used for virus control. Separate plates were prepared identically without virus for drug cytotoxicity studies using an XTT assay system. The PBMC cultures were maintained for seven days following infection, at which time cell-free supernate samples were collected and assayed for reverse transcriptase activity as described below.

3. Reverse Transcriptase Activity Assay

A microtiter based reverse transcriptase (RT) reaction was utilized. *See* Buckheit *et al.*, *AIDS Research and Human Retroviruses* 7:295-302 (1991). Tritiated thymidine triphosphate (NEN) (TTP) was resuspended in distilled H_2O at 5 Ci/ml. Poly rA and oligo dT were prepared as a stock solution which was kept at -20°C. The RT reaction buffer was prepared fresh on a daily basis and consists of 125 μl 1M EGTA, 125 μl dH_2O, 110 μl 10% SDS, 50 μl 1M Tris (pH 7.4), 50 μl 1M DTT, and 40 μl 1M $MgCL_2$. These three solutions were mixed together in a ratio of 2 parts TTP, 1 part poly rA:oligo dT, and 1 part reaction buffer. Ten microliters of this reactions mixture was placed at a round bottom microtiter plate and 15 μl of virus containing supernatant was added and mixed. The plate was incubated at 37°C in a water bath with a solid support to prevent submersion of the plate and incubated for 60 minutes. Following reaction, the reaction volume was spotted onto pieces of DE81 paper, washed 5 times 5 minutes each in a 5% sodium phosphate buffer, 2 times 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Opti-Fluor-O (Packard) was added to each sample and incorporated radioactivity was quantified utilizing a Wallac 1450 MicroBeta Plus liquid scintillation counter.

4. Cytotoxicity Measurement By MTS Staining

At assay termination the assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter Reagent, Promega) to determine cell viability and quantify compound toxicity. MTS is metabolized by the mitochondria enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative analysis cell viability and compound cytotoxicity. The MTS is a stable solution that does not require preparation before use. At termination of the assay, 20 µl of MTS reagent was added per well. The wells were incubated overnight for the HIV cytoprotection assay at 37°C. The incubation intervals were chosen based on empirically determined times for optimal dye reduction in each cell type. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490 nm with a Molecular Devices Vmax plate reader.

5. Data Analysis

Indices including %CPE Reduction, %Cell Viability, IC_{50}, TC_{50}, and others were calculated and summarized in Table 4 below. The graphical results for the three peptidic compounds tested are displayed in Figures 4, 5 and 6, respectively. AZT was evaluated in parallel as a relevant positive control compound in the anti-HIV assay, and the graphical result is shown in Figure 7.

Table 4

Compound Name	IC_{50} (µM)	TC_{50} (µM)	Therapeutic Index	Comments
MPI-PEP1	21.7	>200.0	9.2	Active
MPI-PEP2	>200.0	>200.0	N/A	Inactive
MPI-PEP3	>200.0	>200.0	N/A	Inactive
AZT	0.008	>1.0	>125.00	Control; Highly Active

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same

extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQ ID NO:41

CTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAG
GTTTGGGGAAGAGACAACAACTCCCTCTCAGAAGCAGGAGCCGATAGACAA
GGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACCCCTCGT
CACAAT

SEQ ID NO:42

MGARASVLSGGELDKWEKIRLRPGGKKQYKLKHIVWASRELERFAVNPGLLETSEGCRQ
ILGQLQPSLQTGSEELRSLYNTIAVLYCVHQRIDVKDTKEALDKIEEEQNKSKKKAQQAAA
DTGNNSQVSQNYPIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGAT
PQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRLHPVHAGPIAPGQMREPRGSDIAGTT
STLQEQIGWMTHNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRF
YKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPGATLEEMMTACQGVGGPGHKA
RVLAEAMSQVTNPATIMIQKGNFRNQRKTVKCFNCGKEGHIAKNCRAPRKKGCWKCGKEG
HQMKDCTERQANFLGKIWPSHKGRPGNFLQSRPEPTAPPEESFRFGEETTTPSQKQEPID
KELYPLASLRSLFGSDPSSQ

WHAT IS CLAIMED IS:

1. A composition comprising a peptide associated with a transporter capable of increasing the uptake of said peptide by a mammalian cell, wherein said peptide includes a contiguous amino acid sequence of an HIV GAG protein, said contiguous amino acid sequence encompassing the late domain motif of said GAG protein, wherein said peptide is capable of binding the UEV domain of Tsg101.

2. The composition of Claim 1, wherein said peptide is covalently linked to said transporter.

3. The composition of Claim 2, wherein said transporter is selected from the group consisting of penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D-arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histidine oligomers, D-histidine oligomers, L-ornithine oligomers, D-ornithine oligomers, fibroblast growth factor and fragments thereof, Galparan and fragments thereof, and HSV-1 structural protein VP22 and fragments thereof; and peptoid analogs thereof.

4. The composition according to Claim 1, wherein said transporter is selected from the group consisting of liposomes, dendrimers, and siderophores.

5. The composition according to Claim 1, wherein said peptide consists of from about 9 to about 50 amino acid residues.

6. The composition according to Claim 1, wherein said peptide consists of from about 10 to about 20 amino acid residues.

7. The composition according to Claim 1, wherein said peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID

NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

8. The composition according to Claim 1, wherein said contiguous amino acid sequence includes the sequence motif of PTAP or PSAP.

9. An isolated hybrid polypeptide comprising:

a first portion capable of binding the UEV domain of Tsg101 and having a contiguous amino acid sequence of an HIV GAG protein encompassing the late domain motif of said GAG protein; and

a second portion which is a peptidic transporter capable of increasing the uptake of said first portion by a mammalian cell.

10. The isolated hybrid polypeptide according to Claim 9, wherein said first portion consists of from about 9 to about 50 amino acid residues.

11. The isolated hybrid polypeptide according to Claim 9, wherein said first portion consists of from about 10 to about 20 amino acid residues.

12. The isolated hybrid polypeptide according to Claim 9, wherein said first portion consists of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

13. The isolated hybrid polypeptide according to Claim 9, wherein said peptidic transporter is selected from the group consisting of penetratins, HIV tat protein and fragments thereof, retro-inverso isomers of HIV tat protein fragments, L-arginine oligomers, L-lysine oligomers, and L-histidine oligomers.

14. An isolated nucleic acid encoding the isolated hybrid polypeptide according to Claim 9.

15. An isolated nucleic acid encoding the isolated hybrid polypeptide according to Claim 13.

16. A host cell expressing said isolated hybrid polypeptide according to Claim 9.

17. A host cell expressing said isolated hybrid polypeptide according to Claim 13.

18. A method for inhibiting HIV budding from cells, comprising:

administering to cells a composition comprising a peptide associated with a transporter capable of increasing the uptake of said peptide by the cells, wherein said peptide includes a contiguous amino acid sequence of an HIV GAG protein, said contiguous amino acid sequence encompassing the late domain motif of said GAG protein, wherein said peptide is capable of binding the UEV domain of Tsg101.

19. The method of Claim 18, wherein said peptide is covalently linked to said transporter.

20. The method of Claim 19, wherein said transporter is selected from the group consisting of penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D-arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histidine oligomers, D-histidine oligomers, L-ornithine oligomers, D-ornithine oligomers, fibroblast growth factor and fragments thereof, Galparan and fragments thereof, and HSV-1 structural protein VP22 and fragments thereof, and peptoid analogs thereof.

21. The method of Claim 19, wherein said transporter is a penetratin.

22. The method of Claim 19, wherein said transporter comprises a contiguous amino acid sequence of an HIV tat protein.

23. The method of Claim 19, wherein said transporter is an L-arginine oligomer.

24. The method of Claim 19, wherein said transporter has at least six contiguous amino acid residues, all of which are L-arginine, D-arginine, L-lysine, D-lysine, L-histidine, D-histidine, L-ornithine, D-ornithine, or a combination thereof.

25. The method of Claim 19, wherein said transporter has at least nine contiguous amino acid residues, all of which are L-arginine, D-arginine, L-lysine, D-lysine, L-histidine, D-histidine, L-ornithine, D-ornithine, or a combination thereof.

26. The method of Claim 18, wherein said transporter is selected from the group consisting of liposomes, dendrimers, and siderophores.

27. The method of Claim 26, wherein said transporter is a liposome.

28. The method of Claim 18, wherein said transporter is capable of increasing the uptake of said peptide by a human cell by at least 100%.

29. The method of Claim 18, wherein said peptide consists of from about 9 to about 50 amino acid residues.

30. The method of Claim 18, wherein said peptide consists of from about 9 to about 20 amino acid residues.

31. The method of Claim 18, wherein said peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID

NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

32. The method of Claim 18, wherein said contiguous amino acid sequence includes the sequence motif of PTAP or PSAP.

33. The method of Claim 18, furhter comprising administering to the cells another anti-HIV compound.

34. The method of Claim 33, wherein said anti-HIV compound is selected from the group consisting of HIV protease inhibitors, nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV integrase inhibitors, and HIV fusion inhibitors.

35. A method for inhibiting HIV budding from cells, comprising:

introducing into cells infected with HIV a peptide consisting of an amino acid sequence of a contiguous amino acids sequence of 9 to 50 residues of an HIV GAG protein, said region encompassing the late domain motif of said GAG protein, wherein said peptide is capable of binding the UEV domain of Tsg101.

36. The method of Claim 35, wherein said introducing step comprises administering to the cells a nucleic acid encoding said peptide.

37. Use of a peptide in the manufacture of a medicament useful in the treatment of HIV infection or preventing AIDS, wherein said peptide consists of a contiguous amino acid sequence of 9 to 50 amino acid residues of an HIV GAG protein, said contiguous amino acid sequence encompassing the late domain motif of said GAG protein, wherein said peptide is capable of binding the UEV domain of Tsg101.

38. The use according Claim 37, wherein the peptide consists of 10 to 20 amino acid residues.

39. Use of a peptide in the manufacture of a medicament useful in the treatment of HIV infection or preventing AIDS, wherein said peptide is associated with a transporter capable of increasing the uptake of said peptide by the cells, wherein said peptide includes an amino acid sequence region of an HIV GAG protein, said region encompassing the late domain motif of said GAG protein, wherein said peptide is capable of binding the UEV domain of Tsg101.

40. The use according to Claim 39, wherein said peptide is covalently linked to said transporter.

41. The use according to Claim 39, wherein said transporter is selected from the group consisting of penetratins, *l*-Tat_{49-57}, *d*-Tat_{49-57}, retro-inverso isomers of *l*- or *d*-Tat_{49-57}, L-arginine oligomers, D-arginine oligomers, L-lysine oligomers, D-lysine oligomers, L-histidine oligomers, D-histidine oligomers, L-ornithine oligomers, D-ornithine oligomers, fibroblast growth factor and fragments thereof, Galparan and fragments thereof, and HSV-1 structural protein VP22 and fragments thereof, and peptoid analogs thereof.

42. The use according to Claim 39, wherein said transporter is selected from the group consisting of liposomes, dendrimers, and siderophores.

43. The use according to Claim 39, wherein said peptide consists of from about 9 to about 50 amino acid residues.

44. The use according to Claim 39, wherein said peptide consists of from about 10 to about 20 amino acid residues.

45. The use according to Claim 39, wherein said peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

46. The use according to Claim 39, wherein said contiguous amino acid sequence includes the sequence motif of PTAP or PSAP.

47. An isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35.

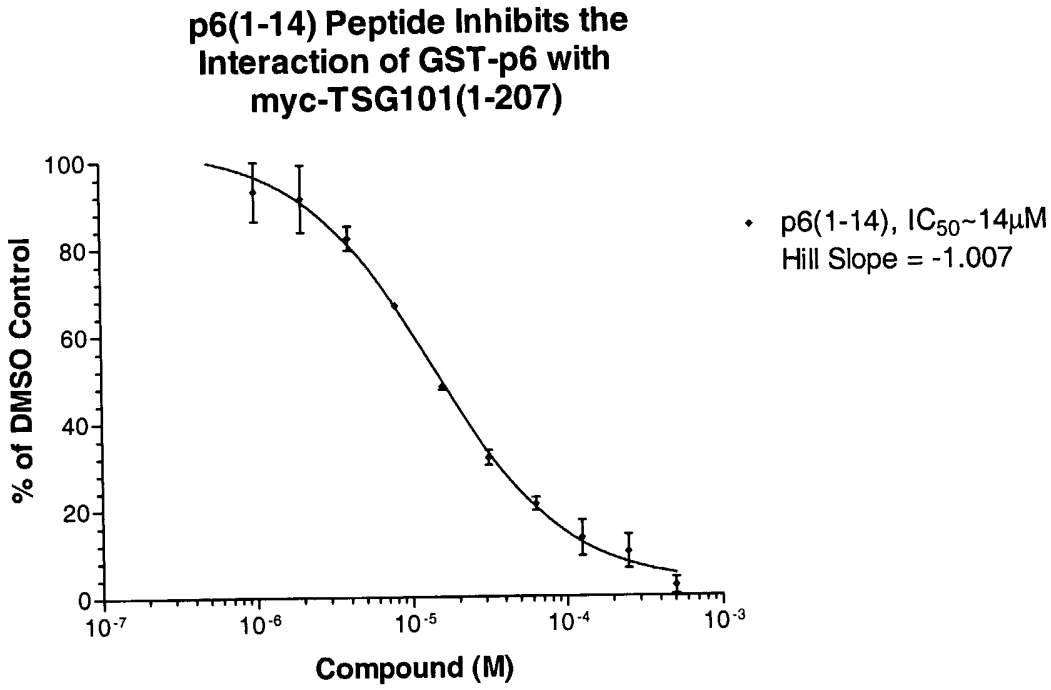
p6(1-14) Peptide Inhibits the Interaction of GST-p6 with myc-TSG101(1-207)
% of DMSO Control
100
80
60
40
20
0
10-7
10-6
10-5
10-4
10-3
Compound (M)
p6(1-14), IC50~14μM
Hill Slope = -1.007

Figure 1

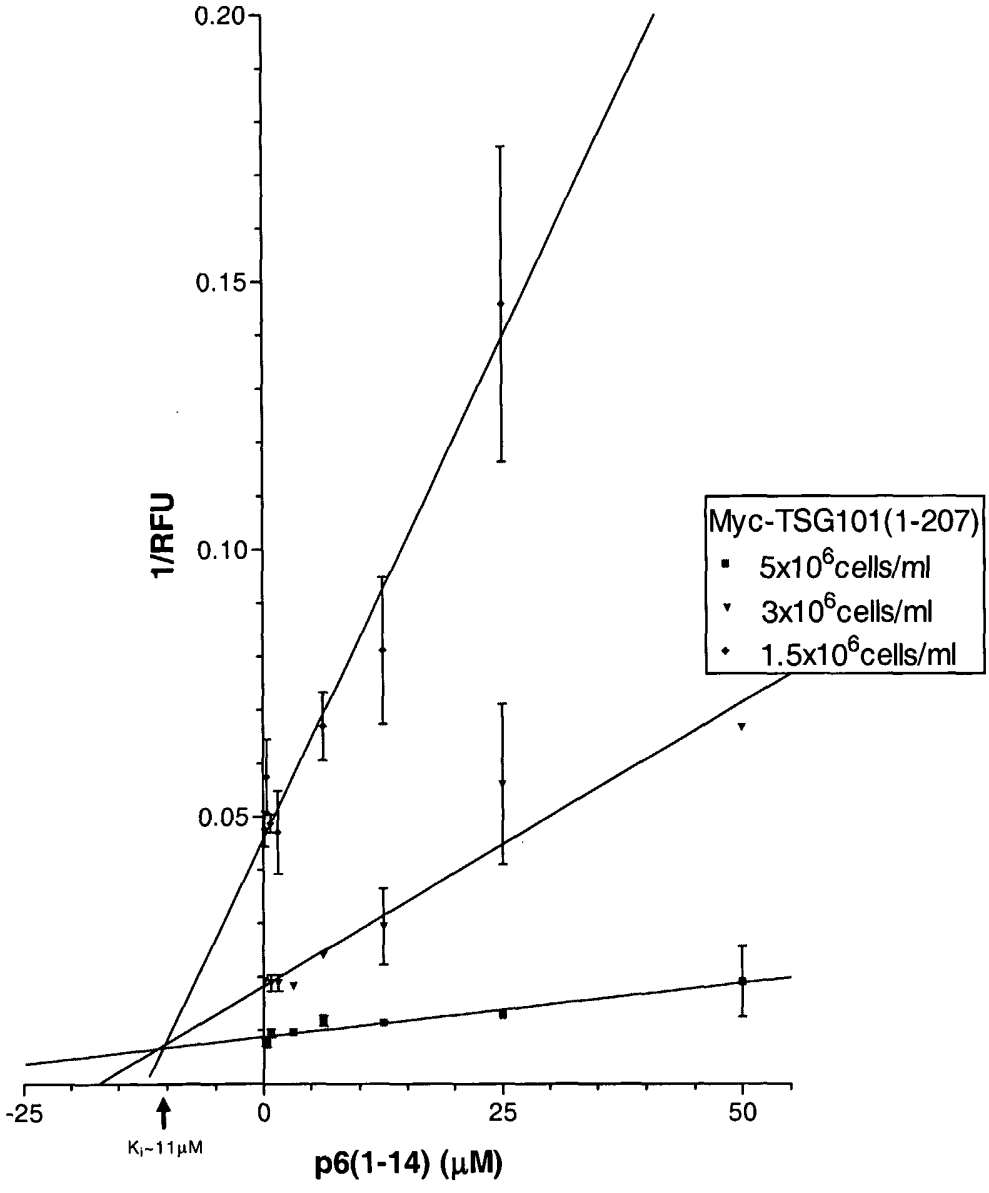
Dixon plot of p6(1-14) on p6/TSG101(1-207)
0.20
0.15
0.10
0.05
1/RFU
Myc-TSG101(1-207)
5x10^6cells/ml
3x10^6cells/ml
1.5x10^6cells/ml
-25
0
25
50
Ki~11μM
p6(1-14) (μM)

Figure 2

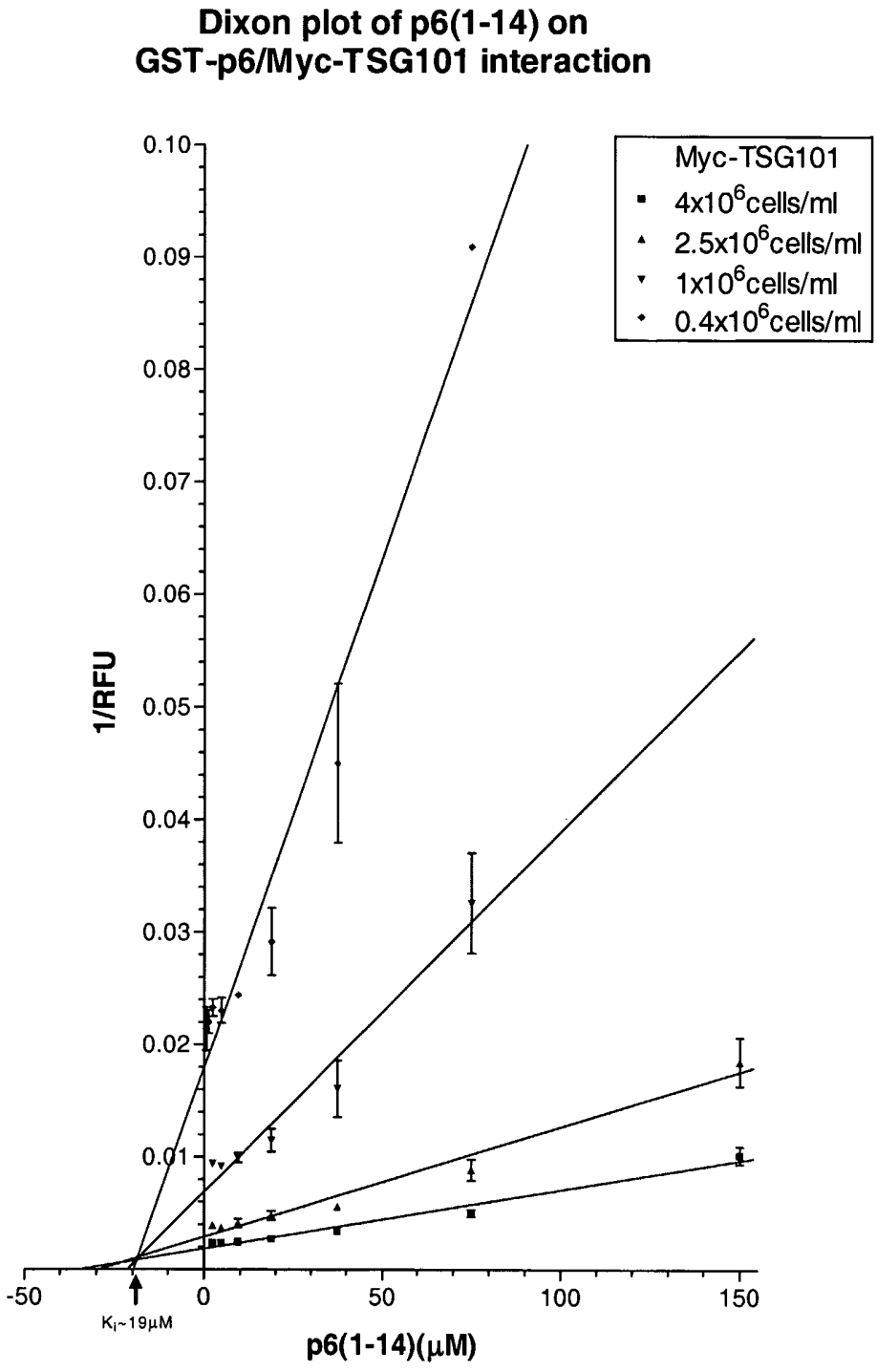
Dixon plot of p6(1-14) on
GST-p6/Myc-TSG101 interaction
Myc-TSG101
4x10^6cells/ml
2.5x10^6cells/ml
1x10^6cells/ml
0.4x10^6cells/ml
1/RFU
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
-50
0
50
100
150
Ki~19μM
p6(1-14)(μM)

Figure 3

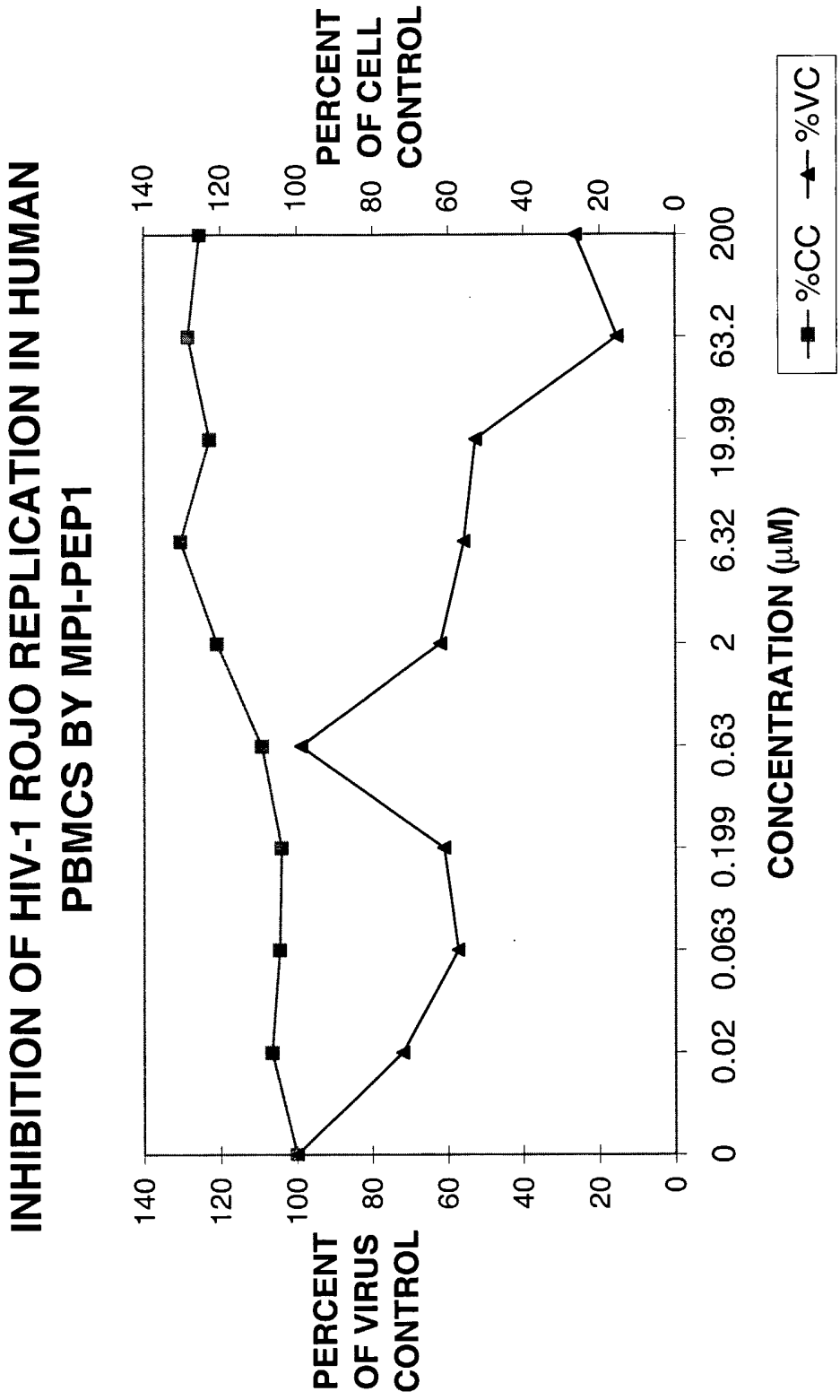
INHIBITION OF HIV-1 ROJO REPLICATION IN HUMAN PBMCS BY MPI-PEP1
PERCENT OF VIRUS CONTROL
PERCENT OF CELL CONTROL
140
120
100
80
60
40
20
0
0
0.02
0.063
0.199
0.63
2
6.32
19.99
63.2
200
CONCENTRATION (μM)
%CC
%VC

FIG. 4

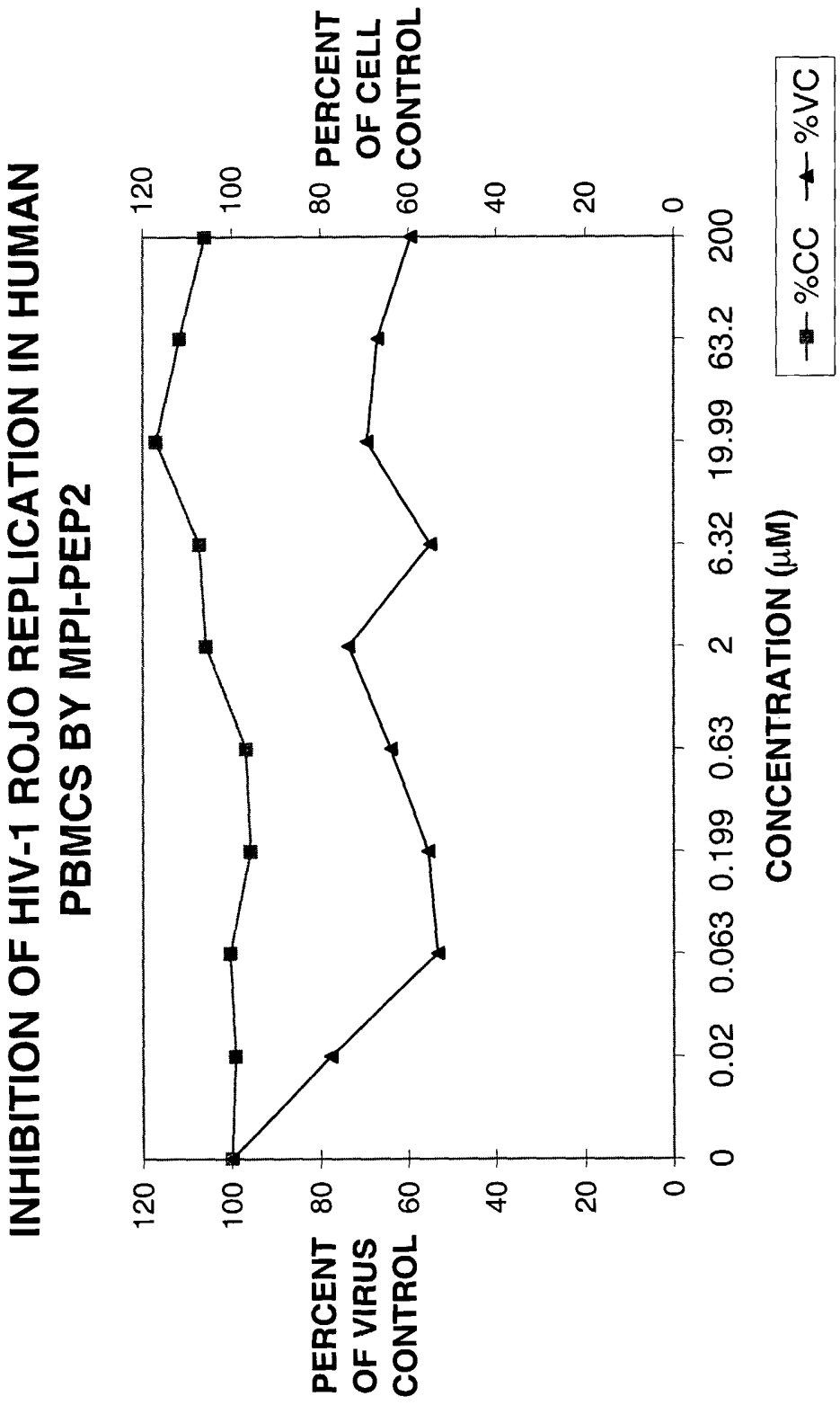
INHIBITION OF HIV-1 ROJO REPLICATION IN HUMAN PBMCS BY MPI-PEP2
PERCENT OF VIRUS CONTROL
PERCENT OF CELL CONTROL
120
100
80
60
40
20
0
0
0.02
0.063
0.199
0.63
2
6.32
19.99
63.2
200
CONCENTRATION (μM)
%CC
%VC

FIG. 5

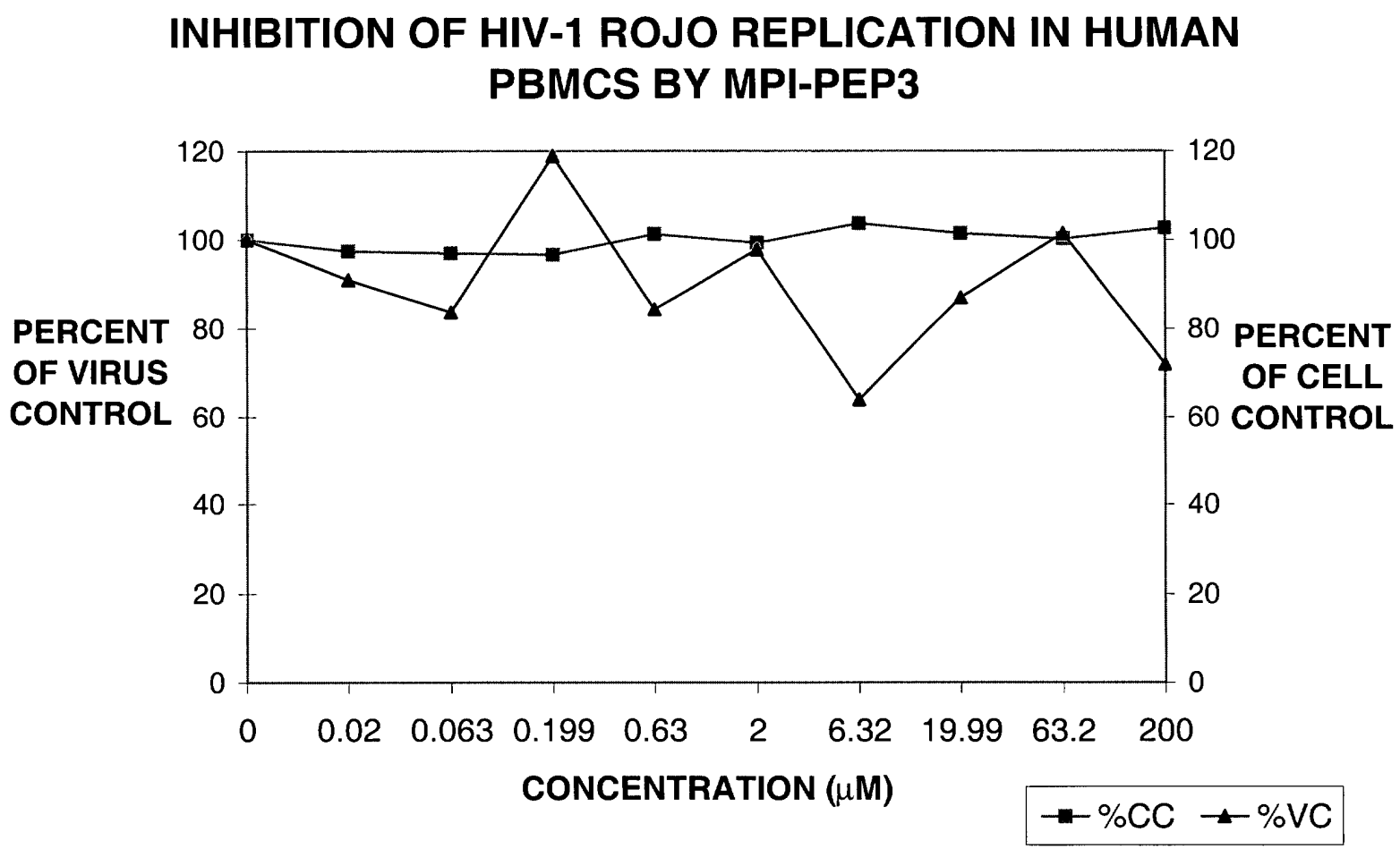
INHIBITION OF HIV-1 ROJO REPLICATION IN HUMAN PBMCS BY MPI-PEP3
PERCENT OF VIRUS CONTROL
PERCENT OF CELL CONTROL
120
100
80
60
40
20
0
0
0.02
0.063
0.199
0.63
2
6.32
19.99
63.2
200
CONCENTRATION (μM)
%CC
%VC

FIG. 6

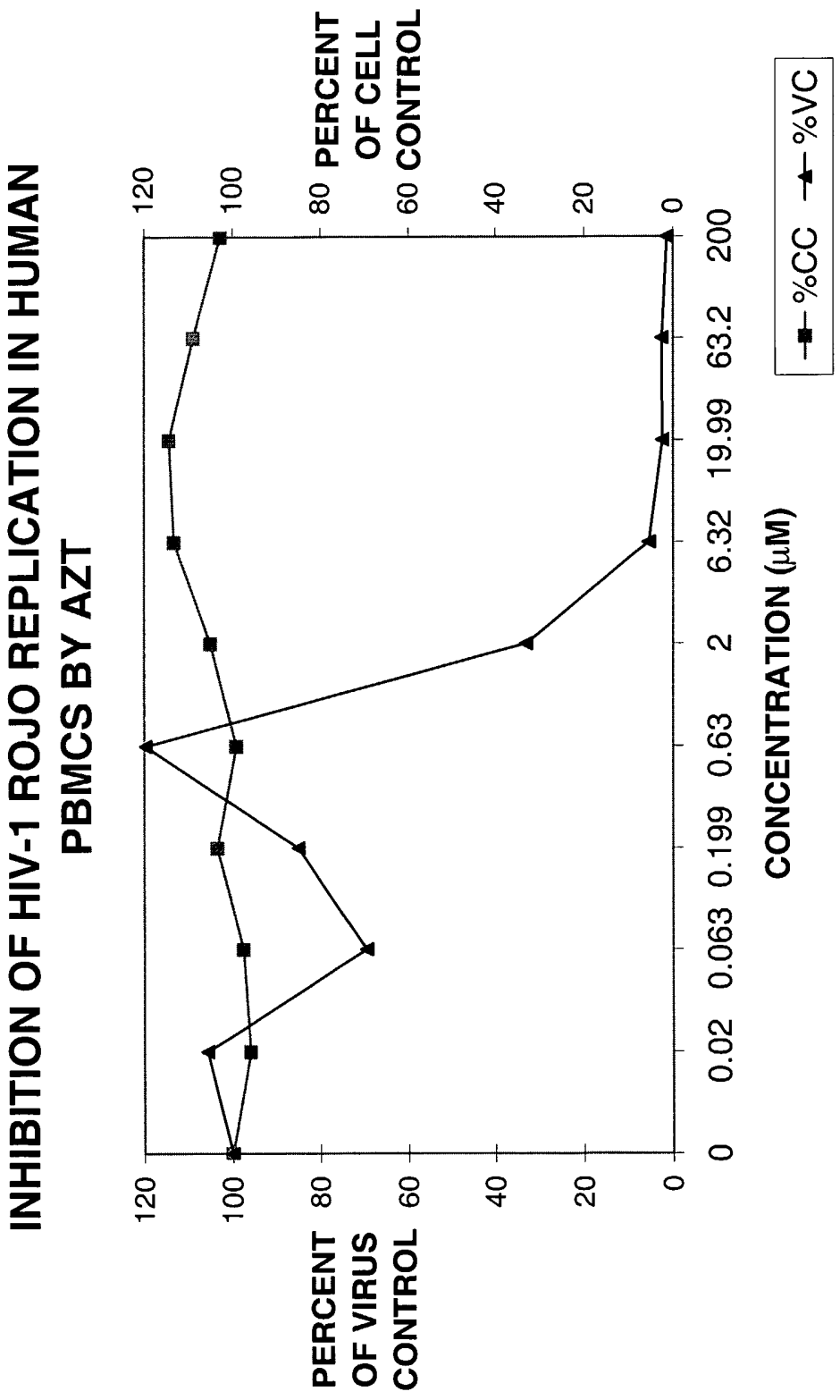
INHIBITION OF HIV-1 ROJO REPLICATION IN HUMAN PBMCS BY AZT
PERCENT OF VIRUS CONTROL
PERCENT OF CELL CONTROL
120
100
80
60
40
20
0
0
0.02
0.063
0.199
0.63
2
6.32
19.99
63.2
200
CONCENTRATION (μM)
%CC
%VC

FIG. 7