Gp160 / HIV-targeted library

Medicinal and Computational Chemistry Dept., ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, CA 92121 USA, Service: +1 877 ChemDiv, Tel: +1 858-794-4860, Fax: +1 858-794-4931, Email: ChemDiv@chemdiv.com

INTRODUCTION

Over the last couple of years, it has become quite clear that HIV-1 infection typically involves an interaction between at least the viral envelope protein gp120/41 and the CD4 molecule followed by a second interaction with a chemokine receptor, usually CCR5 or CXCR4 [1]. However, much remains unknown about basic aspects of HIV-1 infection and cell susceptibility. In the early stages of an HIV-1 infection CCR5 using viruses (R5 viruses) predominate. In some viral subtypes there is a propensity to switch to CXCR4 usage (X4 viruses). The receptor switch occurs in ~ 40% of the infected individuals and is associated with faster disease progression. There are several hypotheses to explain the preferential transmission of R5 viruses and the mechanisms that lead to switching of co-receptor usage; however, there is no definitive explanation for either. One important consideration regarding transmission is that signaling by R5 gp120 may facilitate transmission of R5 viruses by inducing a permissive environment for HIV replication.

The HIV virus genomic material is small and comprises two plus (+) sense single RNA strands that amount to ~9.2 kilobases [2]. Briefly, the viral RNA must be reverse-transcribed into double-stranded complimentary DNA (cDNA) in the host cell cytoplasm and then transported, with the help of the viral p17 matrix protein (MA), integrase (IN), and the viral protein R (Vpr), to the cell nucleus where it is integrated into the host cell genome. Following transcriptional activation of the integrated proviral DNA, with the help of viral protease, early and late viral proteins are translated which are involved in the assembly and packaging of new virions (Fig. 1). The virus also contains an envelope as well as a protein core. The envelope is made up of a lipid bilayer that is derived from the host cell plasma membrane during the budding of newly formed virions. Contained within this viral envelope lipid bilayer is the virus-derived adhesin glycoprotein, gp120. The gp120 and gp41 capsid molecules (jointly - gp160) of the human immunodeficiency virus type-1 (HIV-1) are glycoproteins which form a significant part of the outer layer of the virus. gp160 presents itself as viral membrane spikes consisting of 3 molecules of gp120 linked together and anchored to the membrane by gp41 protein. This protein tandem is essential for viral infection as it facilitates HIV invasion into the host cell and this is its best-known and most researched role in HIV infection.

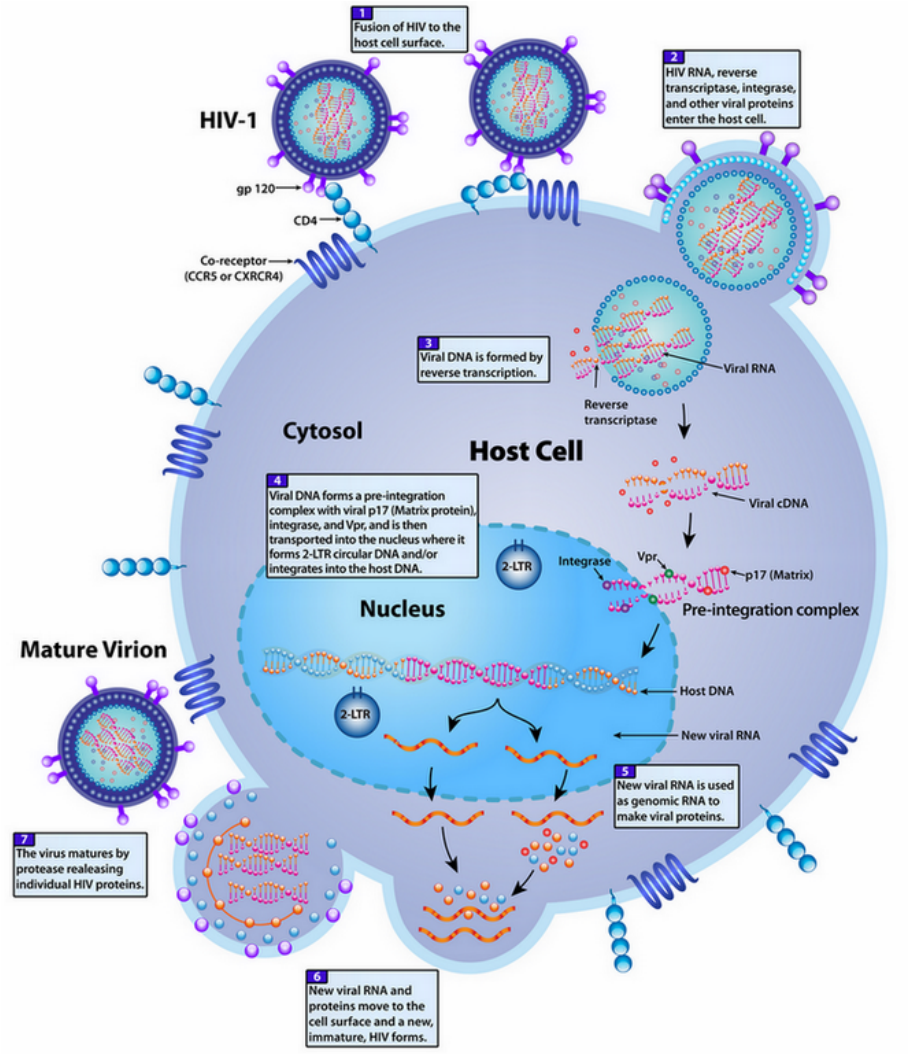


Fig. 1. HIV replication cycle.

Each component of the gp120-gp41 complex has specific functions. For example, anchoring the complex occurs via the gp41, a transmembrane protein [3]. The gp120 V3 variable region binds to CCR5 or CXCR4 cell surface co-receptors and contains conserved regions including a band, arch, and hydrophobic core [4]. HIV-1 gp41 N- and C-domains mediate virus membrane fusion. The HIV-1 gp41 amino-terminal region is a pre-transmembrane domain. It contains an amphipathic-at-interface sequence that is non-polar (aromatic AA-rich), and is conserved among several viral strains. The amphipathic-at-interface sequence also includes a β-turn structure with non-helical extended region. Interaction of the amphipathic-at-interface sequence with the fusion peptide region reduces its fusion ability [5].

However, it is becoming increasingly evident that gp120 might also be facilitating viral persistence and continuing HIV infection by influencing the T cell immune response to the virus [6]. Several mechanisms might be involved in this process of which gp120 binding to the CD4 receptor of T cells is the best known and most important interaction as it facilitates viral entry into the CD4+ cells and their depletion, a hallmark of the HIV infection. Gp120 is shed from the viral membrane and accumulates

in lymphoid tissues in significant amounts, where it can induces apoptosis and severely alters the immune response to the virus by dampening the antiviral CTL response thus impeding the clearance of HIV. The effects of gp120 and how it interacts and influences T cell immune response to the virus is an important topic and this review aims to summarize what has been published so far in hopes of providing guidance for future work in this area.

It has recently been suggested that the affinity of gp120 for integrin α4β7 provides the alternative mechanism for HIV-1 to target a subset of CD4+ T cells that are highly susceptible to infection (Fig. 2); such an activity may be particularly critical during transmission [7]. In contrast to CD4, α4β7 is a more prominent receptor (~3 times the size of CD4) that gp120 can engage independently of CD4 [8]. Unlike CD4, which is expressed uniformly on both resting and activated CD4+ T cells, α4β7 is expressed at high levels primarily on activated cells.

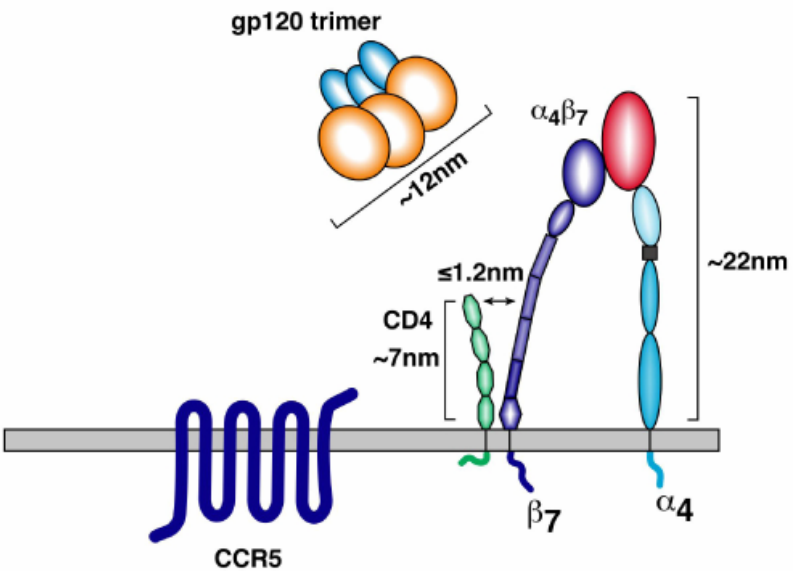


Fig. 2. A schematic depicting approximate sizes of α4β7, CD4, and a gp160.

Several reports are in agreement that HIV-1 transmission in T-lymphocytes cultures occurs predominantly through cell-cell spread with an estimated efficiency 100-1000 times greater than cell free virus replication [9]. The formation of an HIV-1 Virological Synapse (VS) is facilitated by the interaction of envelope with CD4 and the chemokine coreceptor [10]. Integral to HIV-1 VS are adhesion molecules including LFA-1 and its ligand ICAM. Of note, gp120-α4β7 interactions mediate a rapid activation of LFA-1 [11] (Figs. 3A-C). It is important to emphasize that cell-to-cell spread of HIV through VS is far more efficient than cell free infection, and likely to be an important means of viral replication *in vivo*.

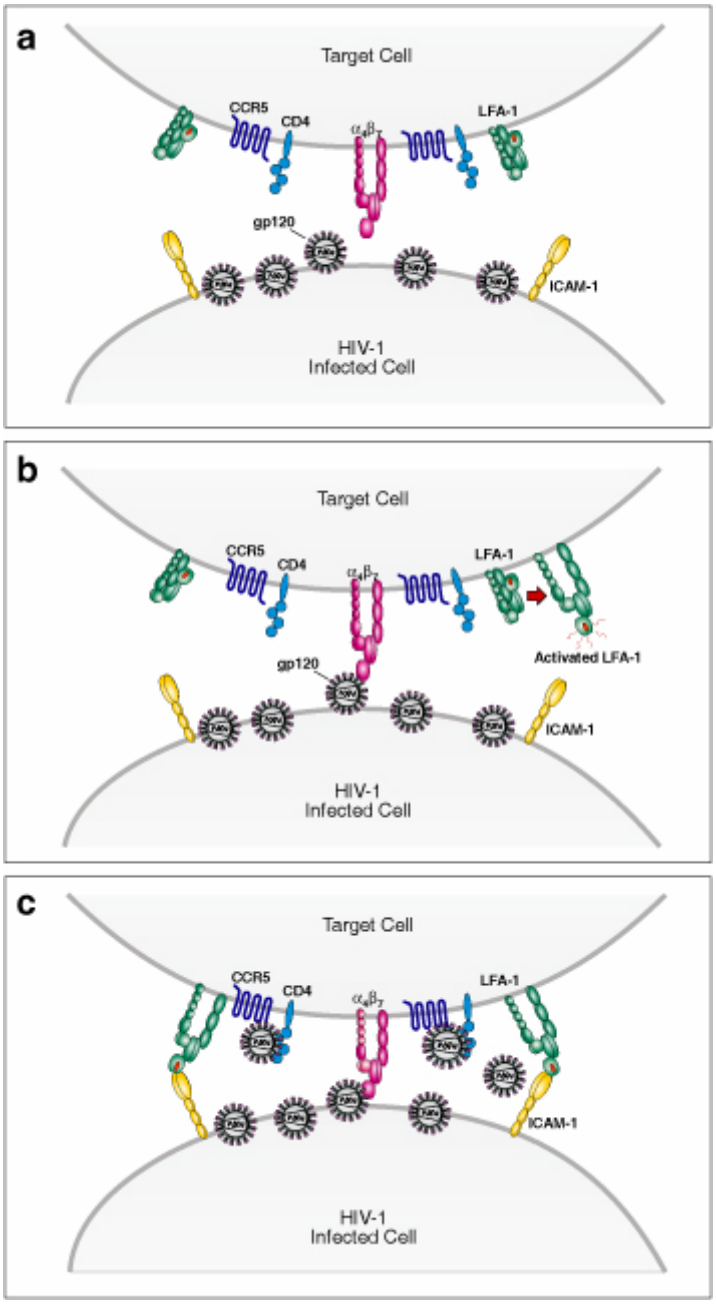


Fig. 3. A schematic depicting the formation of a VS upon engagement of α4β7 by HIV-1 envelope. An HIV-1 infected cell encounters a highly susceptible target cell expressing high levels of α4β7 (panel A). HIV-1 envelope on the surface of the infected cell binds to α4β7 on the target cell and activates the downstream integrin LFA-1 (panel B). LFA-1 binds to its ligand ICAM-1 (panel C) and stabilizes a VS.

The interaction between gp120 and α4β7 triggers a signal, that is not yet fully defined [12]; however, it has been reported that the gp120-mediated signal transduction in several cellular subsets impacts viral replication. In this regard, a number of reports conclude that HIV-1 gp120 mediates signals that facilitate viral replication [13]. Thus, gp120 can be described as a unique ligand that can mediate signals in a near simultaneous manner through CD4, a chemokine receptor and α4β7. The first gp120-mediated signal to be reported involved a protein tyrosine kinase. In response to gp120 treatment, CD4+

T-cells rapidly phosphorylate p56lck, which then dissociates from the cytoplasmic domain of CD4 [14]. The identification of chemokine receptors as HIV coreceptors opened up new questions regarding the role of chemokine receptor signaling in viral infection and pathogenesis [15]. gp120 was shown to trigger rapid calcium fluxes by engaging CCR5 [16]. There is some evidence suggesting that the differential capacity of genetically distinct gp120s to signal correlates with their capacity to facilitate replication [17]. HIV-1 gp120 induces phosphorylation of several proteins, many involved in cytoskeleton rearrangement, including Pyk2 and FAK. Binding of gp120 to both CCR5 and CXCR4, activates several intracellular signaling cascades, mimicking the natural ligands of the chemokine receptors. HIV-1 gp120 has also been shown to trigger signaling in resting cells. In resting cells gp120 mediates the nuclear translocation of the transcription factor NFAT that can enhance viral transcription by binding to NFAT recognition sites on the HIV long terminal repeat (LTR) [18]. gp120 can mediate chemotaxis, actin cytoskeleton rearrangement [19] and the activation of an actin depolymerization factor, cofilin, in resting cells [20]. The density of cell surface CCR5 determines post-entry efficiency of replication of an R5 virus [21] and in unstimulated primary T cells, CCR5 signaling supports HIV-1 infection [22]. Moreover, gp120-CCR5 signaling can induce a distinct gene expression profile in primary cells and a signaling cascade, associated with cellular activation, that favors viral replication in non-proliferating target cells [23]. As noted above, R5 viruses dominate the early stages of infection, largely infecting activated memory CD4+ T cells in the draining lymphoid tissue, particularly the GALT. Both activated and "ostensibly resting" CD4+ T cells are involved in the early stages of infection in the GALT [24]. The capacity of gp120 to trigger signals that promote viral replication in both activated and resting cells, may facilitate infection. This activity may be particularly important during mucosal transmission. Studies of transmission in an SIV macaque model [25] indicate that the first cells infected are not fully activated. It is in these cells that gp120 signals may provide the necessary metabolic stimulus to achieve productive infection. Although the available knowledge about gp120-α4β7 signaling is incomplete, we can speculate that it is in this setting that gp120-α4β7 signal transduction may play an important role and may be a major factor in the transmission of HIV at the mucosal surface.

HIV enters cells directly via plasma membrane penetration for productive infection, which requires fusion of the viral envelope with the host cell membrane. GSLs within the host cell membrane have been proposed to act as HIV-1 fusion receptors [26]. To this effect, several GSLs have been identified that are recognized by HIV gp120 and bind in a receptor-ligand interaction [27]. These glycolipids include galactosylceramide (GalCer) and 3′ sulfogalactosylceramide (SGC), monosialoganglioside (GM3), and globotriaosylceramide (Gb3 or Pk/CD77; see Fig. 4 and Table 1). The lipid moiety of each GSL is a ceramide comprised of a long chain sphingosine base and an amide-linked long chain fatty acid. The alkyl chains anchor the GSL in the cell membrane. Different sugars extend out from the plasma membrane and comprise the recognition unit.

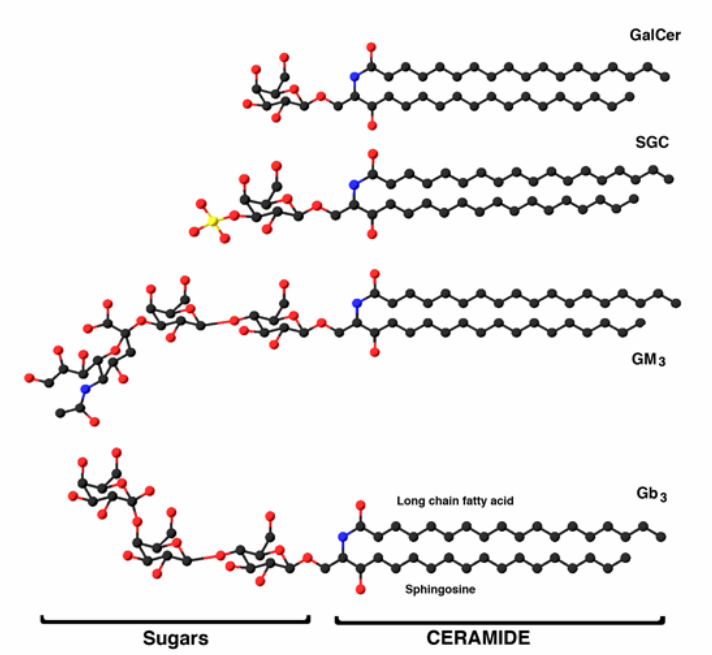


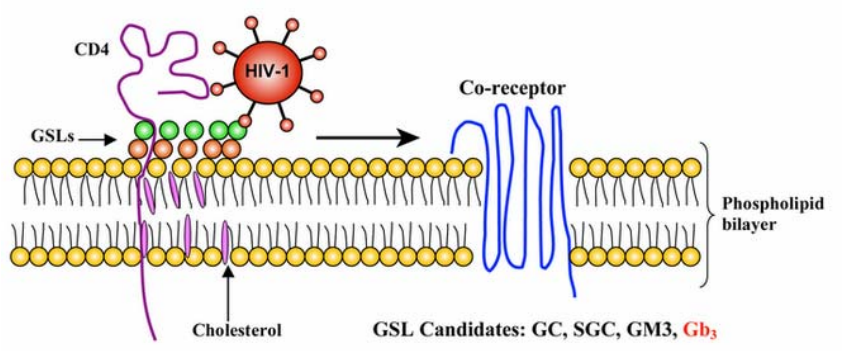
Fig. 4. Schematic of GSL structures involved in HIV infection: A) GalCer (galactoseβ1-1ceramide), B) SGC (3' sulfogalactosyl ceramide), C) GM3 (N-acetyl neuraminic acid β2-3 galactose β1-4glucosyl ceramide), and D) Gb3 (galactose α1-4 galactose α1-4 glucosyl ceramide).

Table 1. Glycosphingolipids Reported to Be Involved in HIV Infection

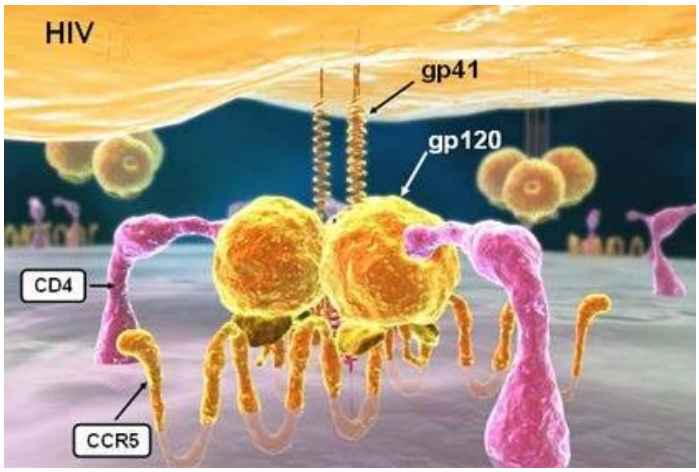
Glycosphingolipid	Role in HIV Infection	References
Galactosyl ceramide	gp120 receptor on CD4 negative cell	Harouse et al., 1995; Fromentin et al., 2011
Galactosyl ceramide	Viral transmission to CD4 T cells	Dezzutti et al., 2001; Magerus-Chatinet et al., 2007
GM3 ganglioside	gp120 receptor, fusion cofactor	Hammache et al., 1998; Hammache et al., 1999; Nehete et al., 2002
GM3 ganglioside	Resistance to HIV	Rawat et al., 2004
Gb_3	gp120 receptor, fusion cofactor	Hug et al., 1998; Hug et al., 2000; Nehete et al., 2002
Gb_3	Resistance to HIV	Lund et al., 2005; Lund et al., 2009; Ramkumar et al., 2009; Harrison et al., 2010

GSLs can interact with HIV gp120 with or without interaction with CD4, although HIV binding to CD4 may allow for increased binding of GSLs to gp120 (Fig. 4) [[28]]. Following binding of GSLs to gp120, they may function differently. GSLs such as GalCer and GM3 may facilitate HIV infection by allowing, through association with lipid rafts, for the fluidic movement of HIV through the plasma membrane to locate a chemokine co-receptor. In contrast, Gb3 has higher binding affinity for gp120 than the other GSLs and may successfully compete for co-receptor binding, and thus inhibit HIV co-receptor interaction and prevent fusion and viral entry [[29]]. Schematic representation of how Gb3 interacts with HIV-1 is shown in Fig. 5A. Current paradigm for HIV infection requires HIV to first bind via gp120 to

CD4 causing a conformational change in gp120 and its binding to a chemokine co-receptor, either CXCR4 or CCR5, triggering gp41 and cell fusion (Fig. 6A). If CD4-negative cells constitutively express or can be made to overexpress Gb3, Gb3 may bind directly to HIV gp120 without HIV binding first to CD4. This may result in diminished HIV fusion as the chemokine binding motif is blocked by Gb3 binding to gp120 (Fig. 6B). If HIV binds to CD4 the binding affinity of Gb3 to gp120 can be increased to result in an inability for HIV gp120 to bind to a chemokine co-receptor, preventing HIV fusion (Fig. 6C). Soluble Gb3 analogue can bind to HIV gp120 independently of CD4 binding and prevent binding to CD4 and/or chemokine co-receptor, preventing HIV infection (Fig. 6D).



(A)



(B)

Fig. 5. gp120-mediated invasion with (**A**) or without (**B**) interaction with CD4.

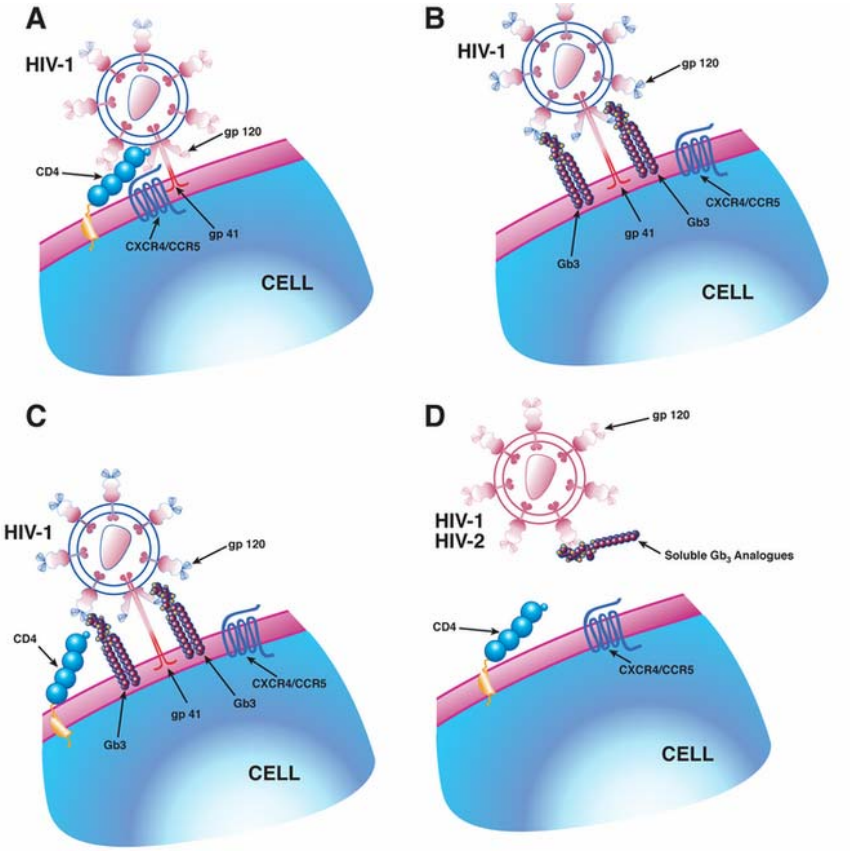


Fig. 6. The HIV invasion models suggested for gp120-gp41/CD4/CXCR4/CCR5/Gb3 interface

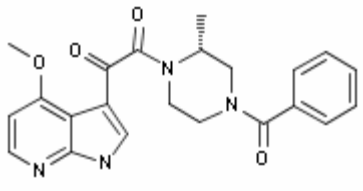
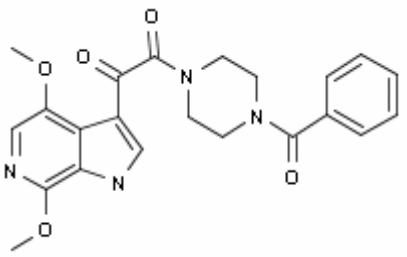
Small-molecule gp120/gp41 Inhibitors

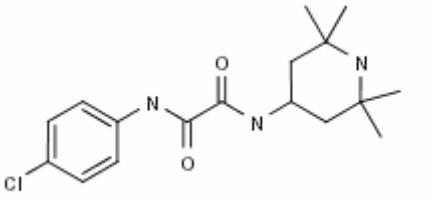
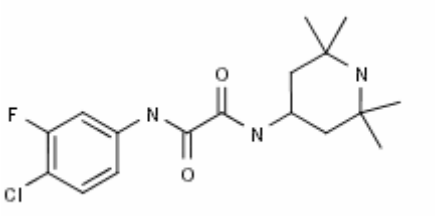
To the present day, more than 50 compounds have been reported to possess a promising activity against gp120-gp41, *in vitro* and *in vivo* [30]. At about 70% of these compounds are peptides with pre-assigned AA-length and conformation, while remaining agents represent structurally diverse small-molecule compounds currently being evaluated in different biological trials. Such compounds are usually assigned to the common group of "HIV attachment and fusion inhibitors". Several, more prominent examples are listed in table 2. For instance, compounds developed by Bristol-Myers Squibb (BMS-378806, BMS-488043 and others from this series) represent attractive drug-candidates against HIV-infection. In HIV envelope surface glycoprotein gp120 assay BMS-378806 showed the IC_{50} value of ~ 0.5 nm [31]; in MT2 human T-lymphoblastoid cells ~ 0.85 nM [32]; in Mononuclear cells (blood), human (phytohemagglutinin-stimulated) ~ 1.50 nm [33]; in U87MG human astrocytoma cells ~ 1.9 nm [34], etc.

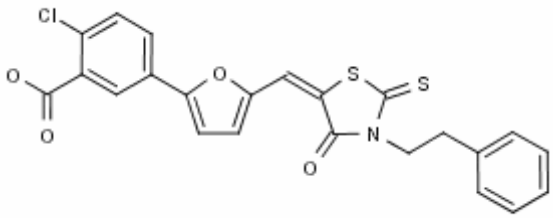
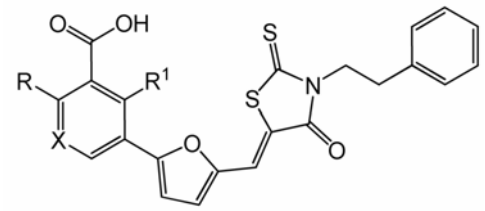
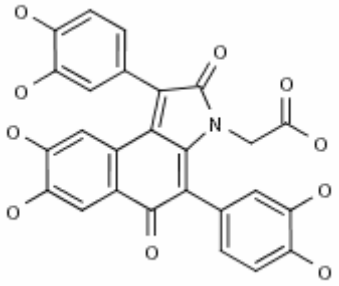
BMS-488043 has also emerged as a lead, exhibiting a Caco-2 permeability of 178 nm/s and a microsomal half-life predictive of a low clearance (4 mL/min/kg) in humans [35]. These *in vitro* characteristics translated well to the *in vivo* setting. The oral bioavailability of BMS-488043 in rats, dogs, and monkeys was 90%, 57%, and 60%, respectively. The clearance was low in all three species tested, with a terminal half-life ranging from 2.4 to 4.7 h. Furthermore, the oral exposure of BMS-488043 was significantly improved (6- to 12-fold in rats and monkeys) compared to the prototype compound BMS-

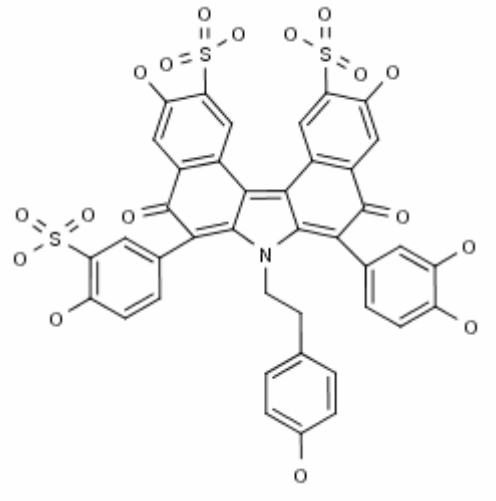
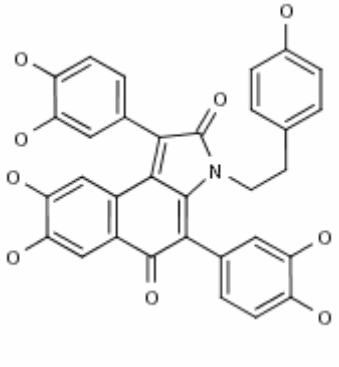
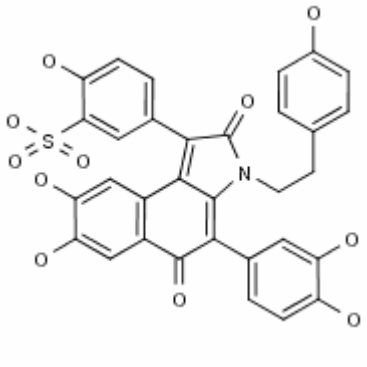
378806 that had a suboptimal Caco-2 permeability (51 nm/s) and microsomal half-life. More importantly, the improvements in preclinical pharmacokinetics translated well to humans, leading to a >15-fold increase in the human oral exposure of BMS-488043 than BMS-378806 and enabling a clinical proof-of-concept for this novel class of anti-HIV agents. These studies have demonstrated the valuable role of *in vitro* ADME screens in improving oral pharmacokinetics at the lead optimization stage. The related SAR for compounds from this series has been thoroughly described by Wang et al [36]. Docking and 3D-QSAR studies of BMS-378806 analogs were shared in [37].

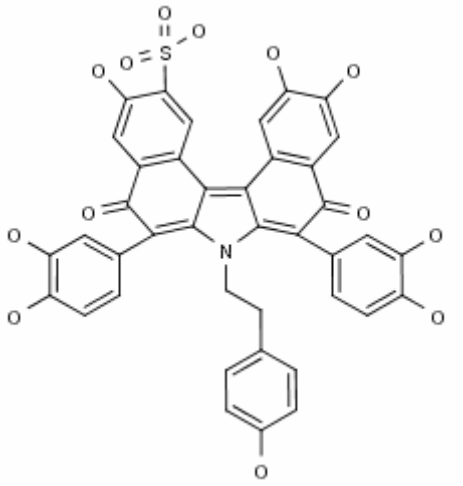
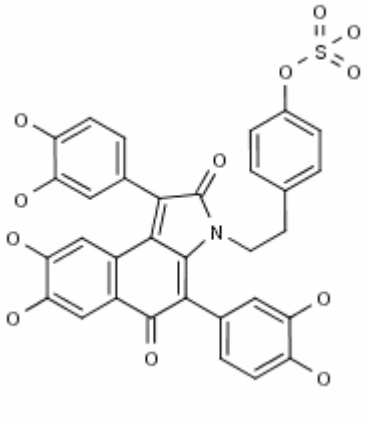
Table 2. Small-molecule compounds with activity against gp160.

Compound Name/Phase	**Structure/Originator**	**Highlighted in the underlying mechanism of action**
BMS-378806 and BMS-488043 / Phase I	 BMS-378806  BMS-488043 Bristol-Myers Squibb	BMS-378806 is a small-molecule HIV-1 inhibitor which had been in early clinical trials at Bristol-Myers Squibb for the treatment of HIV infection. However, no recent development has been reported for this indication. The compound blocks viral entry by binding to the HIV-1 envelope protein gp120 and inhibiting the interaction between gp120 and CD4 receptors. BMS-378806 displayed good oral bioavailability in animals (19, 77 and 24%, respectively, in rats, dogs and cynomolgus monkeys), as well as a prolonged oral half-life (2.1 and 6.5 h in rats and monkeys, respectively). BMS-378806 showed little or no brain penetration and was well tolerated in rats at doses of up to 100 mg/kg/day p.o. for 2 weeks and in dogs at doses of up to 90 mg/kg/day for 10 days; studies in rabbit Purkinje fibers indicated little potential for cardiotoxicity.

MPC-9055 / Preclinical	Structure has not been disclosed yet / Myriad Genetics	MPC-9055 by Myriad Genetics, a small-molecule drug candidate designed to be taken orally and to inhibit viral maturation, for the treatment of AIDS. The company is planning a first phase I trial to assess the pharmacokinetics, absorption and tolerability of the compound. This trial is designed as a single ascending-dose study in healthy volunteers. Assuming successful completion of phase I, Myriad will initiate a phase IIa multiple ascending-dose trial in HIV-infected individuals to evaluate safety, pharmacokinetics and the product's ability to inhibit viral replication. The company also develops a novel, orally-available, small molecule fusion inhibitor against HIV virus, MPI-451936. This compound targets viral Gp41 protein and uniquely inhibits fusion of HIV virus that utilizes the CXCR4 co-receptor, instead of the more common CCR5 co-receptor.
NBD-556 and JRC-II-191 / Biological Testing	 NBD-556 	NBD-556: MT2 human T-lymphoblastoid cells 2.10 μM PM1 human T-lymphocytes (CD4-positive) 5.00 > 30 μM Cf2Th canine thymocytes (CD4+/CCR5+) 73.7 μM HIV envelope surface glycoprotein gp120 affinity 3.70 μM [38]

	JRC-II-191 Harvard Medical School Johns Hopkins University University of Pennsylvania	
681553 / Biological Testing	 The main scaffold is:  New York Blood Center (NYBC) University of Florida (UF)	In viral p24 antigen assay in MT2 human T-lymphoblastoid cells (CD4+/CXCR4+) this compound showed high activity with the IC_{50} value of 17 nM [39]
693604 / Biological Testing	 Peking University (PKU)	Alkaloid isolated from ethanolic extract of the marine sponge Iotrochota baculifera that displayed binding affinity to recombinant viral infectivity factor [vif of HIV-1] and the HIV-1 protein gp41, at 20 mcg/mL, in biacore assays. Compound showed antiviral activity against MT-4 cells infected with HIV-1-IIIB (IC_{50} = 4.363 mcg/mL) in a p24 antigen detection assay and reduced viral titers in HeLa-CD4-TLT-beta-Gal infected with HIV-1-IIIB (IC_{50} =0.012 mcg/mL, 100% inhibitive rate at 125 mcg/mL at 0 h post virus-inoculation) in a MAGI test [40]

693608 / Biological Testing	 Peking University (PKU)	HeLa human cervix adenocarcinoma cells (CD4-LTR/beta-gal-positive) (Microscopic assay) IC_{50}=1.28 mg/l MT4 human T-lymphoblastoid cells Viral p24 antigen assay IC_{50}=1.40 mg/l
693611 / Biological Testing	 Peking University (PKU)	HeLa human cervix adenocarcinoma cells (CD4-LTR/beta-gal-positive) (Microscopic assay) IC_{50}= 0.4 mg/l MT4 human T-lymphoblastoid cells Viral p24 antigen assay IC_{50}= 5.51 mg/l
693614 / Biological Testing	 Peking University (PKU)	[41]

693615 / Biological Testing	 Peking University (PKU)	HeLa human cervix adenocarcinoma cells (CD4-LTR/beta-gal-positive) (Microscopic assay) IC_{50}=0.19 mg/l MT4 human T-lymphoblastoid cells Viral p24 antigen assay IC_{50}=5.01 mg/l
Baculiferin J / Biological Testing	 Peking University (PKU)	[42]

***In silico* approaches to design of novel gp120-gp41 Inhibitors**

Among a range of *in silico* approaches currently applied for drug design & development 3D-molecular docking is considered to be more accurate method. This technique has been effectively used for the design of novel small-molecule gp120-gp41 inhibitors, several examples are below.

It has recently been reported that palmitic acid (PA) is a novel and efficient CD4 fusion inhibitor to HIV-1 entry and infection [43]. Thus, based on *in silico* modeling of the novel CD4 pocket that binds PA, several highly potent PA analogs with increased CD4 receptor binding affinities (Kd) and gp120-to-CD4 inhibition constants (Ki) have been discovered (Fig. 7). The PA analogs were selected to satisfy Lipinski's rule of drug-likeness, increased solubility, and to avoid potential cytotoxicity. Molecular docking software Autodock 4.0 was used for blind docking of flexible PA onto rigid two N-terminal domains of CD4 (PDB code 1GC1, Fig. 7A). The resultant PA-CD4 conformations were ranked and categorized based on the value of free energy of binding. 386 out of 1000 docking runs fell into conformations that are ranked with the highest score (−16 kcal/mol). The root mean square deviation of these conformations was 1.2 A suggesting very similar binding modes. One of the ligand bound

conformations of PA-CD4 with a highest score (−17 kcal/mol) is shown in cyan (PA aliphatic chain) and red (PA carboxylic terminus). Crystal structure of gp120-CD4 (PDB code 1GC1) is presented in Fig. 7B. The backbone of gp120 is shown by using ribbon model. The N-terminal D1 and D2 domains of CD4 are indicated. Comparison between PA-CD4 and gp120-CD4 structures shows the overlapping binding sites for gp120 and PA. Fig. 7C shows the close-up of the PA-CD4 binding cavity shown in A. PA occupies this cavity, which is formed by Phe52, Ile60, Ile62, Leu63, and Leu70 of CD4. Electrostatic potential calculated using DelPhi software (B. Honnig's Lab) was mapped onto the molecular surface of CD4. Positively and negatively charged surfaces are in blue and red, respectively, while non-polar surface is in white.

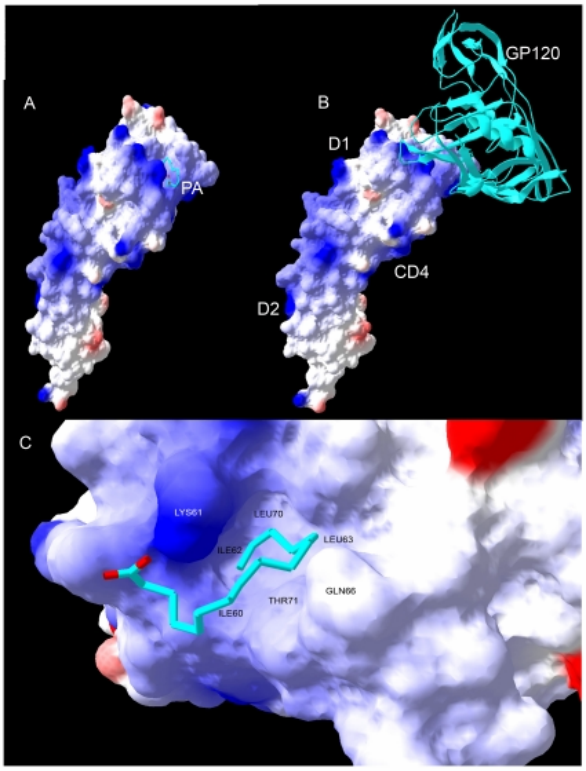
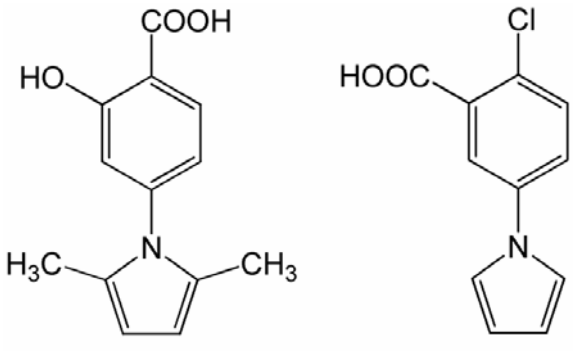


Fig. 7. PA-CD4-gp120 interaction model.

Katritzky et al [44] have previously identified two small molecules targeting the HIV-1 gp41, *N*-(4-carboxy-3-hydroxy) phenyl-2,5-dimethylpyrrole (NB-2) and *N*-(3-carboxy-4-chloro) phenylpyrrole (NB-64) that inhibit HIV-1 infection at low μM level (Fig. 8). Based on molecular docking analysis, authors designed a series of 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans (see Table 2, ID: 681553). Compared with NB-2 and NB-64, these compounds have bigger molecular size (437–515 Da) and could occupy more space in the deep hydrophobic pocket on the gp41 NHR-trimer. Fifteen 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans were synthesized by Suzuki-Miyaura cross coupling, followed by a Knoevenagel condensation and tested for their anti-HIV-1activity and cytotoxicity on MT-2 cells. It has been found that all 15 compounds have improved anti-HIV-1 activity and 3 of them exhibited inhibitory activity against replication of HIV-1 IIIB and 94UG103

at <100 nM range, more than 20-fold more potent than NB-2 and NB-64, suggesting that these compounds can serve as leads for development of novel small molecule HIV fusion inhibitors. Molecular docking analysis revealed that the phenethyl group of compound (X=CH, R=Cl, R^1=H, see Table 2, ID: 681553, the core scaffold) filled the space in the deep hydrophobic pocket of gp41 formed by the NHR trimer (Fig. 9), previously observed to be unoccupied by NB-64.



NB-2 **NB-64**

Fig. 8. Two small molecules targeting the HIV-1 gp41 reported by Katritzky et al [45]

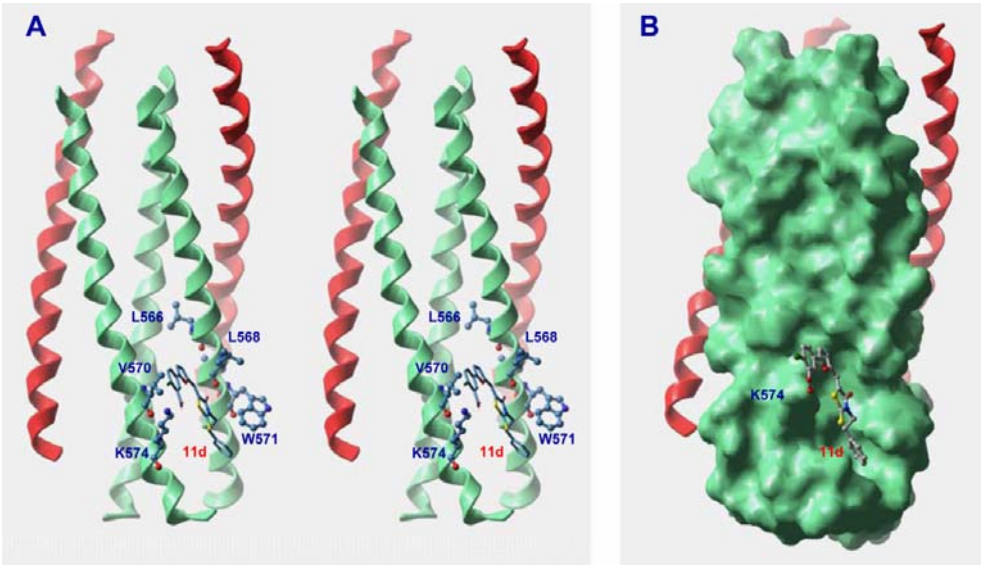


Fig. 9. Docking of 681553 in the gp41 hydrophobic cavity. (A) The stereo view of 681553 docked in the hydrophobic cavity showing possible interactions with the neighboring hydrophobic and charged residue K574. (B) Surface representation of the gp41 core with bound ligand 681553. The compound docked inside the cavity. The negatively charged COOH group is pointing towards the positively charged area contributed by K574.

Recently, 36 analogs compounds of BMS-378806 were synthesized and their biological activity evaluated. Based on these compounds, a molecular docking was performed with BMS-378806 to the gp120 cavity in order to get a representative ligand conformation for the 3D-QSAR process (Fig. 10) [46]. Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were then conducted for these 36 compounds. CoMFA and CoMSIA models give reliable

correlative and predictive abilities but the CoMFA model performance was slightly better than CoMSIA. CoMFA contours were analysed and have been correlated to the gp120 viral protein. The discussion indicates several key fragment positions on the ligands and their implications on the gp120 protein binding. The computational approach used in this paper provides reliable clues for further design of small molecules gp120/CD4 inhibitors based on the BMS-378806.

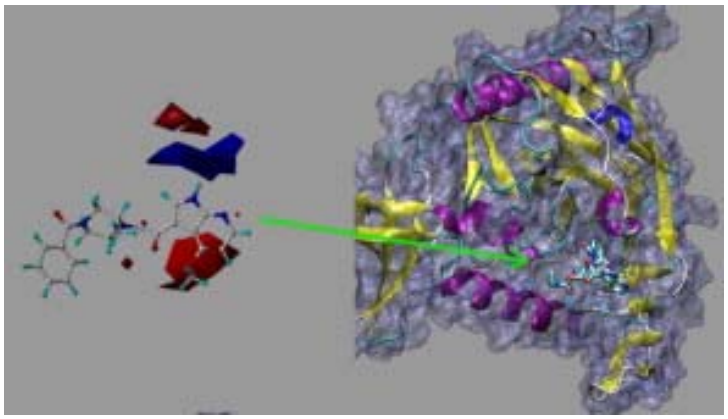


Fig. 10. 3D-Molecular docking performed for BMS-378806 and its analogues.

It has recently been demonstrated that the low-molecular-weight compound JRC-II-191 (see Table 2) inhibits infection of HIV-1 by blocking the binding of the HIV-1 envelope glycoprotein gp120 to the CD4 receptor and is therefore an important lead in the development of a potent viral entry inhibitor. An effective use of two orthogonal screening methods, gold docking and ROCS shape-based similarity searching, to identify amine-building blocks that, when conjugated to the core scaffold, yield novel analogs that maintain similar affinity for gp120 has been described in [47]. The computational approach was used to expand SAR produced analogs of equal inhibitory activity but with diverse capacity to enhance viral infection. The novel analogs provide additional lead scaffolds for the development of HIV-1 entry inhibitors that employ protein-ligand interactions in the vestibule of gp120 Phe 43 cavity.

An interfacial "Phe43 cavity" in gp120, adjacent to residue Phe43 of gp120-bound CD4, has been suggested as a potential target for therapeutic intervention. Xie et al [48] have designed a CD4 mutant (D1D2F43C) for site-specific coupling of compounds for screening against the cavity. Altogether, 81 cysteine-reactive compounds were designed, synthesized, and tested. Eight derivatives exceeded the affinity of native D1D2 for gp120. Structure-activity relationships for derivatized CD4 binding to gp120 revealed significant plasticity of the Phe43 cavity and a narrow entrance. The primary contacts for compound recognition inside the cavity were found to be van der Waals interactions, whereas hydrophilic interactions were detected in the entrance. This first SAR on ligand binding to an interior cavity of gp120 may provide a starting point for structure-based assembly of small molecules targeting gp120-CD4 interaction. Figure 11 below shows the screen for Phe43 cavity-targeting compounds using derivatized CD4. In Fig. 11A, design of modified D1D2F43C for targeting the Phe43 cavity in gp120 is presented.

Thus, the complex of gp120 and the D1D2 domains of CD4 are drawn schematically with gp120 and D1D2 colored in gray and salmon, respectively. Residue Phe43, right at the entrance of the Phe43 cavity, is mutated to a chemically reactive cysteine for specific conjugation of a library of cysteine-reactive compounds (*in green*). The generated D1D2F43C derivatives are then screened for their affinity for gp120. The modification of F43C of D1D2 by haloacetamides or halopropanones (shown on top of the arrows, Y = NH or CH2, respectively) or 5-nitro-2-pyridinesulfenyl reagents (shown below the arrows) is depicted in Fig. 11B.

Figure 12, shows the binding of D1D2 conjugates to cavity-filled gp120. In Fig. 12A, the sliced-open surface representations of the Phe43 cavities of wild type core gp120 (left, PDB code 1RZJ) and cavity-filled core gp120 mutant S375W/T257S (right, PDB code 2NXZ) bound to CD4 are shown. CD4 and gp120 molecules are colored in gray and salmon, respectively. Side chains and Cα atoms of gp120 residues 375 and 257, as well as those of CD4 residue F43, are shown in ball-and-stick model in the coloring scheme: CD4 carbon atoms (salmon), gp120 carbon atoms (gray), nitrogen atom (blue) and oxygen atoms (red). Surfaces in both panels were calculated based on the respective gp120 model in which the side chains of residues 375 and 257 except for Cβ atom were removed. Note that the cavity has a narrow entrance close to the tip of phenyl ring of F43 and that a water channel is located left to the cavity. Side by side comparisons of IC_{50} values of D1D2 variants on the binding of D1D2 to YU2 WT (wild type) gp120 to their IC_{50} values on the binding of D1D2 to YU2 S375W/T257S gp120 are shown in Fig. 12B. Ratios of two IC_{50} values are presented in Fig. 12C.

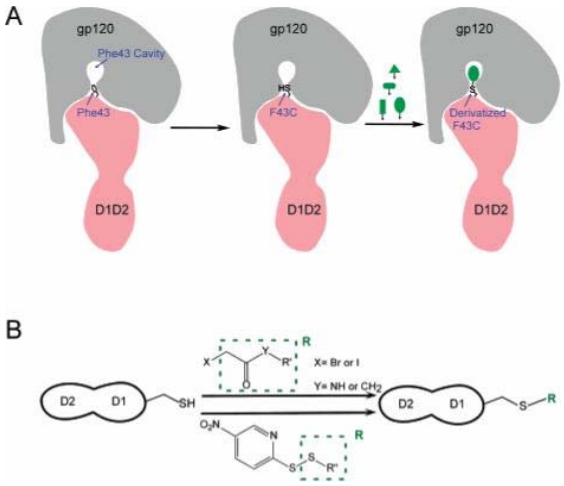


Fig. 11. Design of modified D1D2F43C for targeting the Phe43 cavity in gp120.

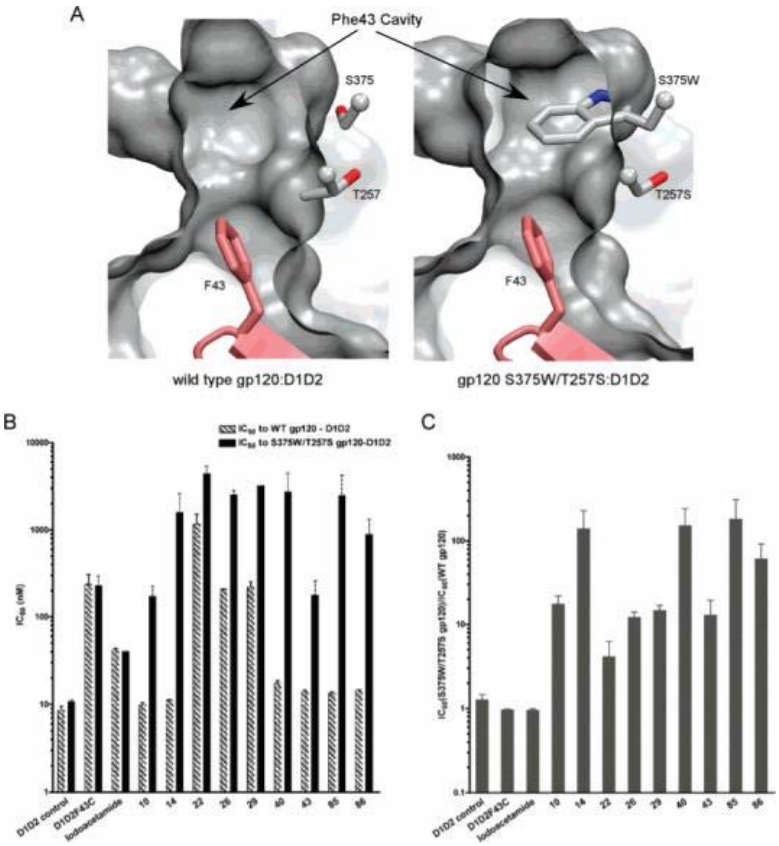


Fig. 12. The binding of D1D2 conjugates to cavity-filled gp120.

Besides molecular docking, several other *in silico* approaches have also been used for the design of novel gp160 inhibitors, these include: 3D-pharmacophore modeling, bioisosteric morphing and structure similarity approach. In addition, advanced molecular technologies have been developed, such as QCM-D.

Thus, Lee et al [49] have evaluated the potential of a quartz crystal microbalance with dissipation monitoring (QCM-D) to provide a sensitive, label-free method for detecting the conformational rearrangement of glycoprotein gp120 upon binding to different ligands (Fig. 13). Tus, gp120 was immobilized on the surface of the sensing element of the QCM-D and was exposed to individual solutions of several different small-molecule inhibitors as well as to a solution of soluble form of the host cell receptor to which gp120 binds. Instrument responses to ligand-triggered changes were in qualitative agreement with conformational changes suggested by other biophysical methods.

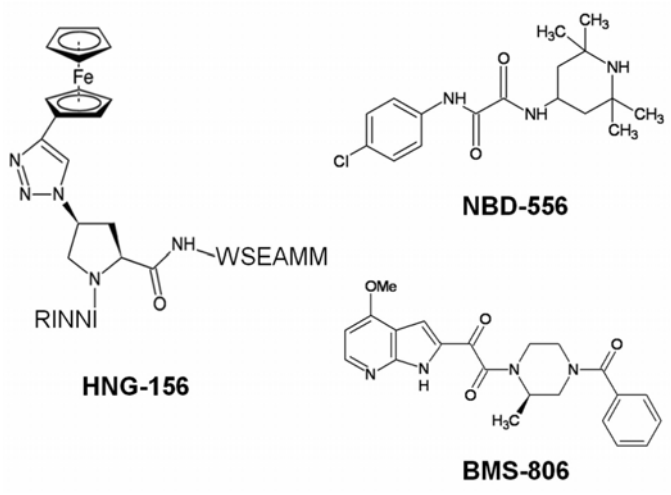


Fig. 13. Small molecules used for binding to gp120 by Lee and co-workers.

As a brief summary, in natural infection the HIV-1 envelope protein is the primary target of neutralizing antibodies. For this reason HIV-1 gp120 has been a central focus of efforts to develop subunit vaccine immunogens that can elicit neutralizing antibodies. The receptor binding epitopes on gp120 are conserved, and antibodies directed against these sites neutralize HIV-1, making receptor binding sites attractive targets in the context of an immunogen. These efforts have proven to be difficult because the viral envelope uses multiple mechanisms to evade and escape neutralizing responses. The envelope protein is hyper-variable in sequence, both within a patient and across each of the major clades. In addition the envelope encodes a shifting pattern of glycosylation. Finally, the flexibility of the variable loops results in conformational masking of conserved epitopes. In particular, the CD4 binding site on gp120, which is structurally conserved, is masked by glycans and variable loops. Thus, efforts to develop an immunogen capable of eliciting broadly cross-reactive Abs against the CD4 binding site have thus far been unsuccessful.

Entry inhibitors mark the beginning of a new era in the history of antiretroviral therapy, opening new therapeutic options for the already large and growing number of patients carrying drug-resistant viruses. Enfuvirtide is the first agent of this class approved for clinical use. Several other compounds are currently in clinical development and may soon be available for use in the treatment of HIV-1. Available evidence indicates that selection of drug resistance may occur with these compounds. However, the pathways leading to resistance to entry inhibitors differ substantially from those causing resistance to the antiretrovirals in current use, and therefore no cross-resistance is anticipated between entry inhibitors and other classes of antiretrovirals, thus allowing salvage therapy with entry inhibitors.

The main mechanism of resistance to enfuvirtide is the selection of changes in a domain consisting of 10 amino acids, between residues 36 and 45 in the HR1 region of gp41. For other entry inhibitors, multiple changes in different gp120 domains (V3, C2, C4 and V4) seem to be responsible for causing loss of susceptibility, although with limited cross-resistance in most cases. Finally, natural susceptibility of

different HIV-1 variants to entry inhibitors warrants further investigation, given that most entry inhibitors target the most variable HIV-1 proteins.

Based on the computational approaches effectively applied for the design of novel gp120-gp41 inhibitors, we have prepared the gp-160-targeted library using small-molecule compounds selected from ChemDiv store; our methodology is presented below.

Concept and Applications

gp160-targeted library design at CDL involves:

• *A combined profiling methodology that provides a consensus score and decision based on various advanced computational tools*:

1. Bioisosteric morphing, structure diversity & similarity concept, topological pharmacophore and funneling procedures in designing novel potential gp160 ligands with high IP value. We apply CDL's proprietary Chemosoft^{TM} software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.

2. Neural Network tools for target-library profiling, in particular Self-organizing Kohonen Maps, performed in SmartMining Software.

3. 3D-molecular docking approach to focused library design.

4. Computational-based `*in silico*` ADME/Tox assessment for novel compounds includes prediction of human CYP P450-mediated metabolism and toxicity as well as many pharmacokinetic parameters, such as Brain-Blood Barrier (BBB) permeability, Human Intestinal Absorption (HIA), Plasma Protein binding (PPB), Plasma half-life time ($T_{1/2}$), Volume of distribution in human plasma (V_d), etc.

The fundamentals for these applications are described in a series of our recent articles on the design of exploratory small molecule chemistry for bioscreening [for related data visit ChemDiv. Inc. online source: www.chemdiv.com].

• *Synthesis, biological evaluation and SAR study for the selected structures*:

1. High-throughput synthesis with multiple parallel library validation. Synthetic protocols, building blocks and chemical strategies are available.

2. Library activity validation via bioscreening; SAR is implemented in the next library generation.

We practice a multi-step approach for building gp160-focused library:

Virtual screening

(1) The small-molecular ligands for gp160 (see Table 1) are compiled into a unique knowledge base (*reference ligand space*) and annotated according to the particular subunit (gp120/gp41). Based on the non-trivial bioisosteric approach and topological pharmacophores more than 40K compounds have been added to the targeted library (Fig. 14).

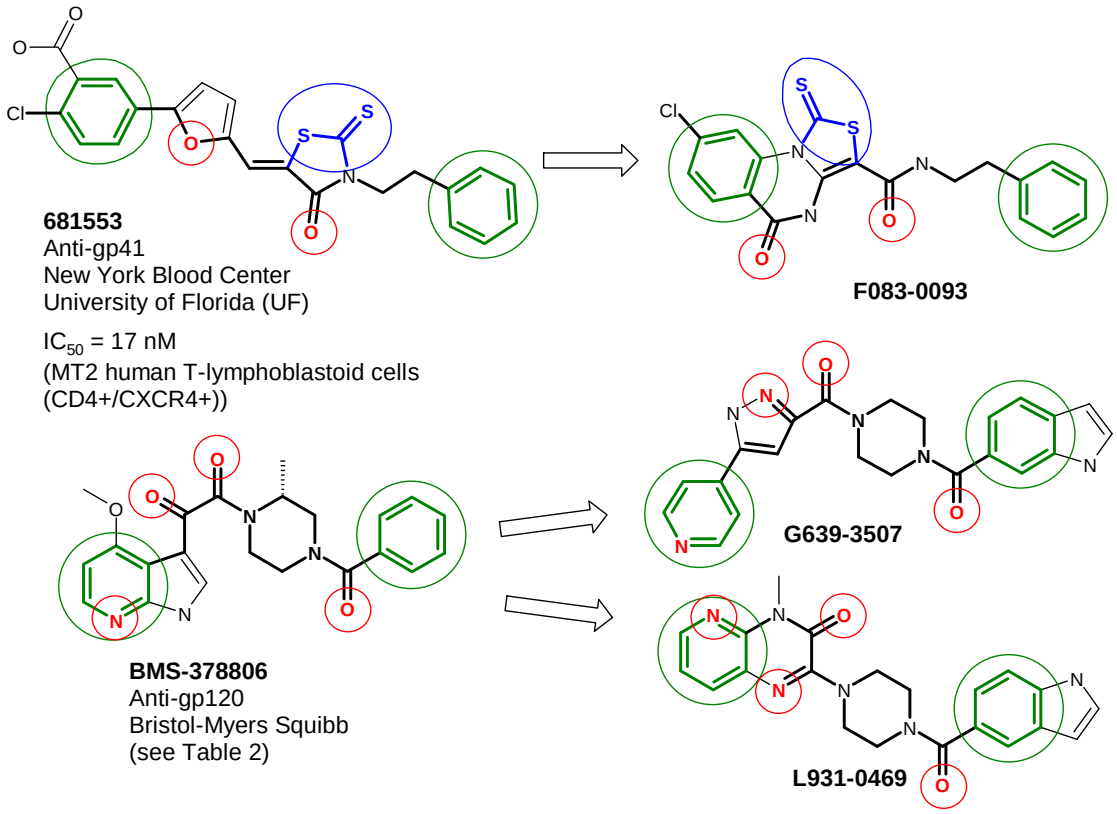


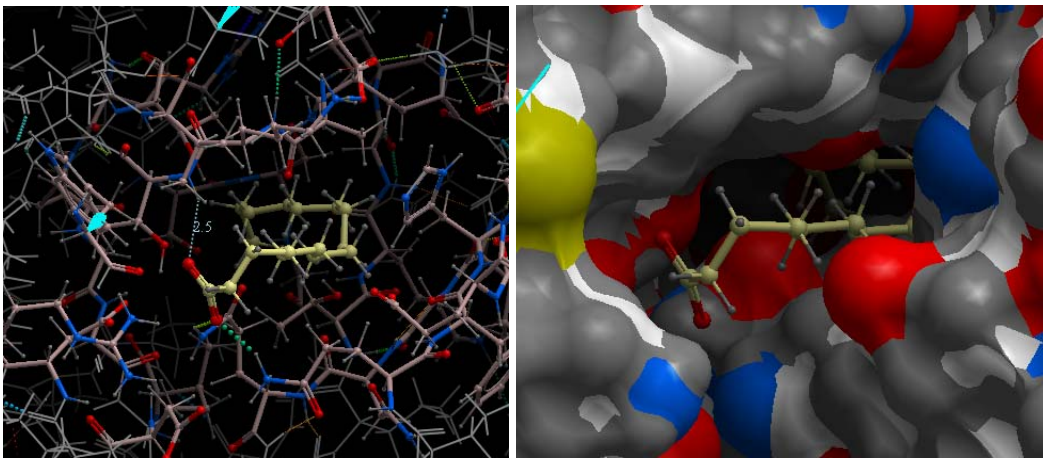
Fig. 14. Examples of bioisosteric modifications and topological phatmacophores for compounds included in the targeted database

3D-molecular Docking

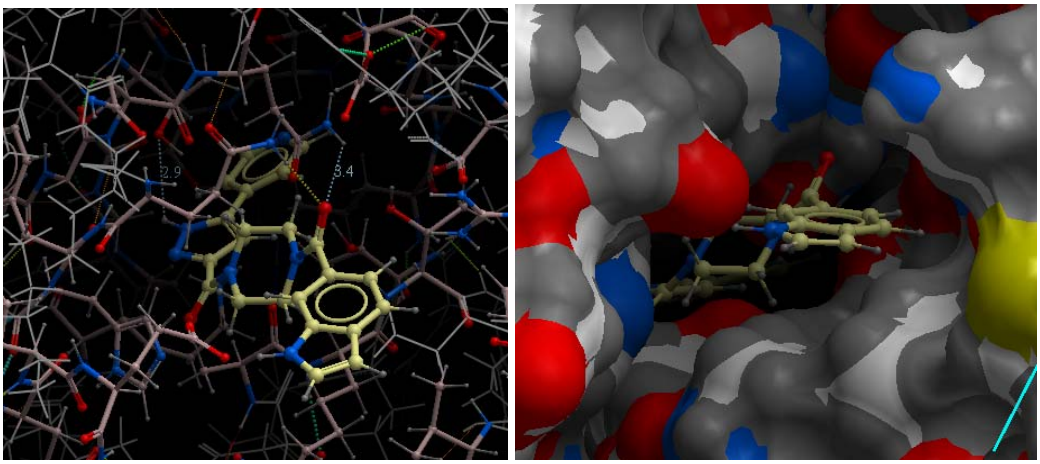
For the gp-160-targeted library design we have been used a molecular docking approach. Currently, several crystallographic complexes of gp-160 with various peptides are available in PDB databank. This data and molecular docking studies described above have been used for the active site construction, 3D-modeling and virtual scoring generation. The constructed gp120- and gp41-binding active sites are shown in Fig. 15A and 16A. The active binding site for gp-120 subunit has been modeled based on PA-CD4-gp120 interaction mode (Fig. 15, pdb code: 1GC1 [50]), while the active binding site for gp-41 (Fig. 16A) has been formed using the reference compound 681553 (see Table 2) [51] and the data reported by Stewart et al [52] (pdb code: 2KP8). Thus, Stewart and colleague have used NMR screening to discover non-peptide leads against this target and resulted in the discovery of a new

benzamide series (Fig 16A). This series is non-peptide, low molecular weight, and analogs have activity in a cell fusion assay with EC_{50} values ranging 3-41 μM. Structural work on the gp41/benzamide complex was determined by NMR spectroscopy using a designed model peptide system that mimics an open pocket of the fusogenic form of the protein.

We have scored the ChemDiv structures outputted from the previous step using the developed models. As a result more than 15K compounds successfully passed through the p120 model and have grouped into the four different categories: *inactive*, *low, medium*, *high*. Compounds from the last three categories were included in the final library (Fig. 15B).



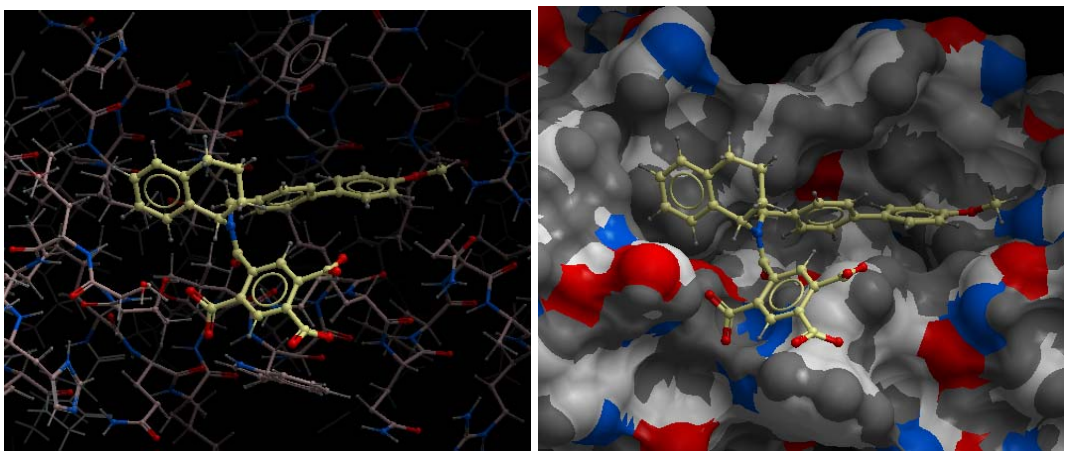
(**A**)



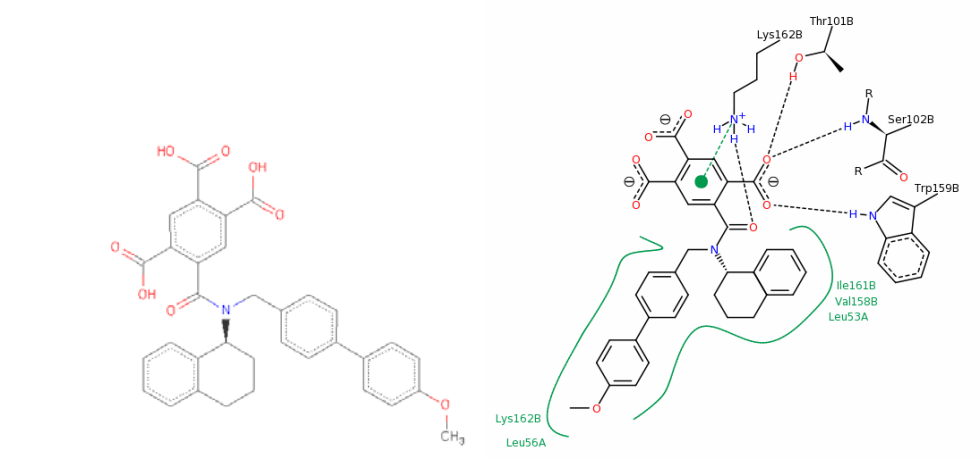
(**B**)

Fig. 15. PA (**A**) and the promising ChemDiv compound - G639-3507 (**B**) form the targeted library in the active binding site of gp120.

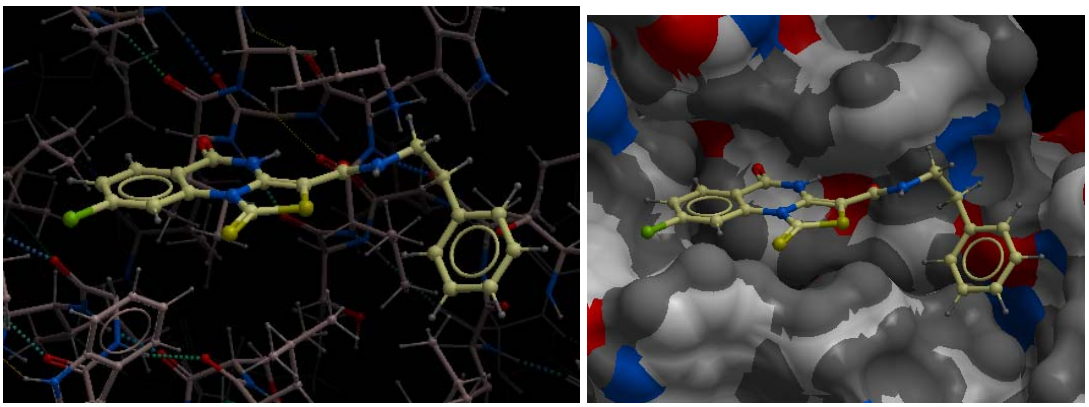
We also have docked the selected structures in the active binding site of gp41 (Fig. 16). The site has been constructed based on the reference compound 681553 [[53]] and compound shown in Fig 16A,B described by Stewart et al [[54]]. The docking procedure has provided more than 7K small molecule "hits" with score ranged from *low* to *high*; representative example is shown in Fig. 16C.



(A)



(B)



(C)

Fig. 16. (**A**,**B**) - the most active molecule from the benzamide series of gp41 inhibitors screened by Stewart [[55]], (**B**) - compound (F083-0093) from the targeted library in the active binding site of gp41.

As a result more than 22K small-molecule compounds have been included in the final gp160-targeted library (Fig. 17).

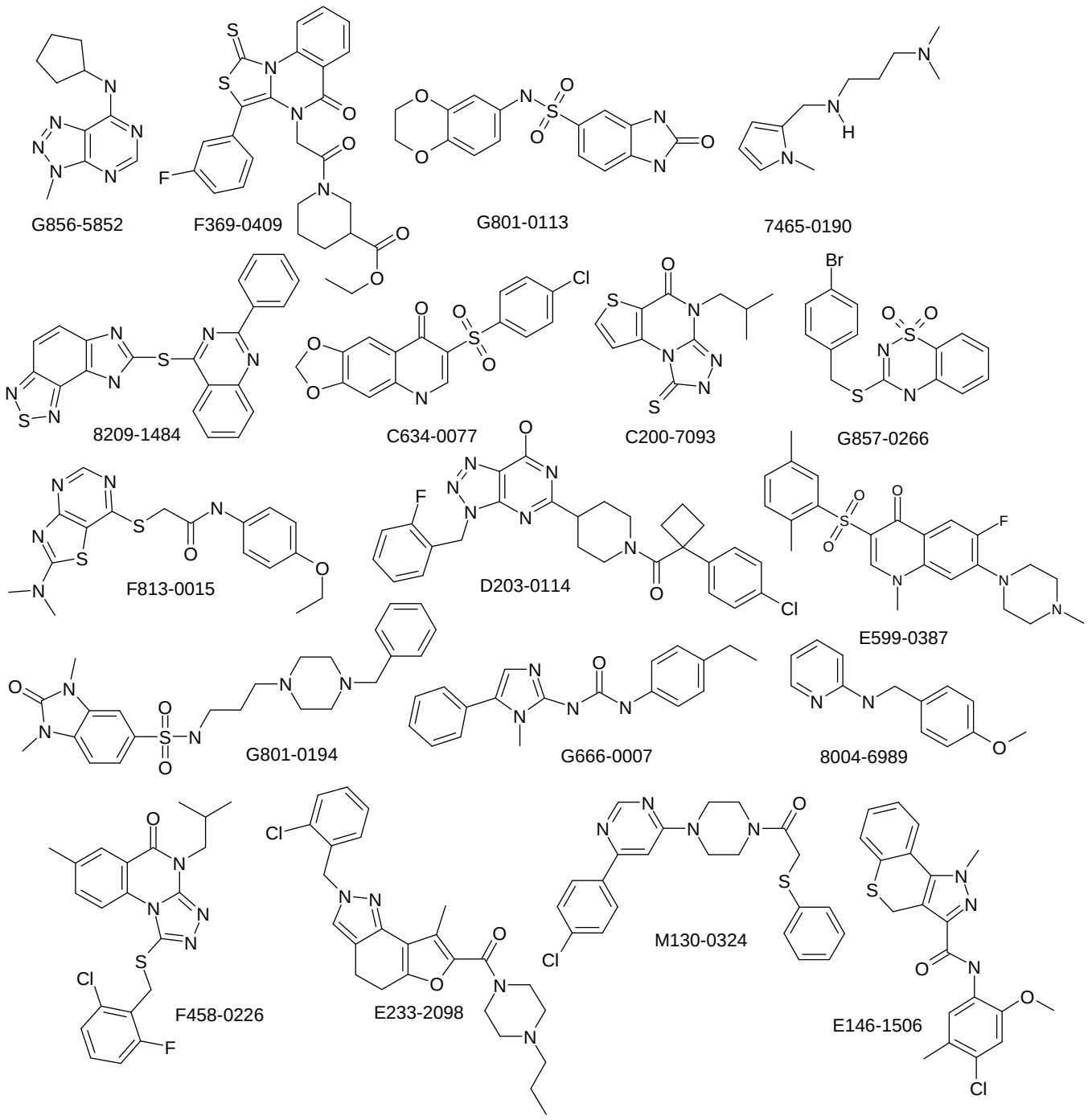


Fig. 17. Representative examples of compounds from the gp160-targeted library.

References

[1] Dalgleish AG, et al.: The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 1984, 312(5996):763-7; Klatzmann D, et al.: T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 1984, 312(5996):767-8; Alkhatib G, et al.: CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996, 272(5270):1955-8; Feng Y, et al.: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996, 272(5263):872-7; Lederman MM, et al.: Biology of CCR5 and its role in HIV infection and treatment. JAMA 2006, 296(7):815-26

[2] Frankel and Young, 1998

[3] Gabuzda D *et al. J Acquir Immune Defic Syndr.* 1991 **4**: 34

[4] Jiang X *et al. Nat Struct Mol Biol.* 2010 **17**: 955

[5] Lorizate M *et al. Biochemistry* 2006 **45**: 14337

[6] V. Yoon, M. Fridkis-Hareli, S. Munisamy, J. Lee, D. Anastasiades, L. Stevceva The GP120 Molecule of HIV-1 and its Interaction with T Cells Current Medicinal Chemistry Volume 17 Issue 8 pp.741-749

[7] Sowmya G, Shamini G, Anita S, Sakharkar M, Mathura V, Rodriguez H, Levine AJ, Singer E, Commins D, Somboonwit C, Sinnott JT, Sidhu HS, Rajaseger G, Pushparaj PN, Kangueane P, Shapshak P. HIV-1 envelope accessible surface and polarity: clade, blood, and brain. Bioinformation. 2011 Mar 22;6(2):48-56.

[8] Arthos J, et al.: HIV-1 envelope protein binds to and signals through integrin alpha(4)beta(7), the gut mucosal homing receptor for peripheral T cells. Nat Immunol 2008; Johansson-Lindbom B, Agace WW: Generation of gut-homing T cells and their localization to the small intestinal mucosa. Immunol Rev 2007, 215:226-42; Cicala C, et al.: The integrin {alpha}4{beta}7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. Proc Natl Acad Sci U S A 2009

[9] Chen P, et al.: Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. J Virol 2007, 81(22):12582-95; Sol-Foulon N, et al.: ZAP-70 kinase regulates HIV cell-to-cell spread and virological synapse formation. EMBO J 2007, 26(2):516-26; Blanco J, et al.: High level of coreceptor-independent HIV transfer induced by contacts between primary CD4 T cells. J Biol Chem 2004, 279(49):51305-14; Dimitrov DS, et al.: Quantitation of human immunodeficiency virus type 1 infection kinetics. J Virol 1993, 67(4):2182-90; Sourisseau M, et al.: Inefficient human immunodeficiency virus replication in mobile lymphocytes. J Virol 2007, 81(2):1000-12.

[10] Jolly C, et al.: HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. J Exp Med 2004, 199(2):283-93; Piguet V, Sattentau Q: Dangerous liaisons at the virological synapse. J Clin Invest 2004, 114(5):605-10.

[11] Arthos J, et al.: HIV-1 envelope protein binds to and signals through integrin alpha(4)beta(7), the gut mucosal homing receptor for peripheral T cells. Nat Immunol 2008.

[12] Arthos J, et al.: HIV-1 envelope protein binds to and signals through integrin alpha(4)beta(7), the gut mucosal homing receptor for peripheral T cells. Nat Immunol 2008; Cicala C, et al.: The integrin {alpha}4{beta}7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. Proc Natl Acad Sci U S A 2009

[13] Arthos J, et al.: CCR5 signal transduction in macrophages by human immunodeficiency virus and simian immunodeficiency virus envelopes. J Virol 2000, 74(14):6418-24; Cicala C, et al.: HIV-1 gp120 induces NFAT nuclear translocation in resting CD4+ T-cells. Virology 2006, 345(1):105-14; Cicala C, et al.: HIV envelope induces a cascade of cell signals in non-proliferating target cells that favor virus replication. Proc Natl Acad Sci U S A 2002, 99(14):9380-5; Kinter AL, et al.: HIV envelope induces virus expression from resting CD4+ T cells isolated from HIV- infected individuals in the absence of markers of cellular activation or apoptosis. J Immunol 2003, 170(5):2449-2455; Yu D, et al.: The HIV envelope but not VSV glycoprotein is capable of mediating HIV latent infection of resting CD4 T cells. PLoS Pathog 2009, 5(10):e1000633.

[14] Juszczak RJ, et al.: Effect of human immunodeficiency virus gp120 glycoprotein on the association of the protein tyrosine kinase p56lck with CD4 in human T lymphocytes. J Biol Chem 1991, 266(17):11176-83

[15] Alkhatib G, et al.: CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996, 272(5270):1955-8; Feng Y, et al.: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996, 272(5263):872-7; Berger EA, Murphy PM, Farber JM: Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 1999, 17:657-700

[16] Weissman D, et al.: Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. Nature 1997, 389(6654):981-5.

[17] Arthos J, et al.: CCR5 signal transduction in macrophages by human immunodeficiency virus and simian immunodeficiency virus envelopes. J Virol 2000, 74(14):6418-24.

[18] Cicala C, et al.: HIV-1 gp120 induces NFAT nuclear translocation in resting CD4+ T-cells. Virology 2006, 345(1):105-14

[19] Balabanian K, et al.: CXCR4-tropic HIV-1 envelope glycoprotein functions as a viral chemokine in unstimulated primary CD4+ T lymphocytes. J Immunol 2004, 173(12):7150-60.

[20] Yoder A, et al.: HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. Cell 2008, 134(5):782-92

[21] Lin YL, et al.: The efficiency of R5 HIV-1 infection is determined by CD4 T-cell surface CCR5 density through G alpha i-protein signalling. AIDS 2006, 20(10):1369-77

[22] Lin YL, et al.: G-protein signaling triggered by R5 human immunodeficiency virus type 1 increases virus replication efficiency in primary T lymphocytes. J Virol 2005, 79(12):7938-41

[23] Cicala C, et al.: HIV-1 gp120 induces NFAT nuclear translocation in resting CD4+ T-cells. Virology 2006, 345(1):105-14; Cicala C, et al.: HIV envelope induces a cascade of cell signals in non-proliferating target cells that favor virus replication. Proc Natl Acad Sci U S A 2002, 99(14):9380-5

[24] Haase AT: Perils at mucosal front lines for HIV and SIV and their hosts. Nat Rev Immunol 2005, 5(10):783-92

[25] Haase AT: Perils at mucosal front lines for HIV and SIV and their hosts. Nat Rev Immunol 2005, 5(10):783-92; Li Q, et al.: Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005, 434(7037):1148-52; Haase AT: Targeting early infection to prevent HIV-1 mucosal transmission. Nature 464(7286):217-23

[26] Fantini et al., 1996; Nehete et al., 2002

[27] Hammache et al., 1998; Hammache et al., 1999; Hug et al., 2000; Rawat et al., 2005

[28] Hammache et al., 1998
[29] Lund et al., 2009
[30] Based on the data obtained from Intergity Database (https://integrity.thomson-pharma.com), patents and available publications.
[31] Yeung, K.S.; Qiu, Z.; Fang, H.; Yin, Z.; et al. C7-Heteroaryl-indoles as potent and orally bioavailable inhibitors of HIV attachment 237th ACS Natl Meet (March 22-26, Salt Lake City) 2009, Abst MEDI 208
[32] Wang, T.; Zhang, Z.; Wallace, O.B.; et al. J Med Chem 2003, 46(20): 4236 Discovery of 4-benzoyl-1-[(4-methoxy-1H-pyrrolo[2,3-b]pyridin-3-yl)oxoacetyl]-2-(R)-methylpiperazine (BMS-378806): A novel HIV-1 attachment inhibitor that interferes with CD4-gp120 interactions.
[33] Lin, P.-F.; Blair, W.; Wang, T.; et al A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding Proc Natl Acad Sci USA 2003, 100(19): 11013
[34] Lin, P.-F.; Blair, W.; Wang, T.; et al Proc Natl Acad Sci USA 2003, 100(19): 11013 A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding
[35] Yang Z, Zadjura LM, Marino AM, D'Arienzo CJ, Malinowski J, Gesenberg C, Lin PF, Colonno RJ, Wang T, Kadow JF, Meanwell NA, Hansel SB Utilization of in vitro Caco-2 permeability and liver microsomal half-life screens in discovering BMS-488043, a novel HIV-1 attachment inhibitor with improved pharmacokinetic properties. J Pharm Sci. 2010 Apr;99(4):2135-52.
[36] Wang T, Yin Z, Zhang Z, Bender JA, Yang Z, Johnson G, Yang Z, Zadjura LM, D'Arienzo CJ, DiGiugno Parker D, Gesenberg C, Yamanaka GA, Gong YF, Ho HT, Fang H, Zhou N, McAuliffe BV, Eggers BJ, Fan L, Nowicka-Sans B, Dicker IB, Gao Q, Colonno RJ, Lin PF, Meanwell NA, Kadow JF Inhibitors of human immunodeficiency virus type 1 (HIV-1) attachment. 5. An evolution from indole to azaindoles leading to the discovery of 1-(4-benzoylpiperazin-1-yl)-2-(4,7-dimethoxy-1H-pyrrolo[2,3-c]pyridin-3-yl)ethane-1,2-dione (BMS-488043), a drug candidate that demonstrates antiviral activity in HIV-1-infected subjects J Med Chem. 2009 Dec 10;52(23):7778-87
[37] Teixeira C, Serradji N, Maurel F, Barbault F. Docking and 3D-QSAR studies of BMS-806 analogs as HIV-1 gp120 entry inhibitors Eur J Med Chem. 2009 Sep;44(9):3524-32
[38] Lalonde, J.M.; Elban, M.A.; Courter, J.R.; et al Design, synthesis and biological evaluation of small molecule inhibitors of CD4-gp120 binding based on virtual screening Bioorg Med Chem 2011, 19(1): 91
[39] Katritzky, A.R.; Tala, S.R.; Lu, H.; Vakulenko, A.V.; Chen, Q.Y.; Sivapackiam, J.; Pandya, K.; Jiang, S.; Debnath, A.K. Design, synthesis, and structure-activity relationship of a novel series of 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans as HIV-1 entry inhibitors J Med Chem 2009, 52(23): 7631
[40] Fan, G.; Li, Z.; Shen, S.; et al. Baculiferins A-O, O-sulfated pyrrole alkaloids with anti-HIV-1 activity, from the Chinese marine sponge Iotrochota baculifera Bioorg Med Chem 2010, 18(15): 5466
[41] Lin, W. et al WO 2010043155 Apr 22, 2010.
[42] Fan, G.; Li, Z.; Shen, S.; et al. Baculiferins A-O, O-sulfated pyrrole alkaloids with anti-HIV-1 activity, from the Chinese marine sponge Iotrochota baculifera Bioorg Med Chem 2010, 18(15): 5466
[43] Elena E. Paskaleva, Jing Xue, David Y-W. Lee, Alexander Shekhtman, Mario Canki. Palmitic Acid Analogs Exhibit Nanomolar Binding Affinity for the HIV-1 CD4 Receptor and Nanomolar Inhibition of gp120-to-CD4 Fusion. PLoS One. 2010; 5(8): e12168
[44] Katritzky AR, Tala SR, Lu H, Vakulenko AV, Chen QY, Sivapackiam J, Pandya K, Jiang S, Debnath AK. Design, synthesis, and structure-activity relationship of a novel series of 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans as HIV-1 entry inhibitors. J Med Chem. 2009 Dec 10;52(23):7631-9
[45] Katritzky AR, Tala SR, Lu H, Vakulenko AV, Chen QY, Sivapackiam J, Pandya K, Jiang S, Debnath AK. Design, synthesis, and structure-activity relationship of a novel series of 2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans as HIV-1 entry inhibitors. J Med Chem. 2009 Dec 10;52(23):7631-9
[46] Teixeira C, Serradji N, Maurel F, Barbault F. Docking and 3D-QSAR studies of BMS-806 analogs as HIV-1 gp120 entry inhibitors Eur J Med Chem. 2009 Sep;44(9):3524-32
[47] Lalonde JM, Elban MA, Courter JR, Sugawara A, Soeta T, Madani N, Princiotto AM, Kwon YD, Kwong PD, Schön A, Freire E, Sodroski J, Smith AB 3rd Design, synthesis and biological evaluation of small molecule inhibitors of CD4-gp120 binding based on virtual screening Bioorg Med Chem. 2011 Jan 1;19(1):91-101
[48] Xie H, Ng D, Savinov SN, Dey B, Kwong PD, Wyatt R, Smith AB 3rd, Hendrickson WA. Structure-activity relationships in the binding of chemically derivatized CD4 to gp120 from human immunodeficiency virus J Med Chem. 2007 Oct 4;50(20):4898-908
[49] Hyun-Su Lee, Mark Contarino, M. Umashankara, Arne Schön, Ernesto Freire, Amos B. Smith, III, Irwin M. Chaiken, and Lynn S. Penn Use of the quartz crystal microbalance to monitor ligand-induced conformational rearrangements in HIV-1 envelope protein gp120 Anal Bioanal Chem. 2010 February; 396(3): 1143–1152
[50] Kwong, P.D. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. (1998) Nature 393: 648-659
[51] Alan R. Katritzky et al, Design, Synthesis, and Structure-Activity Relationship of a Novel Series of 2-Aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl) furans as HIV-1 entry inhibitors, J Med Chem. 2009 December 10; 52(23): 7631–7639

[52] Stewart KD, Huth JR, Ng TI, McDaniel K, Hutchinson RN, Stoll VS, Mendoza RR, Matayoshi ED, Carrick R, Mo H, Severin J, Walter K, Richardson PL, Barrett LW, Meadows R, Anderson S, Kohlbrenner W, Maring C, Kempf DJ, Molla A, Olejniczak ET Non-peptide entry inhibitors of HIV-1 that target the gp41 coiled coil pocket Bioorg Med Chem Lett. 2010 Jan 15;20(2):612-7

[53] Alan R. Katritzky et al, Design, Synthesis, and Structure-Activity Relationship of a Novel Series of 2-Aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl) furans as HIV-1 entry inhibitors, J Med Chem. 2009 December 10; 52(23): 7631–7639

[54] Stewart KD, Huth JR, Ng TI, McDaniel K, Hutchinson RN, Stoll VS, Mendoza RR, Matayoshi ED, Carrick R, Mo H, Severin J, Walter K, Richardson PL, Barrett LW, Meadows R, Anderson S, Kohlbrenner W, Maring C, Kempf DJ, Molla A, Olejniczak ET Non-peptide entry inhibitors of HIV-1 that target the gp41 coiled coil pocket Bioorg Med Chem Lett. 2010 Jan 15;20(2):612-7

[55] Stewart KD, Huth JR, Ng TI, McDaniel K, Hutchinson RN, Stoll VS, Mendoza RR, Matayoshi ED, Carrick R, Mo H, Severin J, Walter K, Richardson PL, Barrett LW, Meadows R, Anderson S, Kohlbrenner W, Maring C, Kempf DJ, Molla A, Olejniczak ET Non-peptide entry inhibitors of HIV-1 that target the gp41 coiled coil pocket Bioorg Med Chem Lett. 2010 Jan 15;20(2):612-7