p24-Targeted Library

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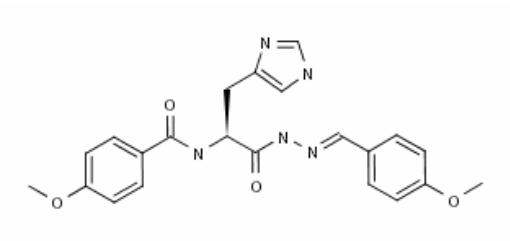
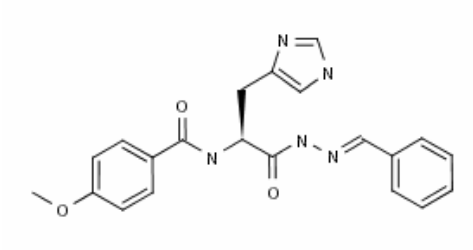
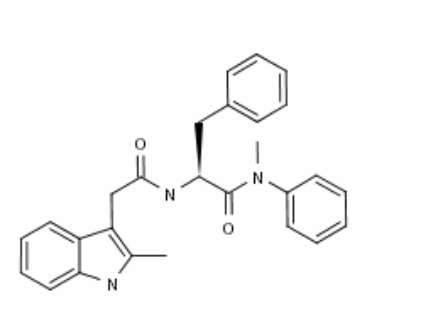
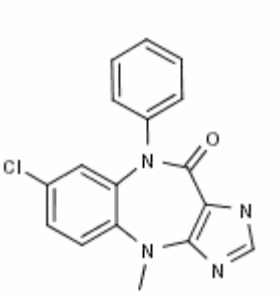
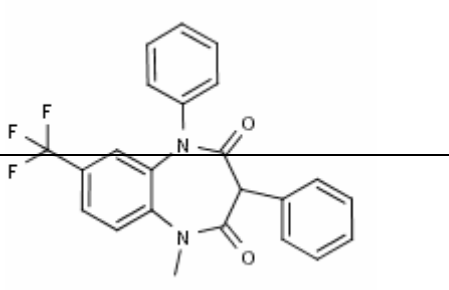
1. Small molecule p24 inhibitors

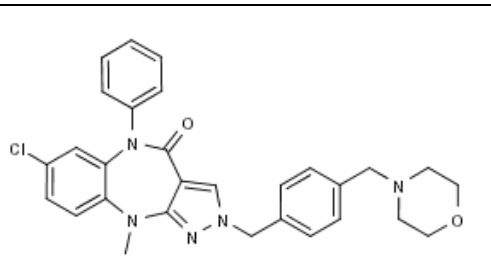
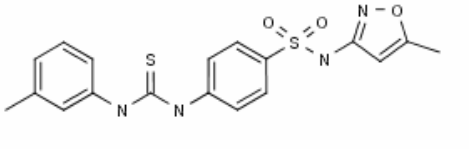
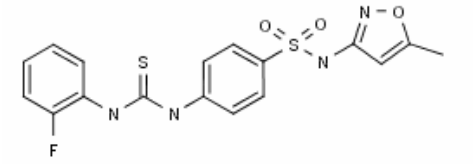
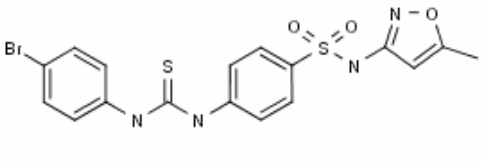
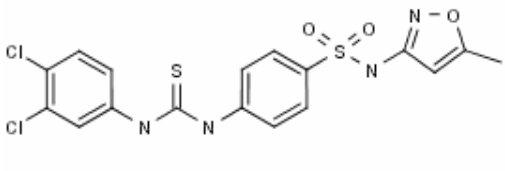
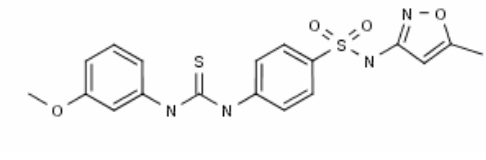
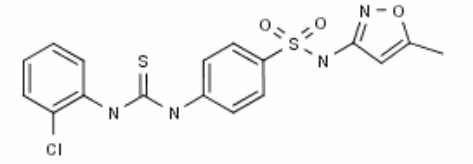
Most of the current drugs in use for the treatment of AIDS work by targeting the enzymatic activities of the human immunodeficiency virus (HIV) reverse transcriptase or protease, although entry and integrase inhibitors are starting to be used, and presently there is also promising development of other novel targets. However, because of the emergence of drug-resistant virus that commonly occurs as the result of treatment, there remains a great need to continue the search for alternative therapies that target other essential viral activities.

The human immunodeficiency virus type 1 (HIV-1) is the main cause of the acquired immunodeficiency syndrome (AIDS) [1]. Diagnosis of HIV infection, especially early diagnosis, and related drug- or immune-based therapy are essential part of AIDS prevention and control. Gag protein of HIV-1, a polyprotein of 55 kDa, is one of the most conserved viral proteins. The Gag protein is cleaved by a viral protease to release p17, p24 and p12 during viral maturation [2]. Among these players, capsid protein - p24 antigen of HIV-1 is the most abundant viral protein, since each virus contains about 1,500 to 3,000 p24 molecules [3]. Therefore, p24 has recently been suggested as a specific target for antiviral strategies [4]. During early and late stages of HIV infection, it is always present at relatively high levels in the blood, making it a potential viral marker for diagnosis, blood donor screening, monitoring disease progression, and evaluating antiretroviral therapy [5].

To the present day, several promising p24 inhibitors have been developed including peptide-based substances and small-molecule compounds (Table 1).

Table 1. Representative examples of small-molecule HIV capsid protein p24 assembly inhibitors currently evaluated in various biological trials

№	Structure/Phase/Name	Originator	Ref	Activity
1	 Biological Testing	Food and Drug Administration (FDA) (Originator) Peking University (PKU) (Originator)	[6]	CEM human acute lymphoblastoid T-cell leukemia cells IC50=0.41 μM
2	 Biological Testing			CEM human acute lymphoblastoid T-cell leukemia cells IC50=0.56 μM
3	 Biological Testing PF-3450074	Pfizer	[7]	-
4	 Biological Testing	Boehringer Ingelheim	[8] Replacement of the enamine functionality of the hit series with either an imidazole or a pyrazole ring led to compounds that inhibited both capsid assembly and reverse transcriptase	-
5				-

	Biological Testing			
6	 Biological Testing			-
7	 Biological Testing	Peking University (PKU)	[9]	-
8	 Biological Testing			-
9	 Biological Testing			-
10	 Biological Testing			-
11	 Biological Testing			-
12	 Biological Testing			-

For instance, cyclophilin A is a peptidyl-propyl isomerase that binds the capsid p24 protein of HIV-1 and facilitates replication. Therefore, Daelemans et al [10] have recently developed a novel cyclophilin inhibitor, a non-immunosuppressive cyclosporine analogue, Debio-025, that is about 15-times more potent than cyclosporine A and less toxic resulting in a selectivity index of more than 300. It was equally active against virus strains that were resistant toward inhibitors of the viral entry, fusion, or reverse transcription while it was not inhibitory to HIV-2 or SIV(MAC). Mechanism of action studies demonstrate that Debio-025 inhibits the HIV-1 replication by interfering with an early stage of the viral replication cycle. Indeed, addition of Debio-025 could be postponed for 2h before loosing its antiviral activity. The compound proved inactive against mutant viruses that are independent of cyclophilin A binding suggesting Debio-025 targets the cyclophilin A-capsid interaction.

Zhang and colleagues [11] reported that two new 7,8-secolignans isolated from the stems of Schisandra wilsoniana inhibited HIV-1(IIIB)-induced syncytia formation with an EC(50) value of 0.55 μg ml(-1). In addition, it was shown that these compounds reduced p24 antigen expression in acutely HIV-1(IIIB)-infected C8166 cells and primary isolate HIV-1(TC-2)-infected peripheral blood mononuclear cells (PBMCs), with EC(50) values of 3.34 and 0.52 μg ml(-1), respectively.

Hagiwara et al [12] have identified new anti-HIV-1 compounds using recombinant Vpr purified from transfected COS-7 cells. Vpr was used to screen compounds by chemical array to identify those that bound Vpr. From this screen, 108 compounds were selected as positive for Vpr binding. Among these, one structurally similar group of four compounds showed anti-HIV activity in macrophages. In particular, compound SIP-1 had high inhibition activity and reduced the levels of p24 by more than 98% in macrophages after 8 or 12 days of infection. SIP-1 had no cytotoxic effects and did not disrupt cell cycle progression or induce apoptosis of Molt-4 and HeLa cell lines as measured by MTT assay, flow-cytometry analysis, and a caspase-3 assay. In addition, SIP-1 specifically bound to Vpr as assessed by photo-cross-linked small-molecule affinity beads. These results suggest that Vpr is a good target for the development of compounds that could potentially inhibit HIV-1 replication.

Stevens and colleagues [13] described the mechanism of antiviral action of the N-aminoimidazole derivatives which exclusively inhibit retroviruses such as HIV-1, HIV-2, SIV and MSV. These antiretroviral compounds, with lead prototype NR-818, were found to inhibit HIV-1 replication at the transcriptional level. Analysis of each individual step of viral transcription, including transcriptional activation mediated by NF-kappaB, the chromatin remodeling process at the viral promoter and viral mRNA transcription mediated by RNAPII, showed that NR-818 was able to prolong the binding of NF-kappaB to its consensus sequence.

The compound also increased the acetylation of histones H3 and H4 within the nucleosome nuc-1 at the transcription initiation site and inhibited the recruitment of viral Tat and the phosphorylation of the RNA polymerase II C-terminal domain (RNAPII CTD) at the viral promoter upon stimulation of latently HIV-1-infected cell lines. As a result, viral mRNA expression and subsequent viral p24 production in stimulated latently HIV-1-infected cell lines was suppressed by NR-818. These data suggest that the N-aminoimidazole derivatives effectively inhibit the reactivation of HIV-1 and may contribute to the control of the latent HIV-1 reservoir.

In [14], key structural determinants of p24-antibody interaction were described. Thus, authors have studied a mouse monoclonal antibody, denoted 13B5, raised against p24, the capsid protein of HIV-1. Moreover, the same authors have previously described the first crystal structure of intact p24 as visualized in the Fab13B5-p24 complex. The structure of the uncomplexed Fab13B5 at 1.8 A° resolution and analysis of the Fab-p24 interface and the conformational changes occurring upon complex formation have also been described. This data can be used for further *in silico* searching for novel small molecule p24 inhibitors using 3D-docking approach or/and 3D-pharmacophore modeling.

2. Concept and Applications

p24-targeted library design at CDL involves:

1. Unique bioisosteric morphing;
2. Structure similarity and funneling procedures in designing novel potential p24 ligands with high IP value. We apply CDL's proprietary Chemosoft™ and SmartMining™ software as well as commercially available solutions from Accelrys (MolSoft™), MOE, Daylight and other platforms;
3. Neural Network tools in particular Self-organizing Kohonen maps performed in SmartMining Software for `*in silico*` ADME/Tox assessment of 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.

A general approach to limiting the space of virtual libraries of combinatorial reaction products consists of implementation of a series of special filtering procedures. The typical filtering stages are briefly summarized in Figure 1. A variety of "Rapid Elimination of Swill" (REOS) filters is used to eliminate compounds that do not meet certain criteria [15].

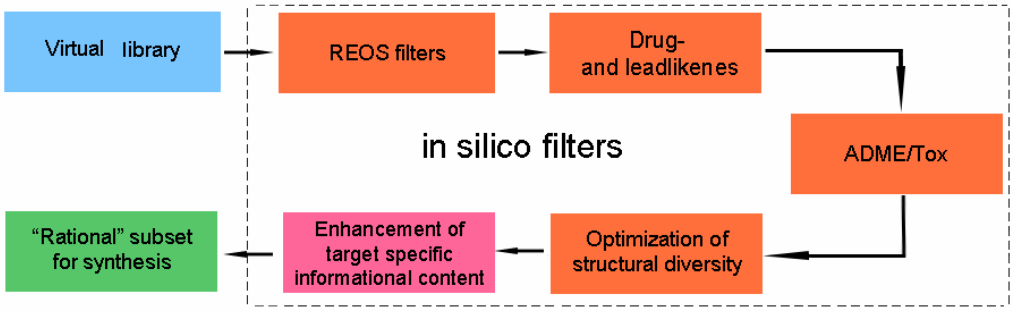


Fig. 1. General procedures of selection of a rational target-specific subset within an initial virtual combinatorial library.

At the first step of our p24-targeted library design we have designed a range of various bioisosteric analogues to known active compounds listed in table 1. These structures (see Fig. 2) were successfully generated using a unique isoster-module integrated in ChemoSoft software. At the output, more than 350 bioisosteric analogues have been suggested. These structures as well as known small molecule p24 inhibitors were included in the common dataset. The prepared database was used as a template for similarity analysis. The Tanimoto similarity measure has been applied to compounds form ChemDiv store (Table 2). Representative examples of compounds from the targeted database are shown in fig. 3 below.

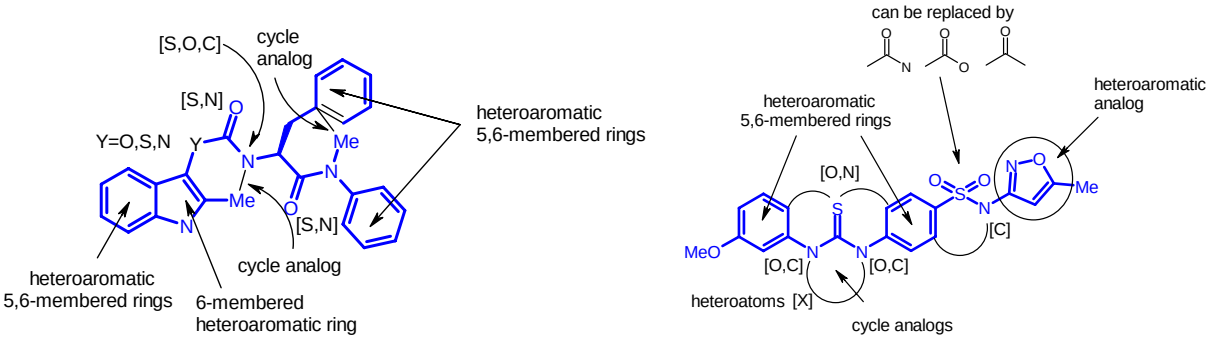
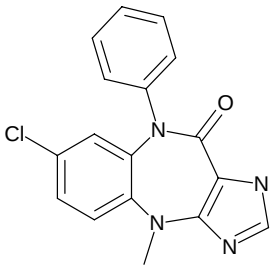
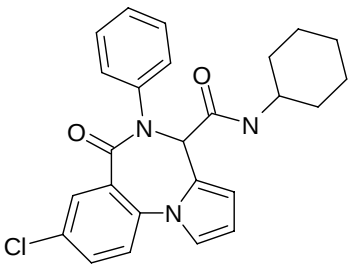
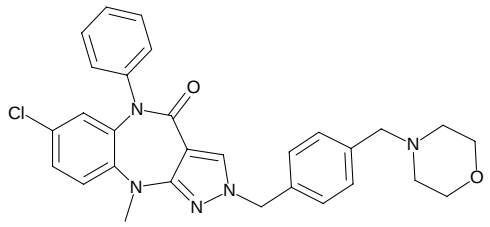
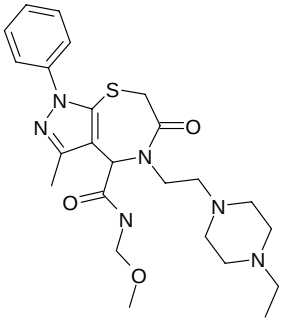
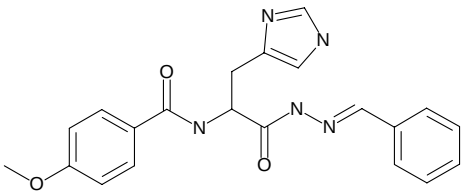
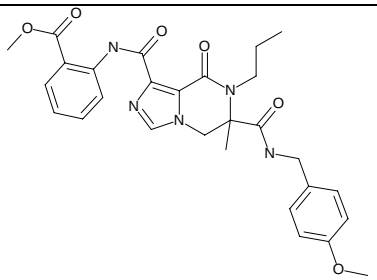
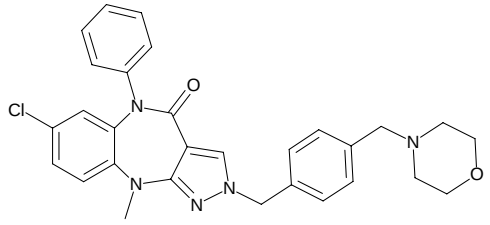
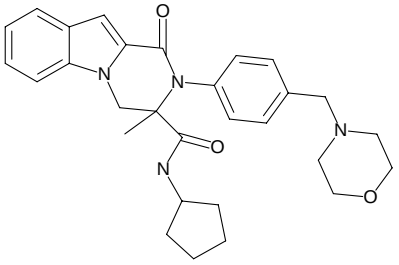
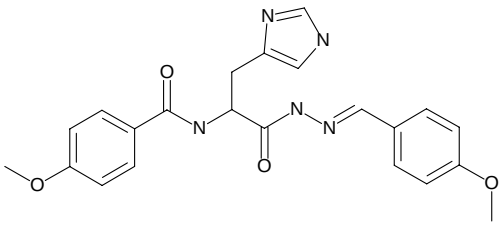
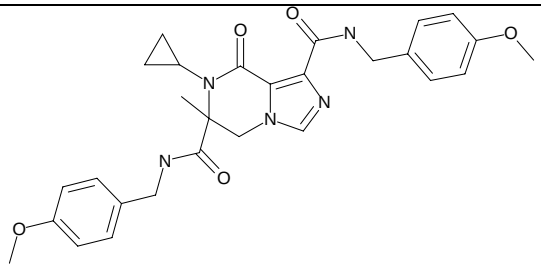


Fig. 2. Representative examples of bioisosteric rules applied for reference compounds.

Table 2. Several examples of ChemDiv compounds with high Tanimoto coefficients to known p24 inhibitors

Reference compound	Tanimoto coefficient	Compound from p24-targeted library
	0.7	

	0.6	
	0.3	
	0.3	
	0.3	

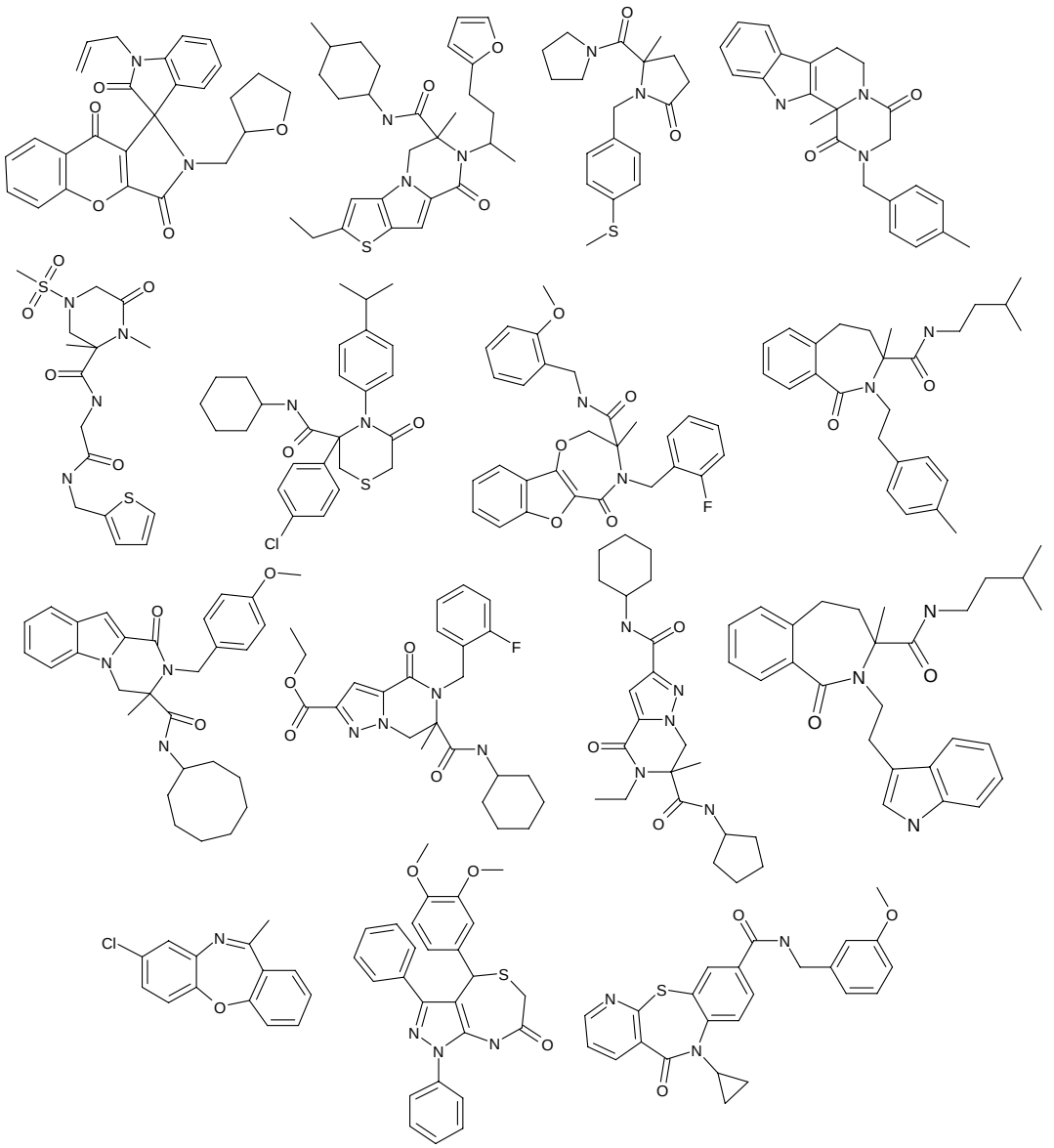


Fig. 3. Representative examples of compounds from p24-targeted library

The developed p24-focused library is updated quarterly based on a "cache" principle. Older scaffolds/compounds are replaced by templates resulting from our in-house development (unique chemistry, literature data, computational approaches) while the overall size of the library remains the same (ca. 15K compounds). As a result, the libraries are renewed each year, proprietary compounds comprising 50-75% of the entire set. Clients are invited to participate in the template selection process prior to launch of our synthetic effort.

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