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Discovery of Novel Non-Cyclam Polynitrogenated CXCR4 Coreceptor Inhibitors

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HIV cell fusion and entry have been validated as targets for therapeutic intervention against infection. Bicyclams were the first low-molecular-weight compounds to show specific interaction with CXCR4. The most potent bicyclam was AMD3100, in which the two cyclam moieties are tethered by a 1,4-phenylenebis(methylene) bridge. It was withdrawn from clinical trials owing to its lack of oral bioavailability and cardiotoxicity. We have designed a combinatorial library of non-cyclam polynitrogenated compounds by preserving the main features of AMD3100. At least two nitrogen atoms on each side of the p*-phenylene moiety, one in the benzylic position and the other(s) in the heterocyclic system were maintained, and the distances between them were similar to the nitrogen atom distances in cyclam. A selection of diverse compounds from this library were prepared, and their in vitro activity was tested in cell cultures against HIV strains. This led to the identification of novel potent CXCR4 coreceptor inhibitors without cytotoxicity at the tested concentrations.*

Introduction

Studies in human immunodeficiency virus (HIV) biology have provided deep knowledge of the molecular events that are involved in the HIV life cycle, which consist of several steps: viral entry,[1,2] reverse transcription,[3–9] integration,[3,10–16] gene expression,[17,18] gene assembly,[19] budding[20] and maturation.[21] There is a need for the development of new drugs that are capable of suppressing HIV strains that are resistant to the currently used reverse transcriptase inhibitors (RTI) or protease inhibitors (PI), and for new drugs that target different stages in the virus life cycle.

HIV cell fusion and entry have been validated as targets for therapeutic intervention against infection.[2] The virus needs a primary receptor (CD4) and a coreceptor, either the chemokine receptor CXCR4 or CCR5, to fuse with the cell. Thus, they became new therapeutic targets for the treatment or prevention of HIV infection.

There are two approved entry and fusion inhibitors: T-20 (Fuzeon or enfuvirtide, developed by Roche–Trimeris), a linear 36 amino acid synthetic peptide with an acetylated N terminus and a carboxamide C terminus that is composed of naturally occurring L-amino acid residues, and maraviroc (Selzentry),[22] a CCR5 inhibitor. The first nonpeptidic CCR5 antagonist was TAK-779[23] (Figure 1), from Takeda Chemicals, although it could not be developed as an anti-HIV-1 drug because of its variable activity and poor oral bioavailability. Later, SCH-D[24] (vicriviroc, Figure 1), was developed by Schering–Plough; it had improved antiviral potency and better pharmacological properties relative to its predecessor SCH-C,[25] and has continued to phase III clinical trials. GW873140 (aplaviroc),[26] a spiroketopiperazine-based agent from Ono Pharmaceutical/GlaxoSmithKline, exhibited potent antiviral activity but has been discontinued for clinical development as an anti-HIV agent. Another class of anti-HIV agents that targets CCR5 includes PRO 140[27] (Progenics Pharmaceuticals), a humanized monoclonal antibody that is designed to block the ability of HIV to enter and infect cells; this antibody is in phase Ib clinical studies. In addition, CCR5 antagonists and monoclonal antibodies have shown potent synergistic antiviral effects by co-binding the receptor.[28]

Bicyclams were the first low-molecular-weight compounds with a specific interaction with CXCR4.[29–32] The most potent bicyclam was AMD3100 (Figure 1) in which the two cyclam moieties are tethered by a 1,4-phenylenebis(methylene) bridge. It has an IC_{50} of 1–10 ng mL^{-1}, which is at least 100 000-fold lower than the cytotoxic concentration. Samples of virus that were recovered from patients whom had been treated with AMD3100 (bicyclam) showed a change in virus phenotype, from X4 to R5; this suggests that AMD3100 blocked selectively those viruses that use CXCR4, although it was not effective in inhibiting CCR5-dependent replication of HIV in vivo. However, AMD3100 has shown poor oral absorption and toxicity, which is related to its high positive charge at physiological

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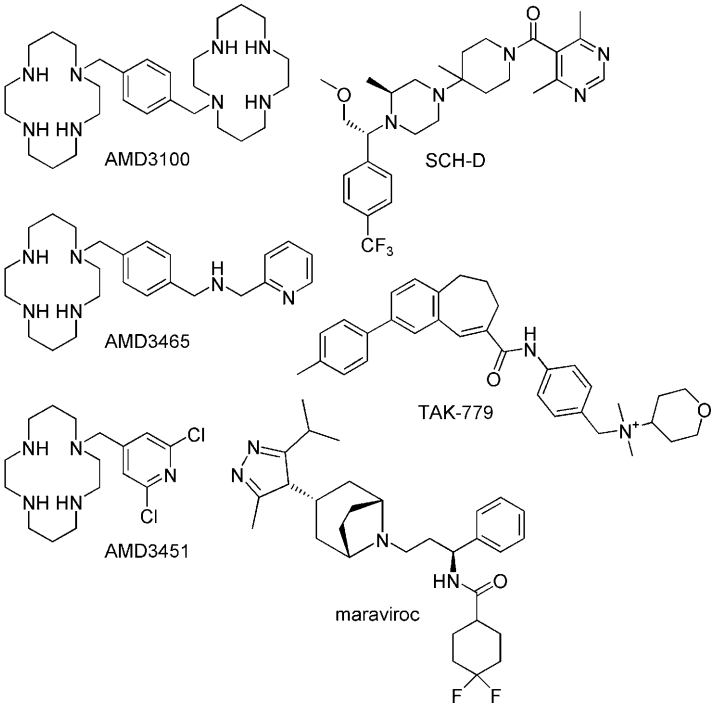


Figure 1. Structures of the main CXCR4 and CCR5 antagonist leads; they have aromatic or aliphatic linkers in polynitrogenated systems.

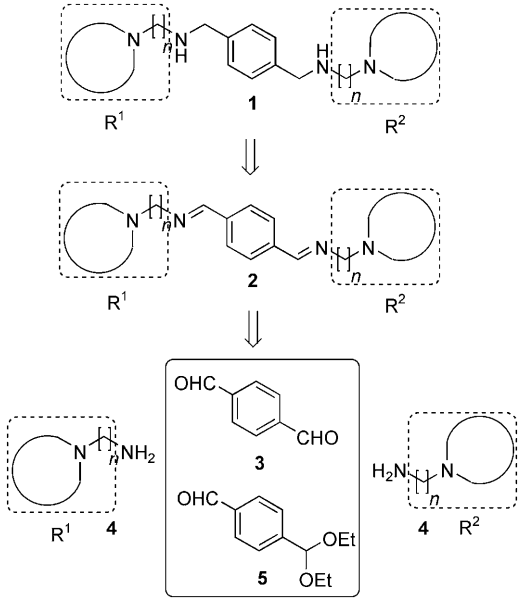


Figure 2. Retrosynthetic analysis for target diamines **1** ($n \geq 1$) and dihydrazones **2** ($n = 0$).

pH, therefore new analogous compounds should improve these characteristics.[33]

CXCR4 and CCR5 antagonist leads, such as AMD3100 (bicyclam), SCH-D or TAK-779 contain aromatic or aliphatic linkers in polynitrogenated systems. Among all of the compounds under study, bicyclams in general, and particularly AMD3100, appear to be the most active. *p*-Phenylenic compounds with a single cyclam moiety have also been developed, such as AMD3465 (Figure 1),[33] which is a CXCR4 antagonist, and AMD3451,[34] which shows antagonist activity against both CXCR4 and CCR5 coreceptors in cell culture studies. This led us to consider the possibility of obtaining symmetrical and nonsymmetrical systems that contain a *p*-phenylenic spacer and nitrogenated cyclic subunits in the search for new compounds that are potentially active against HIV-1. Herein we present the results of these studies.

Library design and compound selection

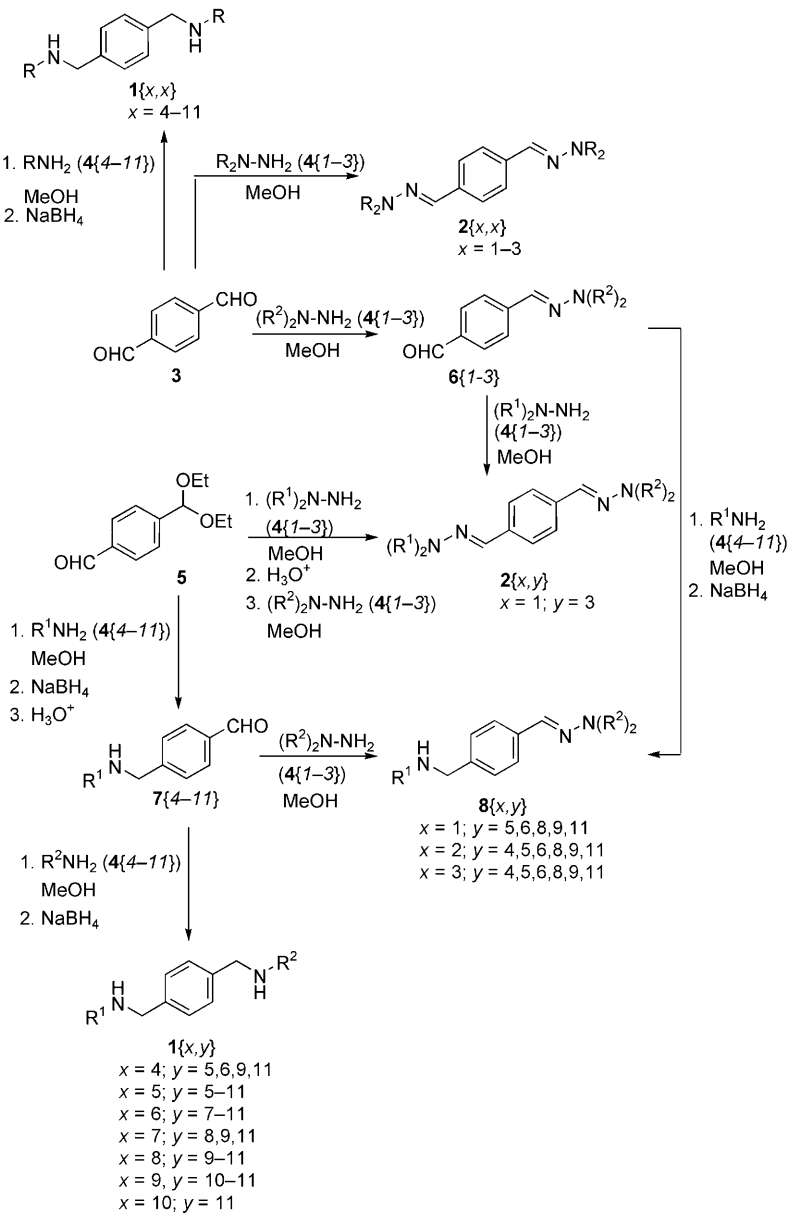
We designed a combinatorial library[35] by preserving the main features of AMD3100: a) at least two nitrogen atoms on each side of the *p*-phenylene moiety, one in the benzylic position and the other(s) in a heterocyclic system and b) similar distances between these nitrogen atoms as those that are present in cyclam. Such considerations led us to diamines **1** as target compounds (Figure 2). Very recently a similar but less restrictive approach was used by Liotta and colleagues[36] to propose a family of compounds whose general structure, $R^3R^4NCH_2C_6H_4CH_2NR^1R^2$, led to a large scaffold diversity. The most active compound in their library blocks in vitro CXCR4/SDF-1-mediated signaling more effectively than AMD3100, but they found it to be weakly active against HIV propagation in cell culture tests.

A retrosynthetic analysis for target compounds **1** in which $R^1 = R^2$ and $n \geq 1$ led to the symmetrical diimines **2** as precursors, which can be further disconnected to terephthalaldehyde (**3**) and two equivalents of the corresponding amine **4** ($n \geq 1$). When $R^1 = R^2$ and $n = 0$, compounds **2** are in fact symmetrical hydrazones, which can be obtained by condensation of terephthalaldehyde and the corresponding hydrazine **4** ($n = 0$). These dihydrazones were also included in our library.

To obtain nonsymmetrical ($R^1 \neq R^2$) diamines **1** ($n \geq 1$) and dihydrazones **2** ($n = 0$), it was necessary to slightly modify our synthetic approach by using 4-(diethoxymethyl)benzaldehyde (**5**) as the core precursor (Scheme 1). Thus, intermediate hydrazono and aminobenzaldehydes **6** and **7** allowed us to include such nonsymmetrical compounds and nonsymmetrical aminohydrazones **8** as target compounds.

The first selection of nitrogenated building blocks was based on commercial availability; the use of synthetic building blocks is currently under development. A search for available building blocks resulted in 18 commercially available nitrogenated building blocks that consist of a nitrogen-containing heterocyclic system (piperidine, piperazine, morpholine, pyrrolidine, imidazole and triazole), a polymethylene spacer, and a terminal amine group (Figure 3). Consequently, the virtual combinatorial library was built by using three hydrazines **4**{*1–3*} and eight amines **4**{*4–11*} as building blocks for substituents R^1 and R^2 of diamines **1**, dihydrazones **2** and aminohydrazones **8**, and it was subsequently enumerated with Cerius2.[37]

In an attempt to explore the chemical space that is covered by the library, we initially decided to select a reduced set of 19 compounds by using PRALINS[38] (*Program for Rational Analysis of Libraries in silico*) and by applying a diversity criteria, which decreases the number of compounds to be synthesized and



Scheme 1. Synthesis of symmetrical and nonsymmetrical diamines **1**, dihydrazones **2**, and aminohydrazones **8**.

strategies that were used to obtain diamines **1**, dihydrazones **2** and aminohydrazones **8** are depicted in Scheme 1. For the synthesis of symmetrical diamines **1** ($R^1 = R^2$), we used a stepwise reductive amination:[41] a) reflux of a mixture of **3** and the corresponding amine **4**{*4–11*} (1:2 molar ratio) in anhydrous methanol by using molecular sieves as a dehydrating agent, and b) subsequent reduction with $NaBH_4$.[35]

Symmetrical dihydrazones **2** ($R^1 = R^2$) were obtained by condensation of **3** with the corresponding hydrazines **4**{*1–3*} in methanol (Scheme 1). Nonsymmetrical ($R^1 \neq R^2$) diamines **1**, dihydrazones **2**, and aminohydrazones **8** needed more complex approaches. Thus, hydrazonobenzaldehydes **6** were synthesized by coupling terephthalaldehyde (**3**) with hydrazines **4**{*1–3*} in a 2:1 molar ratio, followed by chromatographic separation from unreacted **3** and the symmetrical dihydrazone **2** byproduct. The subsequent coupling with a second hydrazine **4**{*1–3*} would afford the nonsymmetrical dihydrazones **2** ($R^1 \neq R^2$) (Scheme 1). This procedure has been used to obtain dihydrazone **2**{*1,3*}.

On the other hand, 4-(diethoxymethyl)benzaldehyde (**5**) was selected as building block to obtain aminobenzaldehydes **7** by reacting equimolar amounts of **5** and the corresponding amine **4**{*4–11*}, followed by reduction of the intermediate iminoacetal and subsequent acetal cleavage with a dilute solution of aqueous hydrochloric acid.[42–44] Treatment of these aminobenzaldehydes **7** with a second amine **4**{*4–11*} in anhydrous MeOH, by using molecular sieves as dehydrating agent, followed by reduction with $NaBH_4$, yielded the nonsymmetrical diamines **1** ($R^1 \neq R^2$) (Scheme 1). Finally, aminohydrazones **8** were accessible either by reductive amination of hydrazonobenzaldehydes **6** with the appropriate amine **4**{*4–11*} or by coupling of the aminobenzaldehydes **7** with the corresponding hydrazine **4**{*1–3*} (Scheme 1).

evaluated without decreasing the chance of hit/lead finding. Thus, a series of molecular 2D (physicochemical, topological and topological based on information theory) and 3D (potential energy, surface, shape and volume) descriptors (computed with MOE[39]) were used for the definition of the chemical space. A subsequent principal component analysis decreased the initial set of descriptors to five components (explaining 90% of the variance) which were used as input for the diversity selection with PRALINS; this resulted in the selection of 19 compounds (Table 1).

Chemistry

Combinatorial approaches have been widely used in the identification of novel anti-HIV drugs;[40] in our case, the synthetic

Results and Discussion

The first subset of compounds synthesized and tested (anti-HIV activity and cytotoxicity) included eight compounds: two dihydrazones (**2**{*1,1*} and **2**{*2,2*}) and six diamines (**1**{*3,4*}, **1**{*5,5*}, **1**{*6,6*}, **1**{*6,11*}, **1**{*9,9*} and **1**{*11,11*}) chosen among the 19 compounds result of the diversity selection. Three of them (**1**{*5,5*}, **1**{*9,9*} and **1**{*6,11*}) showed very promising anti-HIV activities, EC_{50} in the range 0.9–18 μg mL^{-1} (Table 1), so we decided to include structural modifications of them together with the remaining initial 19 candidates.

Thus, the second subset of compounds synthesized and tested was formed by seventeen compounds: one dihydrazone (**2**{*1,3*}), ten diamines (**1**{*6,7*}, **1**{*5,8*}, **1**{*5,10*}, **1**{*5,11*}, **1**{*4,5*}, **1**{*5,6*}, **1**{*5,7*}, **1**{*5,9*}, **1**{*9,10*} and **1**{*9,11*}) and six amino hydrazones (**8**{*3,5*}, **8**{*3,6*}, **8**{*3,8*}, **8**{*3,9*}, **8**{*1,5*} and **8**{*2,5*}). Twelve of these

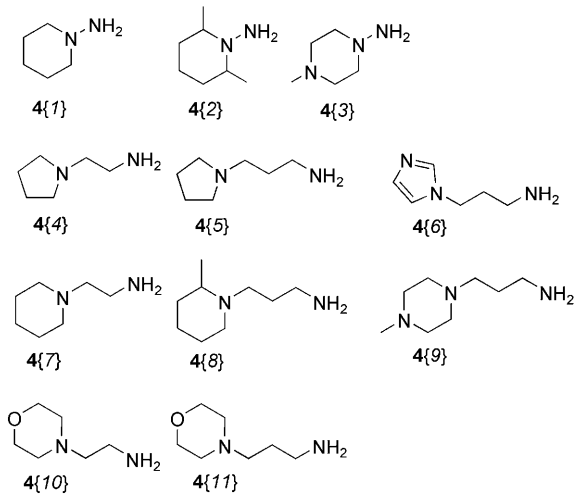


Figure 3. Amine building blocks used in the construction of the virtual combinatorial library.

compounds presented EC_{50} in the range 0.2 to 2.7 μg mL^{-1}, the most potent being **1**{*5,6*} and **1**{*5,8*} (0.2 μg mL^{-1}) (Table 1).

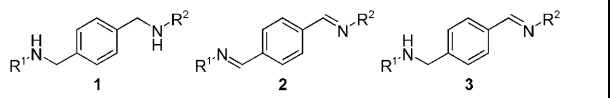
The third and final subset, which included 28 compounds, thus covering the total of 53 compounds synthesized and tested (Table 1), was selected by using computational analysis tools such as quantitative structure–activity relationships (QSAR) techniques and ligand- and structure-based drug design (for CXCR4 and CCR5 modeled HIV-1 entry coreceptors).[45,46] Notable compounds of this subset are **1**{*6,8*} (EC_{50} = 0.03 μg mL^{-1}), **1**{*8,9*} (EC_{50} = 0.03 μg mL^{-1}), and the most active compound in the library, **1**{*8,8*}, which has an EC_{50} value of 0.008 μg mL^{-1} and a CC_{50} > 25 μg mL^{-1}.

Among the different polynitrogenated building blocks, RNH_2, amines **4**{*5*} and **4**{*8*} gave the most active compounds. EC_{50} results suggest that higher activity values could be obtained by using a propylenic spacer between the heterocyclic ring and the nitrogen that supports the *p*-phenylenic moiety, and for heterocyclic systems that contain one nitrogen atom.

To evaluate the results, we determined the EC_{50} and CC_{50} of AMD3100 (EC_{50} = 0.001 μg mL^{-1}; CC_{50} > 5 μg mL^{-1}) and DS (dextran sulfate) (EC_{50} = 0.011 μg mL^{-1}; CC_{50} > 125 μg mL^{-1}) by following the same methodology as for our compounds. As can be seen, compound **1**{*8,8*} presents nearly the same level of activity as the reference compounds, **1**{*8,8*} (EC_{50} = 0.019 μM) and AMD3100 (EC_{50} = 0.002 μM), and shows no cell toxicity at the tested concentrations of up to 25 μg mL^{-1}.

Computational blind docking and ligand binding within the CXCR4 site-directed mutagenesis (SDM)-defined binding pocket[47] were analyzed in detail by using AutoDock[48,49] to study the interactions between **1**{*8,8*} and the CXCR4 coreceptor. To perform these calculations, CXCR4 was first homology modeled with MODELLER[50] and CONGEN[51] by using bovine rhodopsin as a template[52] as described in Pérez-Nueno et al.[53] The compound **1**{*8,8*} structure was built, assigned Gasteiger partial charges,[54] and minimized in MOE with the MMFF94 force field. For the AutoDock blind docking experiment, a 181×181×181 grid with a grid spacing of 0.375 Å was

Table 1. EC_{50} and CC_{50} values of diamines **1**, dihydrazones **2**, and aminohydrazones **8**.



Subset[a]	Compound	R^1NH_2	R^2NH_2	EC_{50} [μg mL^{-1}][b]	CC_{50} [μg mL^{-1}][c]
1	**2**{*1,1*}	**4**{*1*}	**4**{*1*}	> 125	> 125
2	**2**{*1,3*}	**4**{*1*}	**4**{*3*}	> 25	> 25
2	**8**{*1,5*}	**4**{*1*}	**4**{*5*}	2.7	10.1
3	**8**{*1,6*}	**4**{*1*}	**4**{*6*}	> 25	> 25
3	**8**{*1,8*}	**4**{*1*}	**4**{*8*}	> 4.1	4.1
3	**8**{*1,9*}	**4**{*1*}	**4**{*9*}	> 9.8	9.8
3	**8**{*1,11*}	**4**{*1*}	**4**{*11*}	10.6	> 25
1	**2**{*2,2*}	**4**{*2*}	**4**{*2*}	> 125	> 125
3	**8**{*2,4*}	**4**{*2*}	**4**{*4*}	14.7	> 25
2	**8**{*2,5*}	**4**{*2*}	**4**{*5*}	2.0	9.8
3	**8**{*2,6*}	**4**{*2*}	**4**{*6*}	> 25	> 25
3	**8**{*2,8*}	**4**{*2*}	**4**{*8*}	0.6	14.6
3	**8**{*2,9*}	**4**{*2*}	**4**{*9*}	3.8	19.2
3	**8**{*2,11*}	**4**{*2*}	**4**{*11*}	15.7	> 25
3	**2**{*3,3*}	**4**{*3*}	**4**{*3*}	> 125	> 125
1	**8**{*3,4*}	**4**{*3*}	**4**{*4*}	> 84.9	84.9
2	**8**{*3,5*}	**4**{*3*}	**4**{*5*}	1.8	14.3
2	**8**{*3,6*}	**4**{*3*}	**4**{*6*}	11.2	> 25
2	**8**{*3,8*}	**4**{*3*}	**4**{*8*}	1.4	10.3
2	**8**{*3,9*}	**4**{*3*}	**4**{*9*}	11.7	> 25
3	**8**{*3,11*}	**4**{*3*}	**4**{*11*}	8.1	> 25
3	**1**{*4,4*}	**4**{*4*}	**4**{*4*}	10.2	> 25
2	**1**{*4,5*}	**4**{*4*}	**4**{*5*}	1.7	> 25
3	**1**{*4,6*}	**4**{*4*}	**4**{*6*}	4.8	> 25
3	**1**{*4,9*}	**4**{*4*}	**4**{*9*}	8.2	> 25
3	**1**{*4,11*}	**4**{*4*}	**4**{*11*}	> 25	> 25
1	**1**{*5,5*}	**4**{*5*}	**4**{*5*}	0.9	32.4
2	**1**{*5,6*}	**4**{*5*}	**4**{*6*}	0.2	> 25
2	**1**{*5,7*}	**4**{*5*}	**4**{*7*}	1.7	> 25
2	**1**{*5,8*}	**4**{*5*}	**4**{*8*}	0.2	> 25
2	**1**{*5,9*}	**4**{*5*}	**4**{*9*}	0.5	> 25
2	**1**{*5,10*}	**4**{*5*}	**4**{*10*}	2.4	> 25
2	**1**{*5,11*}	**4**{*5*}	**4**{*11*}	1.6	> 25
1	**1**{*6,6*}	**4**{*6*}	**4**{*6*}	> 59.5	59.5
2	**1**{*6,7*}	**4**{*6*}	**4**{*7*}	2.0	> 25
3	**1**{*6,8*}	**4**{*6*}	**4**{*8*}	0.03	> 25
3	**1**{*6,9*}	**4**{*6*}	**4**{*9*}	> 25	> 25
3	**1**{*6,10*}	**4**{*6*}	**4**{*10*}	> 25	> 25
1	**1**{*6,11*}	**4**{*6*}	**4**{*11*}	18.4	> 125
3	**1**{*7,7*}	**4**{*7*}	**4**{*7*}	> 11.7	11.7
3	**1**{*7,8*}	**4**{*7*}	**4**{*8*}	0.5	> 25
3	**1**{*7,9*}	**4**{*7*}	**4**{*9*}	2.5	> 25
3	**1**{*7,11*}	**4**{*7*}	**4**{*11*}	2.7	> 25
3	**1**{*8,8*}	**4**{*8*}	**4**{*8*}	0.008	> 25
3	**1**{*8,9*}	**4**{*8*}	**4**{*9*}	0.03	> 25
3	**1**{*8,10*}	**4**{*8*}	**4**{*10*}	0.4	> 25
3	**1**{*8,11*}	**4**{*8*}	**4**{*11*}	0.5	> 25
1	**1**{*9,9*}	**4**{*9*}	**4**{*9*}	9.5	> 125
2	**1**{*9,10*}	**4**{*9*}	**4**{*10*}	> 25	> 25
2	**1**{*9,11*}	**4**{*9*}	**4**{*11*}	9.1	> 25
3	**1**{*10,10*}	**4**{*10*}	**4**{*10*}	> 85.7	85.7
3	**1**{*10,11*}	**4**{*10*}	**4**{*11*}	> 25	> 25
1	**1**{*11,11*}	**4**{*11*}	**4**{*11*}	> 125	> 125

[a] Subsets of synthesized and tested compounds, in bold for the diversity selection with PRALINS. [b] Effective concentration 50 or the concentration required to inhibit HIV-induced cell death by 50% as evaluated with the MTT method in MT-4 cells. [c] Cytotoxic concentration 50 or the concentration required to induce 50% death of non-infected MT-4 cells as evaluated with the MTT method. Reference compounds: AMD3100: EC_{50} = 0.001 μg mL^{-1}, CC_{50} = > 5 μg mL^{-1}; DS: EC_{50} = 0.011 μg mL^{-1}, CC_{50} = > 125 μg mL^{-1}.

used, and was centered on the SDM-defined ligand-binding site. This grid enclosed the whole protein structure, and the ligand was initially placed far from the protein to include the possibility of finding other binding sites. A smaller (61×61×61) grid was used for the subsequent binding mode analysis calculations. In each case, 100 independent Lamarckian genetic algorithm (LGA) runs were performed and pseudo-Solis and Wets minimization methods were applied by using default parameters. Each docking run was repeated five times. Results from docking analyses were assessed by using the knowledge of the SDM data. They showed two main electrostatic interactions between two positively charged nitrogen atoms in compound **1**{*8,8*} and negatively charged Asp262 and Glu288 residues of the CXCR4 coreceptor (Figure 4). The lowest distances

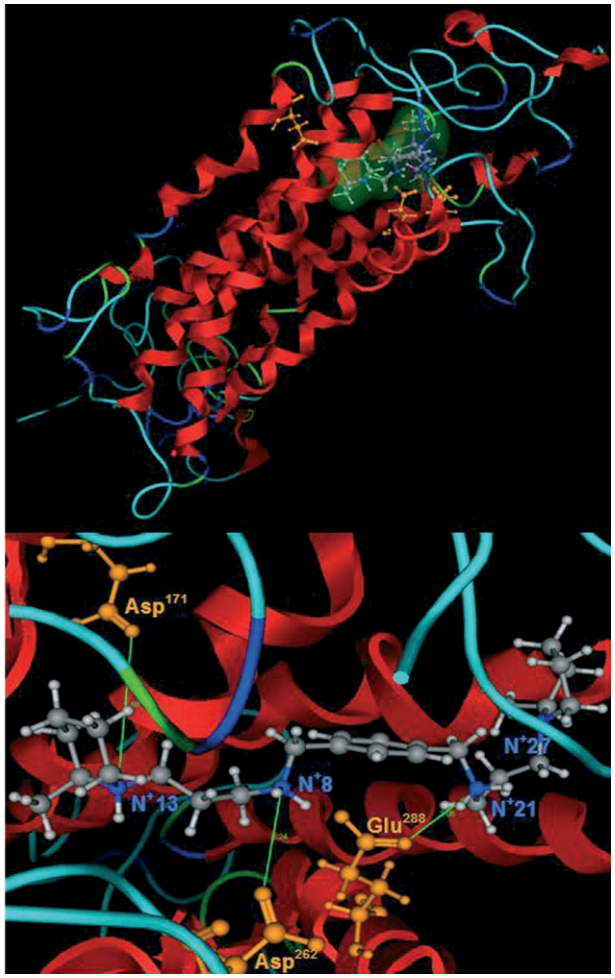


Figure 4. Predicted binding conformation between **1**{*8,8*} and CXCR4 from blind docking analysis. a) Compound **1**{*8,8*} docked within the CXCR4 pocket; b) detailed view of the calculated binding conformation.

from the carboxylic oxygen atoms of the three key binding residues Asp171, Asp262, and Glu288, to the four nitrogen atoms (N27, N21, N8, N13) of **1**{*8,8*} are shown in green: 6.88 Å between N^+13 and O(sp2) Asp171, 5.24 Å between N^+8 and O(sp2) Asp262, and 3.06 Å between N^+21 and O(sp2) Glu288.

Our docking results for **1**{*8,8*} agree with those of Gerlach et al.[55] on the mutagenic substitution of 16 CXCR4 amino acids, in which the three acidic residues, Asp171, Asp262, and Glu288, were identified as the main electrostatic interaction points for positively charged AMD3100 bicyclam rings binding. Moreover, the same study was performed with the AMD3100 ligand as is described in Pérez-Nueno et al.[53] Results with this known active ligand, by using only the same docking protocol as mentioned above, also agree with the **1**{*8,8*} docking results. It is worth mentioning that by using molecular dynamics (MD) it is possible to refine the CXCR4 docking poses to obtain ligand conformations that are closer to the key SDM residues.[56–58] For example, applying 200 ps of AMBER MD to our docking poses by using a protocol as described by Orozco and co-workers[59] gives ligand conformations with an average distance of 2 Å closer to the key binding residues. However, in this work we were more interested in the possibility of predicting a binding site and a binding mode of our more active synthesized molecules by using a docking tool only.

Finally, we carried out time-of-drug-addition experiments to identify the time and site of interaction of our anti-HIV compounds. It is known that the time delay before the addition of a drug is an estimate of its mode of action. Consequently, we determined the time of drug addition for the four most active compounds **1**{*8,8*}, **1**{*6,8*}, **1**{*8,9*} and **1**{*8,10*} compared with a CXCR4 antagonist (AMD3100), a reverse transcriptase inhibitor (AZT; azidothymidine), a fusion inhibitor (C34) and an adhesion inhibitor (DS; dextran sulfate) to confirm the initial hypothesis that the designed compounds act as CXCR4 inhibitors. The results that were obtained (Figure 5) clearly show that these compounds share a time/site of interaction that is similar to that of AMD3100 and act as blockers of the CXCR4 coreceptor. Furthermore, compounds **1**{*8,8*}, **1**{*6,8*}, **1**{*8,9*} completely blocked the binding of the 12G5 monoclonal antibody that targets CXCR4 at 25 μg mL^{-1} as measured by flow cytometry analysis in CXCR4+ cells, but failed to block the binding of antibodies that target CD4, CCR5 or CD45 (data not shown); this

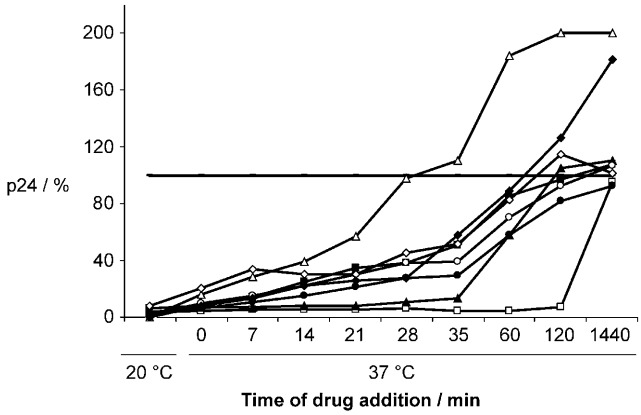


Figure 5. Time of drug addition in MT-4 cells of **1**{*8,8*} (◇), **1**{*6,8*} (●), **1**{*8,9*} (○), and **1**{*8,10*} (◆) in comparison with AZT (□), AMD3100 (■), DS (△), and C34 (▲); control: (——). Virus production was measured by p24 antigen determination in the cell supernatant 30 h post-infection, and is expressed as percentage of control values.

suggests that this class of compounds is very selective for the CXCR4 receptor.

Conclusions

We have designed a combinatorial library of non-cyclam AMD3100 analogues that preserves the main features of AMD3100: a) at least two nitrogen atoms on each side of the *p*-phenylene moiety, one in the benzylic position and the other(s) in a heterocyclic system, and b) similar distances between these nitrogen atoms as those present in cyclam. A diversity-oriented selection has allowed the synthesis of diamines **1**, dihydrazones **2** and aminohydrazones **8**; these compounds cover a broad range of activity values and are useful for calculating QSAR models. This approach led to the synthesis of compounds **1**{*6,8*}, **1**{*8,9*} and **1**{*8,8*}, which show anti-HIV activity values below 0.03 μg mL^{-1} but have displayed no cytotoxic effects at the tested concentrations. Studies on the mode of action of these compounds showed that they inhibited the CXCR4 coreceptor, thus validating the initial target compound design. A combinatorial optimization of the anti-HIV activity of this new family of compounds by using noncommercial amines of general structure **4** is currently on the way.

Experimental Section

Chemistry

IR spectra were recorded in a Nicolet Magna 560 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in a Varian Gemini 300 spectrometer that was operating at a field strength of 300 and 75.5 MHz, respectively. Chemical shifts were reported in parts per million (δ) and coupling constants (J) were in Hz by using, in the case of ^{1}H NMR spectroscopy, TMS as an internal standard, and in the case of ^{13}C NMR spectroscopy the solvent at 77.0 ppm ($CDCl_3$) as an internal reference. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad signal. MS data (*m/z* (%), EI, 70 eV) were obtained by using a Hewlett–Packard HP5988A spectrometer, and HRMS data were obtained by using a Micromass Autospec instrument. Elemental microanalyses were obtained on a Carlo–Erba CHNS-O/EA 1108 analyzer. Thin-layer chromatography (TLC) was performed on precoated sheets of silica 60 Polygram SIL N-HR/UV_{254} (Macherey–Nagel, art. 804023). Flash chromatography was performed with silica gel 35–70 μm (SDS, art. 2000027).

***N*-(4-((2-(Pyrrolidin-1-yl)ethylamino)methyl)benzyl)-2-(pyrrolidin-1-yl)ethanamine (1{*4,4*})**. Terephthalaldehyde (**3**) (0.61 g, 4.5 mmol), 1-(2-aminoethyl)pyrrolidine **4**{*4*} (1.04 g, 9.0 mmol) and molecular sieves (4 Å) were mixed in anhydrous MeOH (30 mL) and held at reflux under a N_2 atmosphere for 24 h. The molecular sieves were filtered, and the intermediate imine in MeOH was cooled to 0 °C and treated with solid $NaBH_4$ (0.34 g, 9.0 mmol). The mixture was stirred at RT overnight. Then H_2O was added and the product was extracted with CH_2Cl_2 The organic layers were combined, washed with brine, dried over $MgSO_4$ and the solvent was removed to give **1**{*4,4*} as a yellow oil (1.32 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.27 (s, 4H; Ph), 3.79 (s, 4H; C*H*$_2$Ph), 2.73 (t, $^3J_{H,H}$ = 6.0 Hz, 4H; C*H*$_2$NH), 2.59 (t, $^3J_{H,H}$ = 6.0 Hz, 4H; CH_2N), 2.47 (m, 8H; CH_2N), 2.20 (br s, 2H; NH), 1.75 ppm (quint, $^{33}J_{H,H}$ = 3.3 Hz, 8H; CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 138.8 (Cq), 128.0 (CH), 55.9 (CH_2), 54.2 (CH_2), 53.8 (CH_2), 47.8 (CH_2), 23.5 ppm (CH_2); IR (film): $\tilde{\nu}$ = 3310 (NH), 2962, 2928, 2874, 2794 (CH), 1485, 1444 cm^{-1} (CH); MS (FAB): *m/z* (%): 331.3 (100) $[M+H]^+$, 330.3 (18) $[M]^+$, 329.3 (77) $[M-H]^+$; HRMS calcd for $C_{20}H_{35}N_4$: 331.2862 $[M+H]^+$, found: 331.2867.

***N*-(4-((3-(2-Methylpiperidin-1-yl)propylamino)methyl)benzyl)-3-(2-methylpiperidin-1-yl)propan-1-amine (1{*8,8*})**. The procedure was the same as that stated above for **1**{*4,4*}, but was carried out by using terephthalaldehyde (**3**) (0.43 g, 3.2 mmol), 1-(3-aminopropyl)-2-methyl-piperidine **4**{*8*} (1.04 g, 6.4 mmol) and $NaBH_4$ (0.25 g, 6.4 mmol) to give **1**{*8,8*} as a pale-brown oil (1.33 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.27 (s, 4H; Ph), 3.77 (s, 4H; C*H*$_2$Ph), 2.87 (m, 2H; CH_{eq}N), 2.73 (m, 2H; CH_{eq}), 2.63 (t, $^3J_{H,H}$ = 6.9 Hz, 4H; C*H*$_2$NH), 2.36 (m, 2H; CH_{ax}N), 2.26 (m, 2H; C*H*CH_3), 2.14 (br s, 2H; NH), 2.11 (m, 2H; CH_{ax}N),1.68 (quint, $^3J_{H,H}$ = 6.9 Hz, 4H; CH_2), 1.61 (m, 2H; CH_{eq}), 1.58 (m, 2H; CH_{eq}), 1.52 (m, 2H; CH_{eq}), 1.44 (m, 2H; CH_{ax}), 1.28 (m, 4H; CH_{ax}), 1.05 ppm (d, $^3J_{H,H}$ = 6.3 Hz, 6H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 138.8 (Cq), 128.0 (CH), 55.9 (CH), 53.7 (CH_2), 52.3 (CH_2), 52.1 (CH_2), 48.3 (CH_2), 34.7 (CH_2), 26.2 (CH_2), 25.7 (CH_2), 24.0 (CH_2), 19.1 ppm (CH_3); IR (film): $\tilde{\nu}$ = 3282 (NH), 2929, 2854, 2793 (CH), 1449, 1372 cm^{-1} (CH); MS (EI): *m/z* (%): 415.4 (0.4) $[M+H]^+$, 112.1 (100) $[C_7H_{14}N]^+$; Anal. ($C_{26}H_{46}N_4$) C, H, N.

((4-(*N*-(Piperidin-1-yl)imino)methyl)phenyl)-*N*-(piperidin-1-yl)methanamine (2{*1,1*}). Terephthalaldehyde (**3**) (0.99 g, 7.3 mmol), 1-aminopiperidine **4**{*1*} (1.51 g, 14.7 mmol) and 4 Å molecular sieves were mixed in anhyd MeOH (30 mL) and held at reflux under a N_2 atmosphere for 16 h. The molecular sieves were filtered, and the solvent was partially removed. The crude oil was cooled, and the resulting precipitate was filtered and rinsed with cold MeOH to give **2**{*1,1*} as a yellow solid (1.42 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.55 (s, 4H; Ph), 7.53 (s, 2H; CH=N), 3.16 (m, 8H; CHN), 1.79–1.71 (m, 8H; CH_2), 1.58–1.50 ppm (m, 4H; CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 136.0 (Cq), 134.3 (CH), 125.9 (CH), 52.1 (CH_2), 25.3 (CH_2), 24.2 ppm (CH_2); IR (film): $\tilde{\nu}$ = 1576 cm^{-1} (C=N); Anal. ($C_{18}H_{26}N_4$) C, H, N.

4-((2-(Pyrrolidin-1-yl)ethylamino)methyl)benzaldehyde (7{*4*}). 4-(Diethoxymethyl)benzaldehyde (**5**) (2.01 g, 9.3 mmol), 1-(2-aminoethyl)pyrrolidine **4**{*4*} (1.09 g, 9.3 mmol) and 4 Å molecular sieves were mixed in anhyd MeOH (30 mL) and held at reflux under a N_2 atmosphere for 36 h. The molecular sieves were filtered and the intermediate imine in MeOH was cooled to 0 °C and treated with solid $NaBH_4$ (0.36 g, 9.3 mmol). The mixture was stirred at RT for 5 h. Then H_2O was added, and the product was extracted with CH_2Cl_2. The organic extracts were combined, washed with brine, dried over $MgSO_4$, and the solvent was removed to give the corresponding 4-(diethoxymethyl)benzylamine as a yellow oil (2.66 g, 93%). This intermediate aminoacetal (2.64 g, 8.6 mmol) was treated with 2 M HCl (20 mL) at RT for 2 h. The resulting mixture was basified with NaOH and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with brine, dried over $MgSO_4$, and the solvent was removed to give the product **7**{*4*} as a brownish oil (1.79 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 10.00 (s, 1H; CHO), 7.84 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 7.51 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 3.90 (s, 2H; C*H*$_2$Ph), 2.75 (t, $^3J_{H,H}$ = 6.0 Hz, 2H; C*H*$_2$NH), 2.64 (t, $^3J_{H,H}$ = 6.0 Hz, 2H; CH_2N), 2.51 (m, 4H; CH_2N), 2.01 (br s, 1H; NH), 1.77 ppm (m, 4H; CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 191.8 (CH), 147.6 (Cq), 135.1 (Cq), 129.7 (CH), 128.4 (CH), 55.8 (CH_2), 54.2 (CH_2), 53.7 (CH_2), 47.8 (CH_2), 23.5 ppm (CH_2); IR (film): $\tilde{\nu}$ = 3309 (NH), 2961, 2930, 2875, 2799 (CH), 1700 (C=O), 1606 (CC), 1459, 1446 cm^{-1} (CH); MS (EI): *m/z* (%): 233.2 (1) $[M+H]^+$, 232.2 (22) $[M]^+$

, 84.1 (100) $[C_5H_{10}N]^+$; HRMS: *m/z* calcd for $C_{14}H_{20}N_2O$: 232.1576 $[M+H]^+$, found: 232.1572.

4-((3-(1*H*-Imidazol-1-yl)propylamino)methyl)benzaldehyde (7{*6*}). The procedure was the same as that stated above for **7**{*4*} but by using 4-(diethoxymethyl)benzaldehyde (**5**) (2.01 g, 9.3 mmol), 1-(3-aminopropyl)imidazole **4**{*6*} (1.20 g, 9.3 mmol) and $NaBH_4$ (0.36 g, 9.3 mmol). The intermediate aminoacetal was obtained as a yellow oil (2.68 g, 90%). This acetal (2.68 g, 9.3 mmol) was deprotected to afford **7**{*6*} (1.76 g, 86%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 10.00$ (s, 1H; CHO), 7.85 (d, $^3J_{H,H} = 8.1$ Hz, 2H; Ph), 7.48 (d, $^3J_{H,H} = 8.1$ Hz, 2H; Ph), 7.46 (s, 1H; CHN), 7.05 (s, 1H; CHN), 6.90 (s, 1H; CHN), 4.07 (t, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2N), 3.85 (s, 2H; CH_2Ph), 2.62 (t, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2NH), 1.95 (quint, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2), 1.75 ppm (br s, 1H; NH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 191.7$ (CH), 147.2 (Cq), 137.0 (CH), 135.3 (Cq), 129.8 (CH), 129.3 (CH), 128.4 (CH), 118.7 (CH), 53.6 (CH_2), 45.8 (CH_2), 44.6 (CH_2), 31.3 ppm (CH_2). IR (film): $\tilde{\nu} = 3268$ (NH), 3108, 2936, 2831, 2738 (CH), 1696 (C=O), 1606 (C-C), 1508 cm^{-1} (imidazole); MS (EI): *m/z* (%): 244.1 (17) $[M+H]^+$, 243.1 (65) $[M]^+$, 119.0 (100) $[C_8H_7O]^+$; HRMS: *m/z* calcd for $C_{14}H_{17}N_3O$: 243.1372 $[M]^+$, found: 243.1366.

4-((3-(2-Methylpiperidin-1-yl)propylamino)methyl)benzaldehyde (7{*8*}). The procedure was the same as that stated above for **7**{*4*} but by using 4-(diethoxymethyl)benzaldehyde (**5**) (2.01 g, 9.3 mmol), 1-(3-aminopropyl)-2-methylpiperidine **4**{*8*} (1.52 g, 9.3 mmol) and $NaBH_4$ (0.36 g, 9.3 mmol). The intermediate aminoacetal was obtained as a yellow oil (3.18 g, 98%). This acetal (3.18 g, 9.1 mmol) was deprotected to afford **7**{*8*} (2.45 g, 98%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 10.00$ (s, 1H; CHO), 7.85 (d, $^3J_{H,H} = 8.1$ Hz, 2H; Ph), 7.50 (d, $^3J_{H,H} = 8.1$ Hz, 2H; Ph), 3.87 (s, 2H; CH_2NH), 2.87 (m, 1H; $CH_{eq}N$), 2.77 (m, 1H; $CH_{eq}N$), 2.65 (t, $^3J_{H,H} = 6.8$ Hz, 2H; CH_2NH), 2.36 (m, 1H; $CH_{ax}N$), 2.27 (m, 1H; $CHCH_3$), 2.11 (m, 1H; $CH_{ax}N$), 2.00 (br s, 1H; NH), 1.75–1.48 (m, 6H; CH_2), 1.29 (m, 2H; CH_{ax}), 1.06 ppm (d, $^3J_{H,H} = 6.0$ Hz, 3H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 191.8$ (CH), 147.7 (Cq), 135.2 (Cq), 129.8 (CH), 128.4 (CH), 56.1 (CH), 53.8 (CH_2), 52.3 (CH_2), 52.0 (CH_2), 48.5 (CH_2), 34.6 (CH_2), 26.1 (CH_2), 25.9 (CH_2), 23.9 (CH_2), 19.0 ppm (CH_3); IR (film): $\tilde{\nu} = 3271$ (NH), 2930, 2852, 2793, 2732 (CH), 1702 (C=O), 1606 (C–C), 1449, 1372 cm^{-1} (CH); MS (EI): *m/z* (%): 275.2 (9) $[M+H]^+$, 274.2 (37) $[M]^+$, 112.1 (100) $[C_7H_{14}N]^+$; HRMS: *m/z* calcd for $C_{17}H_{26}N_2O$: 274.2045 $[M]^+$, found: 274.2046.

4-((Piperidin-1-ylimino)methyl)benzaldehyde (6{*1*}). Terephthalaldehyde (**3**) (1.00 g, 7.4 mmol) was dissolved in anhydrous MeOH (30 mL) with 4 Å molecular sieves, followed by the dropwise addition of a solution of 1-aminopiperidine **4**{*1*} (0.38 g, 3.8 mmol) in anhydrous MeOH (5 mL) under a N_2 atmosphere. The mixture was held at reflux for 36 h. Upon removal of the solvent, the residue was separated by chromatography on silica gel by eluting with hexane/EtOAc (5:1). The resulting product was once again separated by chromatography on silica gel by eluting with CH_2Cl_2 /EtOAc (gradient 25:1 to 1:1) to give **6**{*1*} (0.48 g, 60%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.96$ (s, 1H; CHO), 7.83 (d, $^3J_{H,H} = 8.4$ Hz, 2H; Ph), 7.71 (d, $^3J_{H,H} = 8.4$ Hz, 2H; Ph), 7.48 (s, 1H; CH=N), 3.25 (t, $^3J_{H,H} = 5.7$ Hz, 4H; CH_2N), 1.76 (quint, $^3J_{H,H} = 5.7$ Hz, 4H; CH_2), 1.61–1.54 ppm (m, 2H; CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 191.6$ (CH), 142.8 (Cq), 135.0 (Cq), 131.0 (CH), 130.0 (CH), 125.8 (CH), 51.7 (CH_2), 25.1 (CH_2), 24.0 ppm (CH_2); IR (film): $\tilde{\nu} =$ 2938, 2854, 2818, 2731 (CH), 1694 (C=O), 1605 (C–C), 1579 (C=N), 1549 (C–C), 1448 cm^{-1} (CH); MS (EI): *m/z* (%): 217.0 (17) $[M+H]^+$, 216.0 (100) $[M]^+$; Anal. ($C_{13}H_{16}N_2O$) C, H, N.

4-((2,6-Dimethylpiperidin-1-ylimino)methyl)benzaldehyde (6{*2*}). The procedure was the same as that stated above for **6**{*1*} but terephthalaldehyde (**3**) (3.82 g, 28.2 mmol) and 1-amino-2,6-dimethylpiperidine **4**{*2*} (2.01 g, 14.1 mmol) were used. Upon removal of the solvent, the residue was separated by chromatography on silica gel by eluting with hexane/EtOAc (3:1) to afford **6**{*2*} (2.71 g, 78%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 9.95$ (s, 1H; CHO), 7.81 (d, $^3J_{H,H} = 8.3$ Hz, 2H; Ph), 7.69 (d, $^3J_{H,H} = 8.3$ Hz, 2H; Ph), 7.35 (s, 1H; CH=N), 3.92 (m, 2H; CH-CH_3), 1.87–1.56 (m, 6H; CH_2), 1.15 ppm (d, $^3J_{H,H} = 6.6$ Hz, 6H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 191.5$ (CH), 143.5 (Cq), 134.4 (Cq), 130.0 (CH), 129.5 (CH), 125.3 (CH), 53.1 (CH), 30.8 (CH_2), 18.3 (CH_3), 15.6 ppm (CH_2); IR (film): $\tilde{\nu} = 2967$, 2935, 2869, 2820, 2728 (C–H), 1693 (C=O), 1604 (C–C), 1572 (C=N), 1539 (C–C), 1468, 1372 cm^{-1} (C-H); MS (EI): *m/z* (%): 245.2 (5) $[M+H]^+$, 244.2 (16) $[M]^+$, 229.2 (100) $[C_{14}H_{17}N_2O]^+$, Anal. ($C_{15}H_{20}N_2O$) C, H, N.

***N*-(4-((2,6-Dimethylpiperidin-1-ylimino)methyl)benzyl)-2-(pyrrolidin-1-yl)ethylamine (8{*2,4*}).** 1-(2-Aminoethyl)pyrrolidine **4**{*4*} (0.52 g, 2.1 mmol) and **6**{*2*} (0.52 g, 2.1 mmol) were dissolved in anhyd MeOH (30 mL). 4 Å Molecular sieves were added, and the mixture was held at reflux under a N_2 atmosphere for 36 h. The molecular sieves were filtered, and the intermediate imine in MeOH was cooled to 0 °C and treated with solid $NaBH_4$ (0.08 g, 2.1 mmol). The reaction was stirred at RT for 16 h. Then H_2O was added, and the product was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over $MgSO_4$, and the solvent was removed to afford **8**{*2,4*} as a yellow oil (0.61 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.07$ (s, 1H; CH=N), 7.64 (d, 2H, $^3J = 8.1$ Hz; Ph), 7.34 (d, 2H, $^3J = 8.1$ Hz; Ph), 3.83 (s, 2H; CH_2Ph), 3.06 (m, 2H; $CHCH_3$), 2.77 (t, $^3J_{H,H} = 6.0$ Hz, 2H; CH_2NH), 2.63 (t, $^3J_{H,H} = 6.0$ Hz, 2H; CH_2N), 2.50 (m, 4H; CH_2N), 2.34 (br s, 1H; NH), 1.77 (m, 8H; CH_2), 1.50 (m, 2H; CH_2), 1.00 ppm (d, $^3J_{H,H} = 6.3$ Hz, 6H; CH_3); IR (film): $\tilde{\nu} = 3311$ (NH), 2962, 2931, 2872, 2794 (CH), 1624 (CC), 1584 (C=N), 1556 (C–C), 1459, 1447, 1369 (CH) cm^{-1}; MS (EI): *m/z* (%): 342.3 (0.5) $[M]^+$, 84.0 (100) $[C_5H_{10}N]^+$; HRMS: *m/z* calcd for $C_{21}H_{34}N_4$: 342.2783 $[M]^+$, found: 342.2786.

***N*-(4-((3-(1*H*-Imidazol-1-yl)propylamino)methyl)benzyl)-3-(2-methylpiperidin-1-yl)propan-1-amine (1{*6,8*}).** The procedure was the same as that stated above for **8**{*2,4*}, but 1-(3-aminopropyl)-2-methyl-piperidine **4**{*8*} (0.59 g, 3.6 mmol), **7**{*6*} (0.88 g, 3.6 mmol) and $NaBH_4$ (0.14 g, 3.6 mmol) were used to give **1**{*6,8*} (1.20 g, 86%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.44$ (s, 1H; CHN), 7.27 (s, 4H; Ph), 7.03 (s, 1H; CHN), 6.89 (s, 1H; CHN), 4.04 (t, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2N), 3.77 (s, 2H; CH_2Ph), 3.74 (s, 2H; CH_2-Ph), 2.87 (m, 1H; $CH_{eq}N$), 2.74 (m, 1H; $CH_{eq}N$), 2.65 (t, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2NH), 2.60 (t, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2NH), 2.37 (m, 1H; $CH_{ax}N$), 2.27 (m, 1H; $CHCH_3$), 2.12 (m, 1H; $CH_{ax}N$), 2.09 (br s, 2H; NH), 1.92 (quint, $^3J_{H,H} = 6.9$ Hz, 2H; CH_2), 1.72–1.53 (m, 6H; CH_2), 1.29 (m, 2H; CH_{ax}), 1.05 ppm (d, $^3J_{H,H} = 6.3$ Hz, 3H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 138.9$ (Cq), 138.7 (Cq), 137.1 (CH), 129.2 (CH), 128.2 (CH), 128.0 (CH), 118.7 (CH), 56.0 (CH), 53.7 (CH_2), 52.3 (CH_2), 52.0 (CH_2), 48.3 (CH_2), 45.7 (CH_2), 44.7 (CH_2), 34.6 (CH_2), 31.4 (CH_2), 26.1 (CH_2), 25.7 (CH_2), 23.9 (CH_2), 19.1 ppm (CH_3); IR (film): $\tilde{\nu} = 3277$ (N–H), 3104, 2929, 2853, 2802 (C–H), 1508 (imidazole), 1450, 1373 cm^{-1} (CH); MS (EI): *m/z* (%): 385.3 (2) $[M+2H]^+$, 384.3 (26) $[M+H]^+$, 383.3 (4) $[M]^+$, 112.0 (100) $[C_7H_{14}N]^+$; HRMS: *m/z* calcd for $C_{23}H_{37}N_5$: 383.3049 $[M+H]^+$, found: 383.3048.

***N*-(4-((3-(4-Methylpiperazin-1-yl)propylamino)methyl)benzyl)-3-(2-methylpiperidin-1-yl)propan-1-amine (1{*8,9*}).** The procedure was the same as that stated above for **8**{*2,4*} but 1-(3-aminopropyl)-2-methylpiperidine **4**{*8*} (0.45 g, 2.7 mmol), **7**{*9*} (0.75 g, 2.7 mmol) and $NaBH_4$ (0.10 g, 2.7 mmol) were used to give **1**{*8,9*} (0.45 g, 39%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.28$ (s, 4H; Ph), 3.77 (s, 4H; CH_2Ph), 2.88 (m, 1H; $CH_{eq}N$), 2.75

(m, 1H; $CH_{eq}N$), 2.67 (t, $^3J_{H,H}$ = 6.6 Hz, 2H; CH_2NH), 2.65 (t, $^3J_{H,H}$ = 6.6 Hz, 2H; CH_2NH), 2.43 (brs, 12H; CH_2N, $CH_{ax}N$, $CHCH_3$), 2.27 (s, 3H; CH_3N), 2.23 (brs, 2H; NH), 2.12 (m, 1H; $CH_{ax}N$), 1.76–1.53 (m, 8H; CH_2), 1.32–1.21 (m, 2H; $CH_{ax}N$), 1.05 ppm (d, $^3J_{H,H}$ = 6.3 Hz, 3H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 138.8 (Cq), 138.6 (Cq), 128.1 (CH), 128.0 (CH), 57.0 (CH_2), 56.0 (CH), 55.1 (CH_2), 53.7 (CH_2), 53.2 (CH_2), 52.3 (CH_2), 52.0 (CH_2), 48.3 (CH_2), 48.1 (CH_2), 46.0 (CH_3), 34.5 (CH_2), 26.9 (CH_2), 26.0 (CH_2), 25.6 (CH_2), 23.9 (CH_2), 19.0 ppm (CH_3); IR (film): $\tilde{\nu}$ = 3280 (N–H), 2931, 2875, 2852, 2793 (C–H), 1458, 1448, 1372 cm^{-1} (C–H); MS (EI): *m/z* (%): 415.4 (0.3) $[M]^+$, 112.2 (100) $[C_7H_{14}N]^+$; HRMS: *m/z* calcd for $C_{25}H_{45}N_5$: 415.3675 $[M]^+$, found: 415.3660.

((4-(*N*-(4-Methylpiperazin-1-yl)imino)methyl)phenyl)-*N*-(piperidin-1-yl)methanamine (2{*1*,*3*}). 1-Amino-4-methylpiperazine **4**{*3*} (0.24 g, 2.3 mmol) and **6**{*3*} (0.53 g, 2.3 mmol) were dissolved in anhydrous MeOH (30 mL). Molecular sieves (4 Å) were added and the mixture was held at reflux under a N_2 atmosphere for 36 h. The molecular sieves were filtered, and the solvent was removed to afford **2**{*1*,*3*} as a yellow solid (0.69 g, 95%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.56 (s, 4H; Ph), 7.53 (s, 1H; CH_2Ph), 7.52 (s, 1H; CH_2-Ph), 3.22 (t, $^3J_{H,H}$ = 5.1 Hz, 4H; CH_2N), 3.17 (t, $^3J_{H,H}$ = 5.6 Hz, 4H; CH_2N), 2.62 (t, $^3J_{H,H}$ = 5.1 Hz, 4H; CH_2N), 2.36 (s, 3H; CH_3), 1.75 (m, 4H; CH_2), 1.54 ppm (m, 2H; CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 136.4 (Cq), 135.6 (CH), 135.5 (Cq), 134.0 (CH), 126.2 (CH), 126.0 (CH), 54.6 (CH_2), 52.1 (CH_2), 51.0 (CH_2), 46.0 (CH_3), 25.3 (CH_2), 24.2 ppm (CH_2); IR (film): $\tilde{\nu}$ = 2934, 2837, 2798 (C–H), 1577 (C=N), 1452, 1365 cm^{-1} (C–H); MS (EI): *m/z* (%): 315.2 (2) $[M+2H]^+$, 314.2 (22) $[M+H]^+$, 313.2 (100) $[M]^+$; HRMS: *m/z* calcd for $C_{18}H_{27}N_5$: 313.2266 $[M-H]^+$, found: 313.2266.

4-((2-(Piperidin-1-yl)ethylamino)methyl)benzaldehyde (7{*7*}). 4-(diethoxymethyl)benzaldehyde (**5**) (0.91 g, 4.2 mmol) and 1-(2-aminoethyl)piperidine **4**{*7*} (0.55 g, 4.2 mmol) were dissolved in anhydrous MeOH (3 mL) in a 5 mL microwave reaction vessel. Na_2SO_4 was added and the vessel was sealed. The mixture was heated for 2 h at 100 °C in the microwave. The mixture was filtered and the solvent removed to yield *N*-(4-(diethoxymethyl)benzyliden)-2-(piperidin-1-yl)ethanamine as a reddish oil (1.35 g, 100%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 8.31 (s, 1H; CH=N), 7.71 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 7.52 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 5.53 (s, 1H; CH), 3.79 (t, $^3J_{H,H}$ = 7.1 Hz, 2H; CH_2N), 3.57 (m, 4H; CH_2CH_3), 2.68 (t, $^3J_{H,H}$ = 7.1 Hz, 2H; CH_2N), 2.51 (brs, 4H; CH_2N), 1.61 (m, 4H; CH_2), 1.45 (m, 2H; CH_2), 1.24 ppm (t, $^3J_{H,H}$ = 7.1 Hz, 6H; CH_3). This imine (1.32 g, 4.1 mmol) was dissolved in anhyd MeOH (30 mL), cooled to 0 °C, and treated with solid $NaBH_4$ (0.16 g, 4.1 mmol). The mixture was stirred at RT for 5 h. Then H_2O was added, and the product was extracted with CH_2Cl_2. The organic extracts were combined, washed with brine, dried over $MgSO_4$, and the solvent was removed to give *N*-(4-(diethoxymethyl)benzyl)-2-(piperidin-1-yl)-ethanamine as a yellow oil (1.23 g, 92%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.42 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 7.31 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 5.49 (s, 1H; CH), 3.80 (s, 2H; CH_2-Ph), 3.57 (m, 4H; CH_2CH_3), 2.71 (t, $^3J_{H,H}$ = 6.3 Hz, 2H; CH_2NH), 2.46 (t, $^3J_{H,H}$ = 6.3 Hz, 2H; CH_2N), 2.36 (br, 4H; CH_2N), 2.24 (brs, 1H; NH), 1.56 (quint, $^3J_{H,H}$ = 5.7 Hz, 4H; CH_2), 1.43 (m, 2H; CH_2), 1.23 ppm (t, $^3J_{H,H}$ = 7.1 Hz, 3H, CH_3). This aminoacetal (2.64 g, 8.6 mmol) was treated with 2 M HCl (20 mL) at RT for 2 h. The resulting mixture was basified with NaOH and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with brine, dried over $MgSO_4$, and the solvent was removed to afford aldehyde **7**{*7*} as a brownish oil (0.87 g, 94%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 10.00 (s, 1H; CHO), 7.84 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 7.50 (d, $^3J_{H,H}$ = 8.1 Hz, 2H; Ph), 3.89 (s, 2H; CH_2-Ph), 2.70 (t, $^3J_{H,H}$ = 6.2 Hz, 2H; CH_2NH), 2.47 (t, $^3J_{H,H}$ = 6.2 Hz, 2H; CH_2N), 2.36 (br, 4H; CH_2N), 2.18 (brs, 1H; NH), 1.57 (quint, $^3J_{H,H}$ = 5.7 Hz, 4H; CH_2), 1.43 ppm (m, 2H; CH_2).

***N*-(4-((2-(Piperidin-1-yl)ethylamino)methyl)benzyl)-3-(2-methylpiperidin-1-yl)propan-1-amine (1{*7*,*8*})**. 1-(3-aminopropyl)-2-methylpiperidine **4**{*8*} (0.44 g, 2.7 mmol) and **7**{*7*} (0.66 g, 2.7 mmol) were dissolved in anhyd MeOH (3 mL) in a 5 mL microwave vessel, Na_2SO_4 was added, and the vessel was sealed. The mixture was heated for 2 h at 100 °C in the microwave. Then it was filtered, diluted with MeOH (10 mL), cooled to 0 °C, and treated with solid $NaBH_4$ (0.10 g, 2.7 mmol). The mixture was stirred at RT for 4 h. Then H_2O was added and the product was extracted with CH_2Cl_2. The organic extracts were combined, washed with brine, dried over $MgSO_4$ and the solvent was removed to give **1**{*7*,*8*} as a yellow oil (0.98 g, 95%). ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.27 (s, 4H; Ph), 3.78 (s, 2H; CH_2-Ph), 3.77 (s, 2H; CH_2-Ph), 2.86 (m, 1H; $CH_{eq}N$), 2.78 (m, 1H; $CH_{eq}N$), 2.69 (t, $^3J_{H,H}$ = 6.3 Hz, 2H; CH_2NH), 2.63 (t, $^3J_{H,H}$ = 6.9 Hz, 4H; CH_2NH), 2.44 (t, $^3J_{H,H}$ = 6.3 Hz, 2H; CH_2N), 2.34 (m, 5H; $CH_{ax}N$, CH_2N), 2.25 (m, 1H; $CHCH_3$), 2.11 (m, 1H; $CH_{ax}N$), 2.07 (brs, 2H; NH), 1.68 (quint, $^3J_{H,H}$ = 6.9 Hz, 2H; CH_2), 1.55 (m, 8H; CH_2), 1.42 (m, 2H; CH_2), 1.28 (m, 2H; CH_{ax}), 1.05 ppm (d, $^3J_{H,H}$ = 6.3 Hz, 3H; CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 139.0 (Cq), 138.8 (Cq), 128.0 (CH), 58.6 (CH_2), 55.9 (CH), 54.7 (CH_2), 53.7 (CH_2), 52.3 (CH_2), 52.1 (CH_2), 48.3 (CH_2), 45.9 (CH_2), 34.7 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 25.7 (CH_2), 24.5 (CH_2), 24.0 (CH_2), 19.1 ppm (CH_3); IR (film): $\tilde{\nu}$ = 3301 (N–H), 2932, 2852, 2802 (C–H), 1467, 1443, 1373 cm^{-1} (C–H); Anal. ($C_{24}H_{42}N_4$) C, H, N.

Biological evaluation

Antiviral activity: HIV-1 strains were titered in MT-4 cells after acute infection, and infectivity was measured by evaluating the cytopathic effect that was induced after 5 day cultures as described.[60] Anti-HIV activity (EC_{50}) and cytotoxicity (CC_{50}) measurements in MT-4 cells were based on the viability of cells that had been infected or not infected with HIV-1, all were exposed to various concentrations of the test compound. After the MT-4 cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric method (MTT method) as described.

Time-of-drug-addition studies: MT-4 cells were infected with HIV-1 NL4–3 at a multiplicity of infection of 0.5 and incubated for 1 h at 20 °C in the presence or absence of test compounds. Cells were then washed twice in cool PBS and seeded in 96-well plates at a concentration of 2×10^5 cells per well (final volume 200 μL) at a temperature of 37 °C. Test compounds, dextran sulfate, AMD3100, C34 or AZT were added at various times post-infection, or the cells were cultured in the absence of drug (control). Test compounds were added at concentrations that completely block HIV replication (roughly 100-fold higher than the determined EC_{50}) of each drug in the standard assay performed with MT-4 cells. Virus production was measured by p24 antigen determination in the cell supernatant 30 h post-infection with a commercial p24 antigen ELISA (Innogenetics, Barcelona, Spain).[61]

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