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Progress towards new conformationally constrained HIV-1 protease inhibitors

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Summary — Two series of molecules containing a trisubstituted cyclopentyl group as the central unit were synthesized and evaluated as inhibitors of HIV-1 protease (HIV PR). In the first series of molecules (**13–20**), the central unit A, 3-(*N*-acyl)amino-2-hydroxy-1-cyclopentylacetyl, was designed so as to reproduce three of the central interactions found in the 'classical' complex HIV PR-JG365 inhibitor. Significant inhibitions (IC_{50} ~ 10 μM) were obtained with compound **20** in which the central unit was elongated by Z-Ile-Phe at the N-terminus and by Val-OMe at the C-terminus. In the second series of molecules (**21–28**), the central unit B, 3-hydroxy-2-(*N*-acyl)amino-1-cyclopentylacetyl, was obtained in the first steps of the synthesis. Unexpectedly better inhibitions were observed with these derivatives (K_i = 2 μM for compound **28**). Docking and molecular dynamics simulations performed with compound **28** into HIV PR suggested that the HIV PR-**28** complex should have a structure analogous to that of the recently described HIV PR-urea complex.

HIV-1 / aspartyl protease / inhibitor / constrained species

Introduction

Inhibition of the HIV-1 protease (HIV PR), which is essential for the synthesis of the functional proteins responsible for viral maturation [1, 2], continues to be a therapeutic target for the treatment of AIDS. The HIV PR is a member of the well-known aspartyl protease family [3], but its specificity lies in its dimeric structure made of two identical 99 residue subunits with only one active site [4], which is C_2-symmetric in the apo-form [5–7]. The intensive effort over the past two decades to find inhibitors of human renin, another aspartyl protease, has provided a great deal of information about the design of HIV PR inhibitors. Generally, these inhibitors are peptide analogues [8–11] which are not convenient for oral delivery due to their rapid proteolytic degradation and their subsequent poor bioavailability [12]. To overcome this drawback, a search for new non-peptidic inhibitors is needed [13–18]. Two different strategies may be developed. One is essentially based on shape complementarity between the receptor and the ligand and arises from the computational screening of three-dimensional structures available in databases [19, 20]. The other relies on rational ligand design based on essential enzyme–ligand interactions and structural features [5, 13, 17, 18]. In the past three years, there has been an explosion of X-ray crystal structures of HIV PR and HIV PR–inhibitor complexes [11], which provided useful information about the rational design of inhibitors. Common features observed include the interaction of the central hydroxyl group of inhibitors with side chain carboxylates of residues Asp 25 and Asp 125, together with the presence of a tetracoordinated structural water molecule linking the bound inhibitor to the flexible flaps of HIV PR. This water molecule accepts two hydrogen bonds from backbone amide hydrogens of residues Ile 50 and Ile 150, and donates two hydrogen bonds to carbonyl oxygens of the inhibitor.

This paper reports on a tentative design of a new, structurally constrained, basic moiety which allows the formation of the three essential interactions found in most of the HIV PR–inhibitor complexes.

We hypothesized that incorporation of a favorable arrangement of the inhibitor groups concerned with these interactions into a cyclic structure with restricted conformation should provide a positive entropic effect with respect to flexible linear inhibitors [21].

For this purpose, we used the molecular modeling tools of Sybyl, taking the inhibitor JG 365 as a refer-

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ence (fig 1) [22] in its geometric structure when complexed to HIV PR [23]. The modeling studies identified a cyclopentanol ring substituted on each side of the hydroxyl group with carbonyl containing arms in a *trans-trans* configuration (basic moiety A, fig 2). This identification was based on a fit analysis on six atoms, between the JG 365 central backbone and our cyclic central unit: the carbons and the oxygens of the two carbonyl groups and the carbon and the oxygen of the hydroxyl group involved in the main interactions (fig 3). The conformations that gave

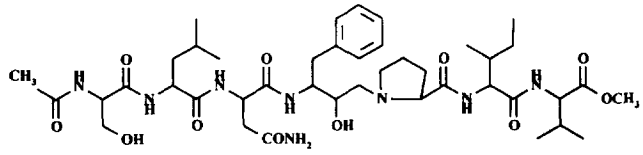


Fig 1. Structure of the inhibitor JG 365.

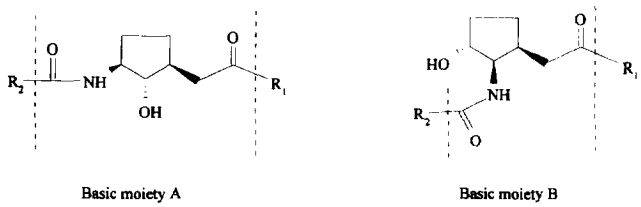


Fig 2. Constrained central units of the studied molecules. The basic moiety A was identified using modeling studies.

the best fits were minimized and fitted again. The *RRR* configuration found to give the best fit (fit 1, RMS: 0.55 Å; fit 2, RMS: 0.48 Å) and the best *SSS* configuration (fit 1, RMS: 0.52 Å; fit 2, RMS: 0.51 Å) are both 1.22 kcal/mol less stable than the lower energy conformation obtained with the randomsearch tool of Sybyl. For synthetic reasons, the two arms of the cyclopentanol ring were designed with different functionalities.

In order to evaluate our hypothesis, molecules **13–20** (scheme 1), which contain the basic moiety A (fig 2), were synthesized. The synthetic route also allowed us to obtain molecules **21–28** (scheme 1), which contain the isomeric basic moiety B (fig 2). The interaction of the molecules **13–28** was evaluated with recombinant HIV PR, and the results were analyzed using molecular modeling and molecular dynamics simulations.

Chemistry

Commercially available 2-cyclopentene-1-acetic acid was the starting material for all the synthesis. The amides **1–3** were synthesized classically using *i*-Bu-chloroformate as a coupling agent and natural (*S*) amino acid methyl ester or *t*-butylamide derivatives. Treatment of these amides with *m*-chloroperbenzoic acid produced the epoxides **4–6** in only one stereoisomeric form. In the literature it has been reported that in the cyclopentyl series a carbonyl group containing a side chain has only a steric directing effect leading to *trans*-epoxides [24]. On this basis and NMR observations, it was reasonably assumed that these

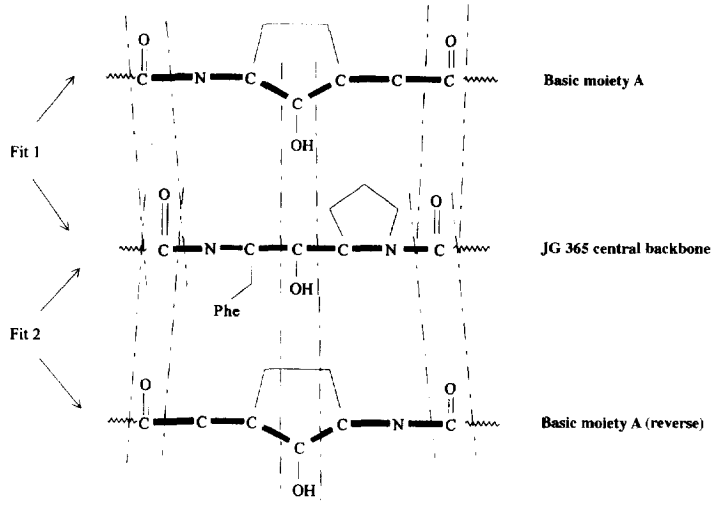
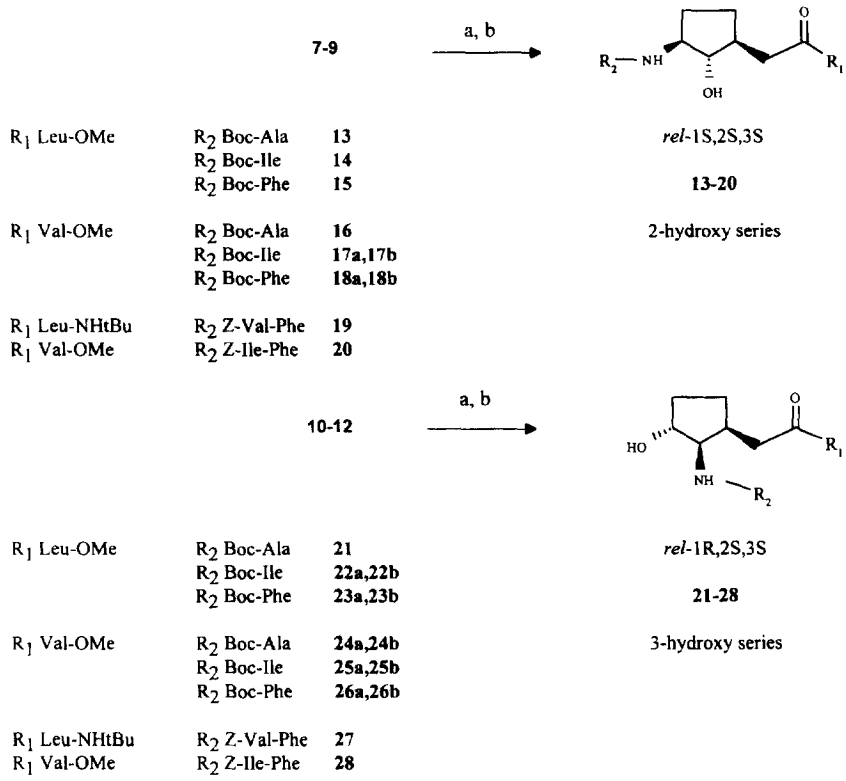


Fig 3. Superposition of the JG365 central backbone and the basic moiety A according to the two possibilities. The atoms fitted are indicated between the dotted lines.



Scheme 1. Reagents and conditions: (a) H_2, Pd/C; (b) ClCOO-*i*-Bu, CH_2Cl_2, −15°C, N-blocked amino acid or dipeptide.

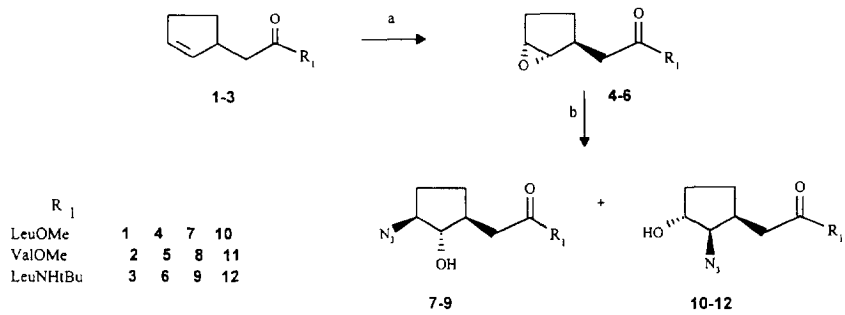
stereoisomers have the *trans* configuration. Treatment of these epoxides with sodium azide afforded two regioisomers: the *trans-trans*-(*rel*-1*S*,2*S*,3*S*)-3-azido-2-hydroxy compounds **7–9** (70–80% of the mixture) corresponding to nucleophilic attack of azide on the carbon 3 of the cyclopentane ring; and the *cis-trans*-(*rel*-1*R*,2*S*,3*S*)-3-hydroxy-2-azido compounds **10–12** (20–30% of the mixture) corresponding to nucleophilic attack on the carbon 2 (scheme 2). These two regioisomers were easily separated by liquid chromatography, and their structure determined by 2D ^{1}H-NMR.

The azido compounds were then catalytically hydrogenated over Pd/C, and the resulting amines used without further purification for the last condensation step (scheme 1). In the 2-hydroxy series, the two diastereomers 1*S*,2*S*,3*S* and 1*R*,2*R*,3*R* of compounds **17** and **18** were well separated by liquid chromatography on silica leading respectively to the homochiral compounds **17a,b** and **18b**. In the 3-hydroxy series, the two diastereomers 1*S*,2*R*,3*R* and 1*R*,2*S*,3*S* of compounds **23** to **26** were separated leading to compounds **23a,b** to **26a,b**. Compounds **23a** and **26a** could be distinguished from compounds **23b** and **26b** by the comparison of their benzylic protons signals. For compounds **23a** and **26a**, the signal appears as a doublet, while it appears as a multiplet for compounds **23b** and **26b**.

Compounds **19**, **20**, **27** and **28** were obtained after condensation of crude amines with dipeptides Z-Val-Phe-OH or Z-Ile-Phe-OH. Chromatographic comparisons (HPLC) of compound **20** with the (D)Phe analog obtained step by step showed that no more than 5% racemization occurred during this condensation step.

Biological results and discussion

Our hypothesis based on the design of inhibitors with a constrained basic moiety containing three of the main interactions of most inhibitor-HIV PR complexes, led us to synthesize molecules **13–20** (2-hydroxy series) with the A moiety. To ensure the validity of our hypothesis, only one amino acid was added on each side of this moiety (**13–18b**). Enzymatic assays (table I) showed no or only weak inhibitory activity (38% at 10 μM for the best inhibitor **15**). These results suggest that the introduction of



Scheme 2. Reagents and conditions: (a) *m*-$ClC_6H_4CO_3H$, CH_2Cl_2, 0°C; (b) NaN_3, NH_4Cl, H_2O/EtOH.

Table I. Inhibition of HIV PR by molecules **13–20** of the 2-hydroxy series (containing the A moiety) and molecules **21–28** of the 3-hydroxy series (containing the B moiety).

Molecule	*Series*	R_1	R_2	*Inhibition (%) (IC_{50} μM)*[a]
13	2-Hydroxy	Leu-OMe	Boc-Ala	13
14	2-Hydroxy	Leu-OMe	Boc-Ile	24
15	2-Hydroxy	Leu-OMe	Boc-Phe	38
16	2-Hydroxy	Val-OMe	Boc-Ala	0
17a	2-Hydroxy	Val-OMe	Boc-Ile	15
17b	2-Hydroxy	Val-OMe	Boc-Ile	21
18a	2-Hydroxy	Val-OMe	Boc-Phe	0
18b	2-Hydroxy	Val-OMe	Boc-Phe	8
19	2-Hydroxy	Leu-NH*t*-Bu	Z-Val-Phe	0
20	2-Hydroxy	Val-OMe	Z-Ile-Phe	50 (12)
21	3-Hydroxy	Leu-OMe	Boc-Ala	19
22a	3-Hydroxy	Leu-OMe	Boc-Ile	18
22b	3-Hydroxy	Leu-OMe	Boc-Ile	8
23a	3-Hydroxy	Leu-OMe	Boc-Phe	30 (20)
23b	3-Hydroxy	Leu-OMe	Boc-Phe	30 (20)
24a	3-Hydroxy	Val-OMe	Boc-Ala	10
24b	3-Hydroxy	Val-OMe	Boc-Ala	0
25a	3-Hydroxy	Val-OMe	Boc-Ile	50
25b	3-Hydroxy	Val-OMe	Boc-Ile	18
26a	3-Hydroxy	Val-OMe	Boc-Phe	25
26b	3-Hydroxy	Val-OMe	Boc-Phe	27
27	3-Hydroxy	Leu-NH*t*-Bu	Z-Val-Phe	37
28	3-Hydroxy	Val-OMe	Z-Ile-Phe	70 (3)[b]

[a]Percentage inhibition was measured at 10 μM inhibitor concentration; [b]K_i = 2 μM.

three main interactions in our constrained structure was not sufficient for reaching good inhibitions. Compound **15** was docked into the HIV PR binding pocket manually, following the classical model of the HIV PR–JG365 complex [23]. The model complex obtained after MD simulations suggested a good fit of Leu into the S'_2 subsite, but the S_1 subsite was partially filled by Phe (fig 4). It was reasonable to assume that the best inhibitions could be obtained by introducing other substituents in order to improve the interactions with protease subsites. In fact, for the same R_1 substituent (Val-OMe), better inhibitions were obtained with the dipeptidic Z-Ile-Phe (**20**) than with the Boc-Phe (**18a,b**) as the R_2 substituent. Docking of compound **20,** following the same procedure as used before, and MD simulations suggested an improvement of the fit of Phe in the S_1 subsite, but Ile was badly positioned in the S_2 subsite and Val partially filled the S'_2 subsite (fig 5).

Compounds with R_1 = Leu-NH*t*Bu and R_2 = Z-Val-Phe were inefficient, probably due to the size of the *t*-Bu group.

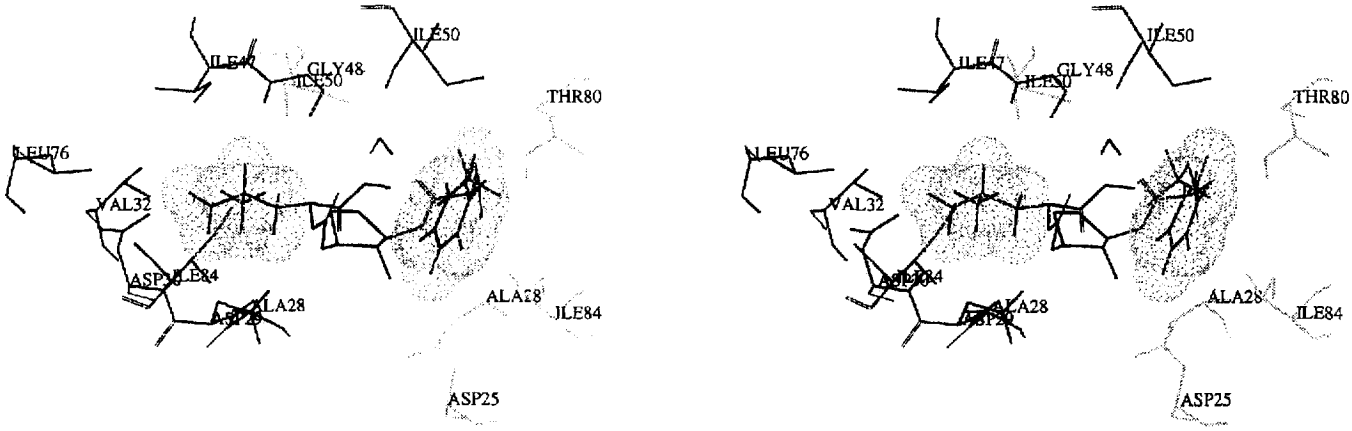


Fig 4. Stereoview of HIV PR–**15** complex obtained after manual docking and MD simulations. The phenyl and isoleucyl groups of compound **15** are represented with their van der Waals dot surface.

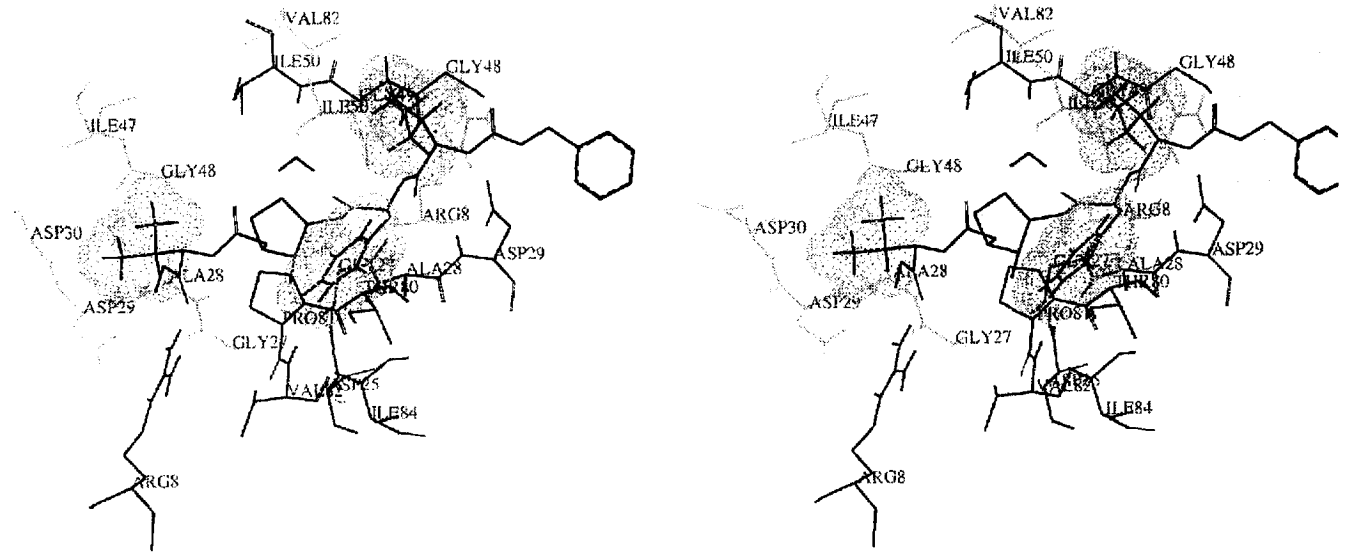


Fig 5. Stereoview of HIV PR–**20** complex obtained after manual docking and MD simulations. The phenyl, isoleucyl and valyl groups of compound **20** are represented with their van der Waals dot surface.

For some compounds, replacement of the A moiety (2-hydroxy series) by the B moiety (3-hydroxy series) led to nearly identical inhibitory potencies (**21/13**; **22a/14**; **23a**, **b/15**). In the other cases, better inhibitions were observed with compounds of the 3-hydroxy series. For example, compounds **27** and **26a,b** behaved as weak inhibitors of HIV PR, in contrast to the corresponding molecules of the 2-hydroxy series **19a,b** and **18a,b**, respectively, which were inactive. In both series, compounds with R_1 = Val-OMe and R_2 = Boc-Ala were devoid of inhibitory effect. The best inhibitor was compound **28**, which behaved as a competitive inhibitor of HIV PR with a K_i of 2 μM (fig 6). The presence of the B moiety in compound **28** led to a three- and fourfold improvement of the inhibitory activity over the corresponding molecule **20** of the 2-hydroxy series.

Docking of compound **28** into the binding pocket of HIV PR following the model of the HIV PR-JG365 complex was unsuccessful since the energy minimization of the complex could not converge. Other modeling studies were performed following the docking model recently described for cyclic ureas inhibitors [15, 18, 25]. In this model, the urea group replaces the structural water hydrogen bonded to the Ile of the two flaps. Compound **28** was docked into the binding pocket of HIV1 PR manually. The oxygen of the hydroxyl of cyclopentane was well positioned for serving as a surrogate of the structural water molecule because it could be hydrogen bonded to the NH hydrogens of both Ile 50 and Ile 150. Moreover, the NH immediately attached to the cyclopentane ring was positioned to allow hydrogen bonding with Asp 25. Molecular dynamics simulation

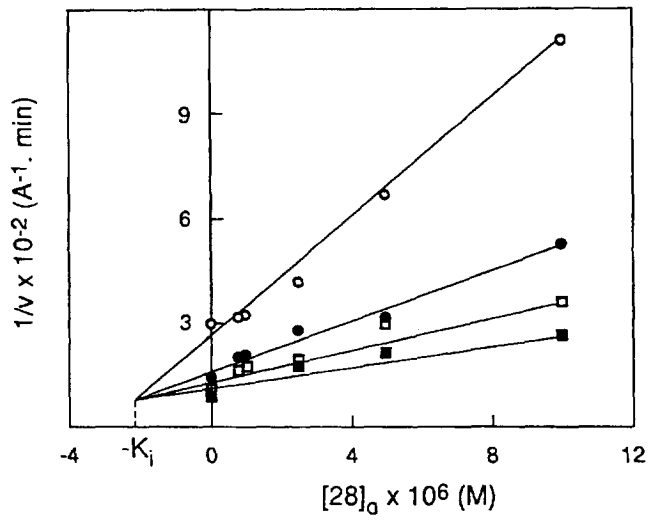


Fig 6. Dixon plot of the inhibition of HIV PR by **28** with V-S-Q-N-F(NO_2)-P-I-VNH_2 as substrate at pH 5.5 and 30°C. The substrate concentrations were 125 (○); 250 (●); 350 (□); 500 (■) μM. The enzyme concentration was 0.24 μM. A: absorbance.

with this model complex suggested that Ile and Phe were well positioned into the S1 and S2 subsites, respectively, but the S'1 subsite was partially filled by Val (fig 7).

From another point of view, our results showed no influence of the stereochemistry (*R,S*) of the cyclopentyl-bound hydroxyl group on the activity of our series of molecules [26, 27].

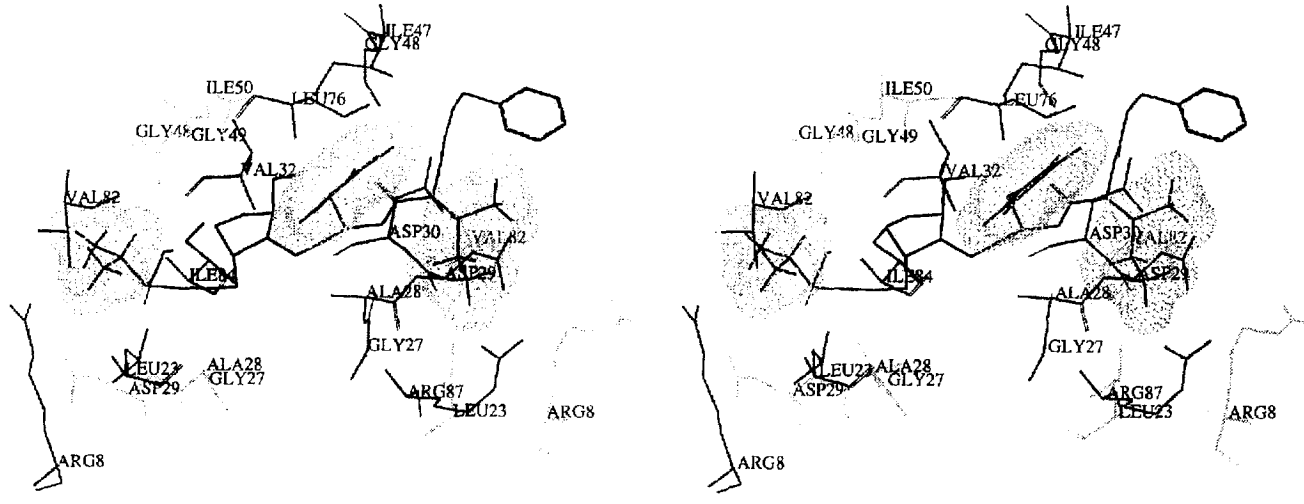


Fig 7. Stereoview of HIV PR–**28** complex obtained after manual docking and MD simulations. The phenyl, isoleucyl and valyl groups of compound **28** are represented with their van der Waals dot surface.

Conclusion

In this work, two constrained structures were demonstrated to be the basic moieties of potentially new HIV PR inhibitors. The first basic moiety reproduces the three central interactions found in the 'classical' inhibitors. The second basic moiety probably reproduces the central interactions previously found in the urea-type protease–inhibitor complex. Micromolar activity was obtained with addition of particular amino acids on each side of the moieties. Further experiments are in progress in order to improve these activities by introducing other substituents which could interact more accurately with subsites S1, S'1 and S2, S'2, and to design new compounds of both series devoid of peptidic character.

Experimental protocols

General

Melting points were determined on a Mettler FP61 apparatus. NMR spectra were recorded using Bruker AC200 and ARX400 spectrometers. Mass spectra were obtained using a Ribermag R10-10 mass spectrometer. IR spectra were performed on a Perkin Elmer 1420 spectrometer. Microanalyses were performed at the CNRS (Vernaison, France), and at the Service de microanalyse of the Faculté de pharmacie in Châtenay-Malabry; all the microanalyses were obtained within ±0.4% of the theoretical values. All the amino acids were purchased from Novabiochem (Meudon, France). Recombinant HIV PR from *Escherichia coli* [28] was used.

Chemistry

N-[(Cyclopent-2-enyl)acetyl]leucine methyl ester ***1*** *(general procedure for the preparation of compounds* ***1–3****)*
To a solution of 2-cyclopentene-1-acetic acid (5.0 g, 39.7 mmol) and 4-methylmorpholine (4.41 mL, 39.7 mmol) in methylene chloride (50 mL) was added dropwise at −15°C *i*-butylchloroformate (5.15 mL, 39.7 mmol). The mixture was stirred for 0.5 h and then a solution of L-leucine methyl ester, HCl (7.21 g, 39.7 mmol) and 4-methylmorpholine (4.41 mL, 39.7 mmol) in methylene chloride (50 mL) was added dropwise. The mixture was stirred for 1 h at −15°C and overnight at room temperature. This mixture was filtered and the residue washed with successively a solution of Na_2CO_3 (10%), citric acid (10%) and water, dried ($MgSO_4$) and evaporated giving 11.2 g of an oily product. Liquid chromatography (silica gel, methylene chloride/*i*–propanol, 95:5) gave 9 g of compound **1** (90% yield). ^{1}H-NMR ($CDCl_3$) δ 5.95 (d, 1H, *J* = 8 Hz), 5.5 (m, 2H), 4.4 (m, 1H), 3.5 (s, 3H), 2.9 (m, 1H), 2.2–1.8 (m, 5H), 1.5–1.15 (m, 4H), 0.8 (d, 6H, *J* = 8 Hz). Anal $C_{14}H_{23}O_3N$ (C, H, N).

N-[(Cyclopent-2-enyl)acetyl]valine methyl ester ***2***. 85% yield; mp 55–56°C; ^{1}H-NMR ($CDCl_3$) δ 5.9 (d, 1H, *J* = 8 Hz), 5.8–5.5 (m, 2H), 4.4 (m, 1H), 3.6 (s, 3H), 2.9 (m, 1H), 2.2–1.8 (m, 6H), 1.3 (m, 1H), 0.8 (m, 6H). Anal $C_{13}H_{21}O_3N$ (C, H, N).

N-[(Cyclopent-2-enyl)acetyl]leucine t-butyl amide ***3***. 85% yield; ^{1}H-NMR ($CDCl_3$) δ 6.2 (m, 1H), 6 (m, 1H), 5.8–5.6 (m, 2H), 4.4 (m, 1H), 3.1 (m, 1H), 2.4–1.6 (m, 9H), 1.3 (s, 9H), 1.1 (m, 6H).

N-[[2(Bicyclo[3.1.0]-5-oxa)hexyl]acetyl]leucine methyl ester ***4*** *(general procedure for the preparation of compounds* ***4–6****)*
To a solution of compound **1** (10.0 g, 39.6 mmol) in methylene chloride (100 mL) was added dropwise at 0°C a solution of *m*-chloroperbenzoic acid (10.7 g, 59.4 mmol) in methylene chloride (50 mL). The mixture was stirred for 24 h at room temperature and was washed with successively Na_2CO_3 (10%), water and brine, and dried ($MgSO_4$). Evaporation of solvent and liquid chromatography of crude product (pentane/ethyl acetate, 50:50) gave 8.4 g (84% yield) of compound **4**. ^{1}H-NMR ($CDCl_3$) δ 6.7 (m, 1H), 4.3 (m, 1H), 3.4 (s, 3H), 3.15 (m, 2H), 2.15 (m, 3H), 1.7 (m, 1H), 1.35 (m, 6H), 0.6 (d, 6H, *J* = 8 Hz). Anal $C_{14}H_{23}O_4N$ (C, H, N). Mass *m/z* 270 (M + 1).

N-[[2(Bicyclo[3.1.0]-5-oxa)hexyl]acetyl]valine methyl ester ***5***. 80% yield; ^{1}H-NMR ($CDCl_3$) δ 6.4 (d, 1H, *J* = 7 Hz), 4.4 (dd, 1H, *J* = 9 Hz, *J* = 5 Hz), 3.6 (s, 3H), 3.4 (m, 2H), 2.5–2.2 (m, 6H), 1.6 (m, 2H), 0.8 (m, 6H). Anal $C_{13}H_{21}O_4N$ (C, H, N). Mass *m/z* 256 (M + 1).

N-[[2(Bicyclo[3.1.0]-5-oxa)hexyl]acetyl]leucine t-butylamide ***6***. 88% yield; ^{1}H-NMR ($CDCl_3$) δ 6.7 (d, 1H, *J* = 9 Hz), 6.1 (s, 1H), 4.3 (m, 1H), 3.4 (m, 2H), 2.5–2.3 (m, 3H), 1.9 (m, 3H), 1.7–1.4 (m, 4H), 1.3 (s, 9H), 0.9–0.7 (m, 6H). Anal $C_{17}H_{30}O_3N_2$ (C, H, N).

N-[rel-(1S,2S,3S)-(3-Azido-2-hydroxy-1-cyclopentyl)acetyl]-leucine methyl ester ***7*** *and N-[rel-(1R,2S,3S)-(3-hydroxy-2-azido-1-cyclopentyl)acetyl]leucine methyl ester* ***10*** *(general procedure for the preparation of compounds* ***7–12****)*
To a solution of epoxide **4** (3 g, 11.2 mmol) in ethanol/water (80:20) (100 mL), were added sodium azide (1g, 15 mmol) and ammonium chloride (0.8 g, 15 mmol). The mixture was stirred at reflux for 1 d, evaporated and extracted with ether. The organic extracts were washed with water, dried ($MgSO_4$) and evaporated giving rise to 2.6 g of crude product. Liquid chromatography (silica gel, methylene chloride/*i*-propanol, 95:5) gave the isomer **7** (1.84 g, 53% yield) as the first eluted product and the isomer **10** as the second (0.55 g, 21% yield). Isomer **7**: IR ν 2100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.2 (m, 1H), 4.45 (m, 1H), 4.11 (m, 1H), 3.67 (m, 1H), 3.59 (s, 3H), 2.42–2.15 (m, 3H), 2.06–1.9 (m, 1H), 1.85–1.68 (m, 1H), 1.57–1.22 (m, 5H), 0.79 (d, 6H, *J* = 6 Hz). Anal $C_{14}H_{24}O_4N_4$ (C, H, N). Isomer **10**: IR ν 2100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.45 (m, 1H), 3.9 (m, 1H), 3.5 (s, 3H), 3.1 (m, 1H), 2.3 (m, 2H), 2 (m, 2H), 1.8 (m, 2H), 1.5–1.1 (m, 4H), 0.7 (d, 6H, *J* = 6 Hz). Anal $C_{14}H_{24}O_4N_4$ (C, H, N).

N-[rel-(1S,2S,3S)-(3-Azido-2-hydroxy-1-cyclopentyl)acetyl]-valine methyl ester ***8*** *and N-[rel-(1R,2S,3S)-(3-hydroxy-2-azido-1-cyclopentyl)acetyl]valine methyl ester* ***11***. From epoxide **5** (3.2 g, 12 mmol); isomer **8** eluted first (1.9 g, 53% yield); IR ν 2090 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.4 (d, 1H, *J* = 8 Hz), 4.5 (dd, 1H, *J* = 5 Hz, *J* = 9 Hz), 4.03 (m, 1H), 3.8 (m, 1H), 3.7 (s, 3H), 2.45 (m, 1H), 2.4 (m, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 2.1 (m, 1H), 1.9 (m, 1H), 1.55 (m, 1H), 1.43 (m, 1H), 0.86 (m, 6H). Anal $C_{13}H_{22}O_4N_4$ (C, H, N). Isomer **11** eluted second (0.63 g, 18% yield); IR ν 2090 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.4 (d, 1H, *J* = 8 Hz), 4.55 (m, 1H), 4.1 (m, 1H), 3.74 (s, 3H), 3.41 (m, 1H), 2.55 (dd, 1H, *J* = 14 Hz, *J* = 6 Hz), 2.3 (m, 1H), 2.2 (m, 1H), 2.13 (m, 1H), 1.9 (m, 2H), 1.65 (m, 1H), 1.55 (m, 1H), 0.9 (m, 6H). Anal $C_{13}H_{22}O_4N_4$ (C, H, N).

N-[rel-(1S,2S,3S)-(3-Azido-2-hydroxy-1-cyclopentyl)acetyl]-leucine t-butylamide ***9*** *and N-[rel-(1R,2S,3S)-(3-hydroxy-2-azido-1-cyclopentyl)acetyl]leucine t-butylamide* ***12***. From

epoxide **6** (5.4 g, 17 mmol); isomer **9** eluted first (3.6 g, 60% yield), mp 118°C; IR ν 2090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.6 (d, 1H, J = 9 Hz), 5.9 (s, 1H), 4.4 (m, 1H), 4 (m, 1H), 3.8 (m, 1H), 2.5–2.4 (m, 3H), 2.2–1.4 (m, 7H), 1.3 (s, 9H), 0.95–0.85 (m, 6H). Anal $C_{17}H_{31}O_3N_5$ (C, H, N); isomer **12** eluted second (0.7 g, 12% yield), mp 80°C; IR ν 2090 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 7.5 (m, 1H), 6.8 (s, 1H), 4.5 (m, 1H), 4.1 (m, 1H), 3.4 (dd, 1H, J = 9 Hz, J = 6 Hz), 3.1 (s, 1H), 2.6–2.2 (m, 3H), 2.1–1.8 (m, 2H), 1.7–1.4 (m, 5H), 1.35 (s, 9H), 1–0.8 (m, 6H). Anal $C_{17}H_{31}O_3N_5$ (C, H, N).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-alanyl]amino-1-cyclopentyl]acetyl]leucine methyl ester ***21*** *(general procedure for the preparation of compounds* ***13–28****)*
A solution of azide **10** (0.55 g, 1.8 mmol) in ethanol (15 mL) over Pd/C (10%) was stirred for 16 h in a Parr apparatus using 30 psi of hydrogen. The mixture was filtered and evaporated giving 0.5 g (90% yield) of crude amine which was used without further purification. To a solution of Boc-alanine (0.14 g, 0.74 mmol) and 4-MM (82 μL, 0.74 mmol) in methylene chloride (15 mL) was added at –15°C i-butylchloroformate (96 μL, 0.74 mmol). The mixture was stirred for 1 h at –15°C, and then a solution of previously prepared amine (0.2 g, 0.74 mmol) in methylene chloride was added. The mixture was stirred for 1 h at –15°C and overnight at room temperature. The mixture was washed with successively 1 N HCl (2 x 10 mL), Na_2CO_3 (5%, 2 x 10 mL) and water (2 x 10 mL), and dried ($MgSO_4$) and evaporated giving 0.17 g of a crude product. Liquid chromatography (silica gel, methylene chloride/*i*-propanol, 95:5) gave 0.15 g (60% yield) of compound **21**; mp 173°C (hexane/ethyl acetate). ^1H-NMR ($CDCl_3$) δ 7.68 (m, 1H), 6.64 (d, 1H, J = 7.6 Hz), 5.35 (d, 1H, J = 7.2 Hz), 4.48 (m, 1H), 4.18 (m, 1H), 3.95 (m, 1H), 3.68 (s, 3H), 3.4 (m, 1H), 2.35–1.56 (m, 10H), 1.37 (s, 9H), 1.33–1.28 (m, 3H), 0.87 (d, 6H, J = 3.6 Hz). Mass m/z 458 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)alanyl]amino-2-hydroxy-1-cyclopentyl]acetyl]leucine methyl ester ***13****.* 60% yield; ^1H-NMR ($CDCl_3$) δ 7.8 (m, 1H), 6.8 (m, 1H), 5 (m, 1H), 4.78 (m, 1H), 4.5 (m, 1H), 4.1 (m, 1H), 3.75 (m, 1H), 3.65 (s, 3H), 2.5–1.56 (m, 10H), 1.37 (s, 9H), 1.18–1 (m, 3H), 0.88– 0.83 (m, 6H). Anal $C_{22}H_{39}O_7N_3$ (C, H, N). Mass m/z 458 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)isoleucyl]amino-2-hydroxy-1-cyclopentyl]acetyl]leucine methyl ester ***14****.* 57% yield; ^1H-NMR ($CDCl_3$) δ 7.1 (m, 1H), 5.5 (m, 1H), 4.5 (m, 1H), 3.9 (m, 2H), 3.7 (m, 1H), 3.6 (s, 3H), 2.5–1.53 (m, 11H), 1.34 (s, 9H), 0.93–0.76 (m, 14H). Anal $C_{25}H_{45}O_7N_3$ (C, H, N). Mass m/z 500 (M+1).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)phenylalanyl]-amino-2-hydroxy-1-cyclopentyl]acetyl] leucine methyl ester ***15****.* 58% yield; mp 113°C; ^1H-NMR ($CDCl_3$) δ 7.3–7.1 (m, 5H), 6.7 (d, 1H, J = 8 Hz), 6 (m, 1H), 5.1 (m, 1H), 4.5 (m, 1H), 4.2 (m, 2H), 3.8 (m, 1H), 3.6 (s, 3H), 3 (m, 2H), 2.5 (m, 1H), 2.2–1.4 (m, 9H), 1.33 (s, 9H), 0.87–0.85 (m, 6H). Anal $C_{28}H_{43}O_7N_3$ (C, H, N). Mass m/z 534 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)alanyl]amino-2-hydroxy-1-cyclopentyl]acetyl] valine methyl ester ***16****.* Crude compound **16** (52% yield) was chromatographed. Compound **16a** eluted first; ^1H-NMR ($CDCl_3$) δ 6.9 (m, 1H), 6.1 (m, 1H), 5 (m, 1H), 4.4 (m, 1H), 4.2 (m, 2H), 3.7 (m, 1H), 3.6 (s, 3H), 2.5–1.6 (m, 8H), 1.4 (s, 9H), 1.3 (m, 3H), 0.8 (m, 6H). Mass m/z 444 (M + 1). Compound **16b** eluted second; mp 124°C; ^1H-NMR ($CDCl_3$) δ 6.9 (d, 1H, J = 9 Hz), 6.8 (d, 1H, J = 9 Hz), 5.4 (m, 1H), 4.2 (m, 1H), 4 (m, 2H), 3.8 (m, 2H), 3.5 (s, 3H), 2.3 (m, 1H), 2.1–1.6 (m, 7H), 1.2 (s, 9H), 1 (d, 3H, J = 7 Hz), 0.68 (d, 3H, J = 6 Hz), 0.65 (d, 3H, J = 6 Hz). Anal $C_{21}H_{37}O_7N_3$ (C, H, N).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)isoleucyl]-amino-2-hydroxy-1-cyclopentyl]acetyl]valine methyl ester ***17****.* Crude compound **17** (62% yield) was chromatographed. Compound **17a** eluted first; ^1H-NMR ($CDCl_3$) δ 7.8 (m, 1H), 5.9 (m, 1H), 5.0 (m, 1H), 4.5 (m, 1H), 3.9 (m, 2H), 3.7 (m, 1H), 3.5 (s, 3H), 2.4–1.4 (m, 9H), 1.3 (s, 9H), 0.9–0.7 (m, 14H). Mass m/z 486 (M + 1). Compound **17b** eluted second; mp 151°C; ^1H-NMR ($CDCl_3$) δ 7.0 (m, 1H), 6.8 (d, 1H, J = 9 Hz), 5.1 (d, 1H, J = 9 Hz), 4.3 (dd, 1H, J = 8 Hz, J = 5 Hz), 3.8 (m, 2H), 3.6 (m, 1H), 3.5 (s, 3H), 2.6–1.7 (m, 9H), 1.3 (s, 9H), 0.7– 0.6 (m, 14H). Mass m/z 486 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(t-Butoxycarbonyl)phenylalanyl]-amino-2-hydroxy-1-cyclopentyl]acetyl] valine methyl ester ***18****.* Crude compound **18** (54%) was chromatographed. Compound **18a** eluted first; mp 142°C; ^1H-NMR ($CDCl_3$) δ 7.2–7 (m, 5H), 6.7 (d, 1H, J = 9 Hz), 5.7 (d, 1H, J = 5 Hz), 4.9 (m, 1H), 4.4 (dd, 1H, J = 9 Hz, J = 5 Hz), 4.2 (m, 2H), 3.7 (m, 1H), 3.6 (s, 3H), 2.9 (m, 2H), 2.5 (m, 1H), 2.2–1.4 (m, 7H), 1.3 (s, 9H), 0.84–0.76 (2d, 6H, J = 7 Hz). Anal $C_{27}H_{41}O_7N_3$ (C, H, N). Mass m/z 520 (M + 1). Compound **18b** eluted second; mp 146°C; ^1H-NMR ($CDCl_3$) δ 7.3–7.1 (m, 5H), 6.9 (d, 1H, J = 9 Hz), 6.0 (m, 1H), 5.1 (m, 1H), 4.5 (m, 1H), 4.4 (m, 1H), 4.3 (m, 1H), 3.9 (m, 1H), 3.7 (s, 3H), 3.0 (m, 2H), 2.6 (m, 1H), 2.25–2 (m, 3H), 1.8–1.4 (m, 4H), 1.4 (s, 9H), 0.96–0.87 (m, 6H). Anal $C_{27}H_{41}O_7N_3$ (C, H, N). Mass m/z 520 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(Benzyloxycarbonyl)valylphenylalanyl]amino-2-hydroxy-1-cyclopentyl]acetyl] leucine t-butylamide ***19****.* 51% yield; ^1H-NMR ($CDCl_3$) δ 7.5–7.1 (m, 10H), 6.9 (m, 1H), 6.5–6.3 (m, 2H), 5.7–5.5 (m, 2H), 5 (s, 2H), 4.5 (m, 1H), 4.3–4.1 (m, 2H), 3.9–3.6 (m, 2H), 3 (m, 2H), 2.6–1.7 (m, 7H), 1.6–1.4 (m, 4H), 1.2 (s, 9H), 1–0.7 (m, 12H). Mass m/z 708 (M + 1).

N-[[rel-(1R,2R,3R)-3-[N-(Benzyloxycarbonyl)isoleucylphenylalanyl]amino-2-hydroxy-1-cyclopentyl]acetyl] valine methyl ester ***20****.* 65% yield; mp 192°C (chloroform, isopropyl ether); ^1H-NMR ($CDCl_3$) δ 7.2–7 (m, 10H), 6.9 (m, 1H), 6.7 (d, 1H, J = 9 Hz), 6.5 (d, 1H, J = 8 Hz), 5.2 (d, 1H, J = 7 Hz), 4.9 (s, 2H), 4.5 (m, 1H), 4.2 (m, 2H), 3.7 (m, 2H), 3.5 (s, 3H), 2.9 (m, 2H), 2.4 (m, 1H), 2.2–1.6 (m, 6H), 1.4–1 (m, 4H), 0.7 (m, 12H). Anal $C_{36}H_{50}O_8N_4$ (C, H, N). Mass m/z 667 (M + 1).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-isoleucyl]amino-1-cyclopentyl]acetyl]leucine methyl ester ***22****.* Crude compound **22** (56% yield) was chromatographed. Compound **22a** eluted first; mp 193°C (chloroform, isopropyl ether); ^1H-NMR ($CDCl_3$) δ 7.9 (m, 1H), 6.4 (m, 1H), 5.2 (m, 1H), 4.5 (m, 1H), 3.95 (m, 2H), 3.7 (s, 3H), 3.3 (m, 1H), 2.4–1.4 (m, 11H), 1.36 (s, 9H), 1.2–1.1 (m, 2H), 0.88–0.81 (m, 12H). Anal $C_{25}H_{47}O_7N_3$ (C, H, N). Mass m/z 500 (M + 1). Compound **22b** eluted second; mp 155°C (chloroform, isopropyl ether); ^1H-NMR ($CDCl_3$) δ 7.8 (m, 1H), 6.4 (d, 1H, J = 8 Hz), 5.1 (d, 1H, J = 9 Hz), 4,5 (m, 1H), 3.95 (m, 2H), 3.67 (s, 3H), 3.4 (m, 1H), 2.36–1.4 (m, 11H), 1.37 (s, 9H), 1.3–1.18 (m, 2H), 0.87–0.78 (m, 12H). Anal $C_{25}H_{47}O_7N_3$ (C, H, N). Mass m/z 500 (M + 1).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-phenylalanyl]amino-1-cyclopentyl]acetyl]leucine methyl ester ***23****.* Crude compound **23** (0.18 g, 54% yield) was chromato-

graphed. Compound **23a** eluted first; mp 163°C; ^{1}H-NMR ($CDCl_3$) δ 7.5 (m, 1H), 7.25–7.1 (m, 5H), 6.3 (d, 1H, J = 7 Hz), 5.2 (m, 1H), 4.4 (m, 2H), 3.8 (m, 1H), 3.7 (s, 3H), 3.3 (m, 1H), 3 (m, 2H), 2.3–1.32 (m, 10H), 1.3 (s, 9H), 0.86 (d, 6H, J = 5 Hz). Anal $C_{28}H_{43}O_7N_3$ (C, H, N). Mass m/z 534 (M + 1). Compound **23b** eluted second; mp 171°C; ^{1}H-NMR ($CDCl_3$) δ 7.6 (m, 1H), 7.24–7.1 (m, 5H), 6.25 (d, 1H, J = 8 Hz), 5.1 (d, 1H, J = 8 Hz), 4.4 (m, 2H), 3.8 (m, 1H), 3.66 (s, 3H), 3.3 (m, 1H), 3 (d, 2H, J = 6 Hz), 2.3–1.4 (m, 10H), 1.3 (s, 9H), 0.87–0.82 (m, 6H). Anal $C_{28}H_{43}O_7N_3$ (C, H, N). Mass m/z 534 (M + 1).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-alanyl]amino-1-cyclopentyl]acetyl]valine methyl ester ***24***. Crude compound **24** (56% yield) was chromatographed. Compound **24a** eluted first; ^{1}H-NMR ($CDCl_3$) δ 6.1 (m, 2H), 4.5 (dd, 1H, J = 9 Hz, J = 5 Hz), 4 (m, 1H), 3.7 (m, 1H), 3.6 (s, 3H), 3.5 (m, 1H), 2.5–1.4 (m, 8H), 1.2 (s, 9H), 1 (m, 3H), 0.9–0.8 (m, 6H). Anal $C_{21}H_{37}O_7N_3$ (C, H, N). Mass m/z 444 (M + 1). Compound **24b** eluted second; mp 162°C; ^{1}H-NMR ($CDCl_3$) δ 6.2 (d, 1H, J = 8 Hz), 5.9 (m, 1H), 4.9 (m, 1H), 4.6 (s, 1H), 4.3 (dd, 1H, J = 8 Hz, J = 5 Hz), 3.9 (m, 1H), 3.63 (m, 1H), 3.57 (s, 3H), 3.2 (m, 1H), 2.3–1.4 (m, 8H), 1.3 (s, 9H), 1.0 (m, 3H), 0.73 (m, 6H). Anal $C_{21}H_{37}O_7N_3$ (C, H, N). Mass m/z 444 (M + 1).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-isoleucyl]amino-1-cyclopentyl]acetyl]valine methyl ester ***25***. Crude compound **25** (53% yield) was chromatographed. Compound **25a** eluted first; ^{1}H-NMR ($CDCl_3$) δ 7.9 (m, 1H), 6.2 (m, 1H), 5.0 (m, 1H), 4.5 (dd, 1H, J = 8.5 Hz, J = 5 Hz), 4–3.75 (m, 2H), 3.7 (s, 3H), 3.3 (m, 1H), 2.4–1.4 (m, 9H), 1.36 (s, 9H), 1.0–0.8 (m, 14H). Anal $C_{24}H_{43}O_7N_3$ (C, H, N). Mass m/z 486 (M + 1). Compound **25b** eluted second; ^{1}H-NMR ($CDCl_3$) δ 7.9 (m, 1H), 6.1 (d, 1H, J = 8.6 Hz), 5.0 (m, 1H), 4.4 (dd, 1H, J = 9 Hz, J = 5 Hz), 4.0 (m, 2H), 3.7 (s, 3H), 3.3 (m, 1H), 2.44–1.74 (m, 9H), 1.38 (s, 9H), 0.87–0.77 (m, 14H). Anal $C_{24}H_{43}O_7N_3$ (C, H, N). Mass m/z 486 (M + 1)

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(t-butoxycarbonyl)-phenylalanyl]amino-1-cyclopentyl]acetyl]valine methyl ester ***26***. Crude compound **26** (55% yield) was chromatographed. Compound **26a** eluted first; ^{1}H-NMR ($CDCl_3$) δ 7.7 (m, 1H), 7.25 (m, 5H), 6.1 (m, 1H), 5.2 (m, 1H), 4.5 (m, 2H), 3.9 (m, 1H), 3.8 (s, 3H), 3.4 (m, 1H), 3.1 (m, 2H), 2.5–1.8 (m, 8H), 1.4 (s, 9H), 0.95 (m, 6H). Anal $C_{27}H_{41}O_7N_3$ (C, H, N). Compound **26b** eluted second; mp 155°C; ^{1}H-NMR ($CDCl_3$) δ 7.7 (m, 1H), 7.05 (m, 5H), 6.5 (d, 1H, J = 8 Hz), 6.2 (d, 1H, J = 8 Hz), 4.4 (m, 2H), 3.9 (m, 1H), 3.7 (s, 3H), 3.3 (m, 1H), 2.8 (d, 2H, J = 6 Hz), 2.2–1.4 (m, 8H), 1.3 (s, 9H), 0.8–0.6 (m, 6H). Anal $C_{27}H_{41}O_7N_3$ (C, H, N). Mass m/z 520 (M + 1).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(benzyloxycarbonyl)valinyl phenylalanyl]amino-1-cyclopentyl]acetyl] leucine t-butyl amide ***27***. 56% yield; ^{1}H-NMR ($CDCl_3$) δ 7.5–7 (m, 10H), 6.8 (m, 1H), 6.3 (m, 2H), 5.7 (m, 1H), 5.5 (m, 1H), 5.1 (s, 2H), 4.8 (m, 1H), 4.35–4 (m, 2H), 3.8 (m, 1H), 3.5 (m, 1H), 3 (m, 2H), 2.3–1.85 (m, 7H), 1.65–1.4 (m, 4H), 1.3 (s, 9H), 0.9–0.7 (m, 12H). Anal $C_{39}H_{57}O_7N_5$ (C, H, N).

N-[[rel-(1R,2S,3S)-3-Hydroxy-2-[N-(benzyloxycarbonyl)-isoleucinyl phenylalanyl]amino-1-cyclopentyl]acetyl] valine methyl ester ***28***. 70% yield; mp 173°C (chloroform, isopropyl ether); ^{1}H-NMR ($CDCl_3$) δ 7.35–7.1 (m, 10H), 5 (s, 2H), 4.6 (m, 1H), 4.2 (m, 2H), 3.8 (m, 1H), 3.6 (s, 3H), 3.5 (m, 1H), 3 (m, 2H), 2.3 (m, 3H), 2.2–1.6 (m, 4H), 1.4 (m, 4H), 0.7 (m, 12H). Anal $C_{36}H_{50}O_8N_4$ (C, H, N). Mass m/z 667 (M + 1).

Biochemistry

The inhibitory potency was determined by the percentage of inhibition against the substrate H-V-S-Q-N-F(NO_2)-P-I-V-NH_2. This chromogenic substrate was prepared and characterized by B Badet (Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, unpublished results). The enzyme was diluted (factor of 3) by addition to the buffer prepared in 1 mM phosphate (pH 8.0) containing bovine serum albumin (1 mg/mL). The initial rates were determined by following the continuous release of H-V-S-Q-N-F(NO_2)-OH at 307 nm and 30°C in 0.1 M acetate, 1 M NaCl, 5 mM EDTA (final pH 4.5). The reaction was initiated by addition of the previously prepared enzyme (dilution of 20). The inhibitors were dissolved in DMSO with a final cosolvent percentage in buffer of 2.5 (v/v). IC_{50} determinations were performed in duplicate and each concentration with mean values used for data analyses. The experimental conditions for these determinations were: $[E]_0$ = 0.24 μM; [H-V-S-Q-N-F(NO_2)-P-I-V-NH_2]$_0$ = 250 μM; [**20a**] = 5–7.5 μM; [**20b**] = 5–15 μM; [**23a**] = 10–30 μM. The inhibition constant K_i value was calculated from a Dixon plot for competitive inhibition (eq [1]).

$$v = V_M \cdot [S]_0 / \{[S]_0 + (K_M \cdot (1 + [I]_0/K_i)\} \quad [1]$$

The concentrations of the chromogenic substrate varied from 125 to 500 μM with [**28**] = 0 to 10 μM ($[E]_0$ = 0.24 μM).

Modeling studies

Modeling and conformational studies were performed using Sybyl 6.03 and 6.1 programs from Tripos. Energies were calculated using Tripos force field and Gasteiger–Marsili charges. Current minimizations were performed using gradient termination of 0.05 kcal·mol^{-1}. Conformational randomsearch studies were performed using 500 cycles. Docking studies were performed with HIV PR in its conformation resulting from the complexed form with JG 365 [23]. An initial energy minimization (1 kcal·mol^{-1} gradient termination) was performed to relieve steric strains associated with the newly docked ligand. MD simulations were then run at 300K for 5 ps with time step of 1 fs. The resulting structure was extracted and energy minimized (1 kcal.mol^{-1} gradient termination). For HIV PR–**15** and HIV PR–**20** complexes, distance constraints were defined between Ile50-Ile150 and a structural water molecule, between the structural water molecule and the carbonyl groups immediately attached to the cyclopentane ring, and between the hydroxyl of the cyclopentane ring and Asp 25-Asp 125. A sphere was also defined as aggregate (centered on oxygen of the hydroxyl with R = 12 Å). For HIV PR–**28** complex, distance constraints were defined between Ile 50-Ile 150 and the hydroxyl of the cyclopentane ring, and between Asp 25-Asp 125 and the NH immediately attached to the cyclopentane ring. The protease backbone was defined as aggregate.

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