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Short and unexpectedly potent 3-pyrrolidinone type inhibitors of HIV-1 replication

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Abstract – Based on the specific PhePro proteolytic cleavage of the HIV protease, short pseudo-peptides incorporating a 3-pyrrolidinone ring have been synthesized. Their potencies to inhibit HIV-1 in MT_4 cell culture have been evaluated and compared to that of the bioisostere dipeptide BocPhePro. Analogues incorporating an aromatic residue have shown to inhibit HIV-1 infection in MT_4 human lymphoid cell with an IC_{50} ranging from 1 to 10 μM. Further experiments are in progress to determine their HIV protease inhibition properties. © Elsevier, Paris

3-pyrrolidinone / HIV inhibitor / proline isostere / HIV protease

1. Introduction

The substrate specificity of HIV protease represents a paradoxical situation in which the enzyme is required to make a number of highly specific cleavages with the *gag* and *gag-pol* polyproteins at sites spanning remarkably heterogeneous amino acid sequences. Although no consensus sequence for HIV protease has been deduced, the common occurrence of an aromatic proline cleavage site in 19 retroviruses has been noted with particular interest since N-terminal hydrolysis to proline is unusual for mammalian endopeptidases [1]. Analysis of the proteolytic processing of HIV-1, HIV-2 and Simian Immunodeficiency Virus has led to a proposal that sequences flanking the cleavage site could be assigned to one of the three following classes [2]. Class 1 contains Phe-Pro or Tyr-Pro at P_1 and P'_1 sites (Schechter and Berger notation) [3]. Class 2 sites have Arg at P_4 and Phe-Leu at P'_1-P'_2 and class 3 sequences contain Gln or Glu at P'_2. Class 1 sequences are represented by the following peptides cleaved at the Tyr/Pro or Phe/Pro sites:

ValSerGlnAsn**Tyr-Pro**IleValGlnAsn [4–7]
AspLysGluLeu**Tyr-Pro**LeuThrSerLeu [4–7]
ValSerPheAsn**Phe-Pro**GlnIleThrLeu [4–7]
CysThrLeuAsn**Phe-Pro**IleSerProIle [4–7]
HisSerSerGlnValSerGlnAsn**Tyr-Pro**IleValGlnAsnIle [5]

It has also been reported that shorter peptides like SerGlnAsn**Tyr-Pro**IleVal were cleaved only 10% more slowly than the corresponding longer peptide HisSerSerGlnValSerGlnAsn**Tyr-Pro**IleValGlnAsnIle. Therefore, since it was possible to introduce subtile structural modifications in the proline heterocycles, class 1 sequences appeared to us suitable for the design of HIV protease inhibitors. Small molecules that possess high affinity and specificity for enzymes or cellular receptors are attractive therapeutic agents. Since the HIV protease displays an unusual preference for Tyr-Pro or Phe-Pro primary cleavage sites [8, 9], our approach was to replace the 1-carboxy-pyrrole ring of the proline residue by a 3-pyrrolidinone. This differs from the classical strategies which rely on incorporating a transition state analogue and which has been successful for producing potent renin [10] and HIV protease inhibitors [11]. These new proline mimicking moieties were coupled to various amino

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acids, including aromatic (Phe, Tyr) and non-aromatic residues (Gly, Leu). In order to evaluate the anti-HIV potencies of this new class of analogues, we selected *syncitia* formation inhibition on HIV-infected cells for the first screening. Indeed, many peptidomimetic inhibitors possessing effective activity in purified enzyme inhibition assays turned out to be sometimes inactive in HIV infected cells.

In this report, we describe the syntheses of short 3-pyrrolidinone and 3-hydroxy-pyrrolidine peptides based on Phe-Pro or Tyr-Pro specific proteolytic processing of HIV-1. These new compounds were tested for their abilities to inhibit HIV-1 infection in MT-4 human lymphoid cells.

2. Chemistry

3-pyrrolidinone analogues were synthesized according to the following method (*figures 1* and *2*). Firstly, Boc-protected L-amino acids (**1a–d**) were coupled to 3-hydroxypyrrolidine using either DCC/HOBt or benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and triethylamine (TEA) dichloromethane (*figure 1*). No significant differences in terms of yields were observed between the two coupling reagents. The resulting coupling products (**2a–d**) were then oxidized using a catalytic amount of 2, 2, 6, 6-tetramethylpiperidinyl-1-oxy (TEMPO) in a mixture of sodium hypochlorite (pH 8.2) and dichloromethane [12] to afford compounds **3a–d**. Compound **3c** incorporating a protected tyrosine was hydrogenated under mild conditions by catalytic transfer hydrogenation [13]. Thus, 1, 4-cyclohexadiene was used as a hydrogen donor and the resulting compound **3e** was obtained in a quantitative yield. In order to optimize the biological activities of these new 3-pyrrolidinone derivatives, various N-terminal protecting groups were incorporated into the dipeptide moiety. These reactions summarized in *figure 2* were performed only on phenylalanine-3-pyrrolidinone analogues. The dipeptide synthon **3d** was deprotected in a solution of trifluoroacetic acid (TFA) in dichloromethane. The resulting crude product **4** was condensed with either acid, carbamoyl or ureyl chloride and compounds **5** to **11** were obtained. The structures were confirmed by ^{1}H-NMR, mass spectra and elemental analyses. It should be outlined that, as it is frequently observed for X-proline bonds, these types of compounds exist as mixture of rotamers around the amide bond [14]. This feature explains why in some cases, complicated ^{1}H-NMR spectra were obtained.

3. Antiviral activity

Representative compounds were tested for their abilities to inhibit HIV-1 infection in MT_4 human lymphoid cells. The fusogenic effect of HIV in MT_4 cell line was determined as described by Rey et al. [15, 16]. Antiviral potencies of the new synthesized compounds are summarized in *table I*. IC_{50} and CC_{50} have been determined as well as Log *P* using ACD software LogP 1.0.

4. Discussion

Under the assay conditions, apart from the reference dipeptide BocPhePro which was found inactive, only the compounds incorporating an aromatic residue elicited potent inhibitory effects. Tyr or Phe derivatives gave IC_{50} ranging from 1 to 10 μM. In contrast, the corresponding Gly or Leu analogues do not exhibit any anti-HIV activity. Although the HIV protease inhibition assays were not performed, we can tentatively assert that these results support the rational

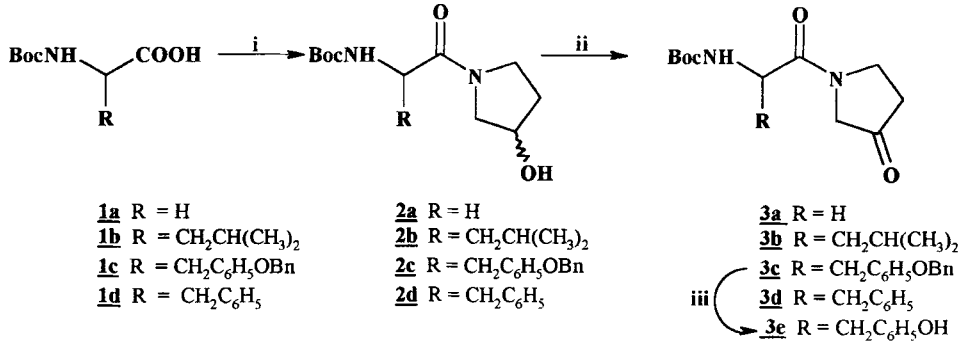

Figure 1. (i) 3-pyrrolidinol, DCC, HOBT, TEA, CH_2Cl_2; (ii) TEMPO, CH_2Cl_2/$NaHCO_3$; (iii) 1,4-cyclohexadiene, Pd-C, EtOH.

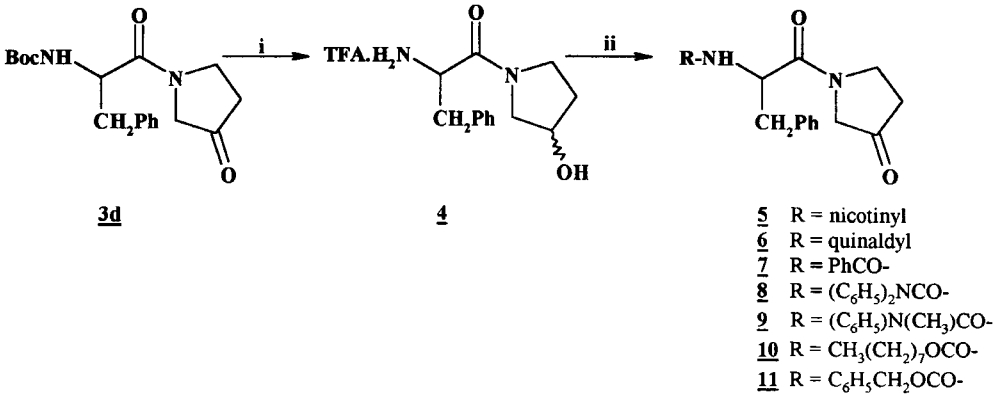

Figure 2. (i) TFA, CH_2Cl_2; (ii) R-OH (**5** and **6**), DCC, HOBT, TEA, CH_2Cl_2 or R-Cl, TEA, CH_2Cl_2.

described in the introduction of this report. Phe-Pro or Tyr-Pro represent a specific sequence recognized by HIV-protease. It is not the first time that short peptides incorporating or mimicking the consensus sequences have been reported to display an anti-HIV protease activity [17]. However, the peptides or pseudo-peptides used in the sequences were all based on the transition state analogue concept. Since the new 3-pyrrolidinone derivatives are not based on this concept, it seems reasonable to assume that this class of compounds could be considered as sterically matching the enzyme active site. Indeed, this mode of inhibition has already been mentioned for the haloperidol compounds [18]. Concerning the aromatic series of the new synthesized derivatives, we thought that the observed differences in HIV inhibitory properties could be attributed to the different lipophilicities which regulate the penetration through the cellular membrane. As reflected by Log *P* values in *table I*, lipophilicity is probably not the only factor that influences the antiviral activity of the new derivatives. For instance, compound **5** which is the less lipo-

Table I. Anti-HIV activities and Log *P* determination.

Compound	IC_{50} (μmol)[a]	CC_{50} (μmol)[b]	Log *P*[c]
BocPhePro	inactive	> 100	3.96 ± 0.53
N-Boc-3-pyrrolidinol	inactive	> 100	–0.35 ± 0.61
2a	inactive	> 100	0.87 ± 0.49
2b	inactive	> 100	2.63 ± 0.50
2c	inactive	> 100	4.72 ± 0.53
2d	50	100	3.15 ± 0.51
3a	inactive	> 100	1.14 ± 0.56
3b	inactive	> 100	2.90 ± 0.57
3c	10	50	4.99 ± 0.59
3d	10	100	3.97 ± 0.58
3e	50	50	2.68 ± 0.58
5	0.1–1	> 100	2.31 ± 0.62
6	10	> 100	3.44 ± 0.63
7	inactive	50	3.17 ± 0.61
8	inactive	> 100	4.86 ± 0.67
9	inactive	50	3.33 ± 0.61
10	inactive	50	5.38 ± 0.57
11	1	50	3.97 ± 0.58

[a]IC_{50} = concentration required to inhibit syncitia formation by 50% on MT_4 cells; [b]CC_{50} = concentration required to cause 50% death on uninfected MT_4 cells; [c]Log *P* determination was performed using ACD (Advanced Chemistry Development)/Log *P* 1.0 base calculations.

philic has the best anti-HIV activity. Further possible Phe series inhibitors were synthesized by the removal of the N-terminal Boc group followed by the addition of different functionalities such as nicotinyl **5**, quinaldyl **6**, benzoyl **7**, N, N-diphenyl carboxamide **8**, octyloxycarbonyl **10**, and benzyloxycarbonyl **11**. IC_{50} values obtained for these series of derivatives showed that the chemical functionalities introduced at the N-terminal position of the new 3-pyrrolidinone analogues played a determinative role in the anti-HIV activity (*table I*). This is not surprising in the light of the known strong dependence of HIV protease inhibitors to fill the P_1-P_3/$P_{1'}$-$P_{3'}$ positions. Although the N-terminal position accomodates a rather large variety of substituents, the preference stands for the groups which exhibit low lipophilicity. N-nicotinyl Phe analogue **5** elicited the highest antiviral activity (IC_{50} = 0.1–1 μM). Concerning the N-terminal position substituted by a Boc group, we have investigated, for the Phe and Tyr series, the influence on anti-HIV activity of the reduced endocyclic ketone. In both cases the resulting alcohol abolished the anti-HIV activity. With the exception of compound **5** having a selective index ranging from 100 to 1000, a majority of the tested compounds elicited a rather high cytotoxicity (*table I*). The cytotoxicity could be due to their lipophilicity which favors higher membrane permeation properties and/or to the fact that these short molecules, possessing a limited number of chiral centers, are not very specific versus the HIV enzymatic pool. This relies on our previous hypothesis suggesting that these new inhibitors could sterically match the enzyme active site. In summary, a novel series of short 3-pyrrolidinone derivatives capable of inhibiting the proliferation of HIV-1 virus in MT_4 infected cells has been developped. These new analogues incorporating a 3-pyrrolidinone cycle, bioisostere of the proline five membered ring, support the idea that the unusual preferences for Tyr-Pro or Phe-Pro primary cleavage sites, are useful in the design of new HIV inhibitors. However, these derivatives were not designed as HIV protease transition state analogues. Since most of the 3-pyrrolidinol derivatives were found inactive, in particular N-Boc-3-pyrrolidinol, it seems that both N-terminal position and the 3-pyrrolidinone type nature are important to elicit anti-HIV activity. Further studies including QSAR and HIV protease inhibition are in progress in order to optimize the therapeutic potential of these compounds.

5. Experimental protocols

5.1. Chemistry

Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC 250 spectrometer. Chemical shifts were expressed in δ values (part per million) relative to tetramethylsilane as an internal standard for ^1H. Mutiplicity is abbreviated as s (singulet), d (doublet), t (triplet), br (broad), or m (multiplet). Coupling constants are expressed in hertz (Hz). FAB^+ mass spectra were recorded on a JEOL DX-100 mass spectrometer at the Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France using a glycerol/thioglycerol matrix. IR spectra were obtained using an 1605 FT-IR Spectrophotometer (Perkin Elmer Instruments). Analyses indicated by the symbols of the elements or functions were within 0.4% of the theoretical values. Preparative flash column chromatography were performed using silica-gel Merck G60 230–240 mesh. Analytical thin-layer chromatography (TLC) was performed on silica-gel plates $60F_{254}$ aluminium (Merck, Darmstadt) of 0.2 mm thickness. Preparative layer chromatography (PLC) were carried out on silica-gel $60F_{254}$ pre-coated PLC plates (20 x 20 cm layer, thickness 1 or 2 mm). All solvents were used from freshly opened sealed bottles purchased from the Aldrich Company. All amino acids used as starting materials were of the L configuration. The reference dipeptide BocPhePro was purchased from Bachem.

5.1.1. General procedure A. Coupling reaction using 1, 3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt)

The amino acid derivative (1.0 equiv.) was dissolved in CH_2Cl_2 at 0 °C under nitrogen atmosphere followed by the addition of DCC (1.2 equiv.), HOBt (1.2 equiv.) and triethylamine (TEA) (3 equiv.). The mixture was stirred for 2 h at 0 °C before the 3-pyrrolidinol derivative (1.0 equiv.) was added. The solution was allowed to warm to room temperature and stirred for 12 h. The DCU (dicyclohexylurea) was filtered and the filtrate was evaporated. A solution of aqueous 5% citric acid and EtOAc were added. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to give after purification by flash chromatography the desired compound.

5.1.2. General procedure B. Oxidation with 2.2.6.6-tetramethyl-1-piperidinyloxy free radical (TEMPO)

A solution containing alcohol derivative (1.0 equiv.), a catalytic amount of TEMPO and KBr, one portion of saturated $NaHCO_3$ solution and 3 portions of CH_2Cl_2 was prepared. To this mixture was added a solution of aqueous sodium hypochlorite (pH 8.2). Addition of NaOCl was performed over a period of 40 min until the starting material was consumed. Brine and CH_2Cl_2 were then added and the product extracted. After drying over Na_2SO_4, filtration and evaporation, the crude product was purified by flash chromatography to give the desired ketone.

5.1.3. General Procedure C

To a solution of **7** N-L-(Phenylalanyl)-3-pyrrolidinone trifluoroacetate salt (1.0 equiv.) in CH_2Cl_2 at 0 °C were added under nitrogen, the chloride derivative (1.2 equiv.) and TEA (3.0 equiv.). The solution was allowed to warm to room temperature and stirred for 6 h. The mixture was then evaporated. An aqueous 5% citric acid solution and EtOAc were added. The organic layer was dried over Na_2SO_4 and evaporated to give after purification by PLC the desired compound. Compounds **2a**, **2b**, **2c**, and **5** were prepared according to general procedure A.

5.1.4. 1-[(tert-butoxycarbonyl)-L-Glycyl]-3(R, S)-pyrrolidinol ***2a***

From (*tert*-butoxycarbonyl)-L-Glycine with 90% yield (1 g). TLC(Hexane/EtOAc 3:7) R_f 0.27. ^1H-NMR ($CDCl_3$) δ: 1.35 (s, 9H, tBu), 1.97 (m, 2H, C_4-H_2), 3.38–3.70 (m, 6H, C_2-H_2 and

C_5-H_2), 3.80 (m, 2H, CH_2 Gly), 4.80 (m, 1H, C_3-H), 5.20 (s, 1H, NH). MS: MH^+ (FAB) *m/z* 245. Anal. $C_{11}H_{20}N_2O_4$ (C, H, N).

5.1.5. 1-[(tert-butoxycarbonyl)-L-Leucyl]-3(R, S)-pyrrolidinol ***2b***

From (*tert*-butoxycarbonyl)-L-Leucine with 78% yield (470 mg). TLC (Hexane/EtOAc 1:1) R_f 0.13. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (m, 6H, CH_3δLeu), 1.17 (s, 9H, tBu), 1.43 (m, 2H, C_4-H_2), 1.70 (m, 1H, CHγ Leu), 1.80 (m, 2H, CH_2β Leu), 3.25–3.40 (m, 4H, C_2-H_2 and C_5-H_2), 3.90–4.20 (m, 2H, C_3-H and CHα Leu), 5.05 (m, 1H, NH). MS: MH^+ (FAB) *m/z* 301. Anal. $C_{15}H_{28}N_2O_4$ (C, H, N).

5.1.6. 1-[(tert-butoxycarbonyl)-L-(O-benzyl)-Tyrosyl]-3(R, S)-pyrrolidinol ***2c***

From (*tert*-butoxycarbonyl)-L-(O-benzyl)-Tyrosine with quantitative yield (1.1 g). TLC (Hexane/EtOAc 1:9) R_f 0.24. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (s, 9H, tBu), 1.75 (m, 2H, C_4-H_2), 2.85–2.90 (m, 4H, C_5-H_2 and CH_2β Tyr), 3.4–3.55 (m, 2H, C_2-H_2), 4.11 (brs, 1H, C_3-H), 4.60 (m, 1H, CHα Tyr), 5.00 (s, 2H, CH_2 Bn), 5.40 (brs, 1H, NH), 6.85–7.10 (m, 4H, ArH Tyr), 7.20–7.32 (m, 5H, ArH Bn). MS: MH^+ (FAB) *m/z* 441. Anal. $C_{25}H_{32}N_2O_5$ (C, H, N).

5.1.7. 1-[(tert-butoxycarbonyl)-L-Phenylalanyl]-3(R, S)pyrrolidinol ***2d***

From (*tert*-butoxycarbonyl)-L-Phenylalanyl with 80% yield (2.6 g). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.50. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (s, 9H, tBu), 1.60 (m, 2H, C_4-H_2), 2.45 (m, 2H, C_2-H_2), 2.70 (m, 2H, CH_2β Phe), 3.25 (m, 2H, C_5-H_2), 4.00 (brs, 1H, C_3-H), 4.30 (m, 1H, CHα Phe), 5.15 (t, 1H, NH), 7.05 (m, 5H, ArH Phe). IR: 3335 (OH), 2959, 1750, 1680 cm^{-1}. MS: MH^+ (FAB) *m/z* 335. Anal. $C_{18}H_{26}N_2O_4$ (C, H, N). Compounds **3a**, **3b**, **3c**, and **3d** were obtained following the general procedure B.

5.1.8. 1-[(tert-butoxycarbonyl)-L-Glycyl]-3(R, S)-pyrrolidinone ***3a***

From **2a** with 27% yield (600 mg). TLC(CH_2Cl_2/MeOH 9:1) R_f 0.51. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (s, 9H, tBu), 2.35 (m, 2H, C_4-H_2), 3.55–3.70 (m, 6H, C_2-H_2 and C_5-H_2 and CH_2 Gly), 5.20 (s, 1H, NH). MS: MH^+ (FAB) *m/z* 243. Anal. $C_{11}H_{18}N_2O_4$ (C, H, N).

5.1.9. 1-[(tert-butoxycarbonyl)-L-Leucyl]-3-pyrrolidinone ***3b***

From **2b** with quantitative yield (470 mg). TLC (Hexane/EtOAc 1:1) R_f 0.38. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (m, 6H, CH_3δ Leu), 1.35 (s, 9H, tBu), 1.46 (m, 1H, CHγ Leu), 1.65 (m, 2H, CH_2β Leu), 2.52 (m, 2H, C_4-H_2), 3.70–3.90 (m, 4H, C_2-H_2 and C_5-H_2), 4.25–4.50 (m, 1H, CHα Leu), 5.30 (m, 1H, NH). MS: MH^+ (FAB) *m/z* 299. Anal. $C_{15}H_{26}N_2O_4$ (C, H, N).

5.1.10. 1-[(tert-butoxycarbonyl)-L-(O-benzyl)-Tyrosyl]-3-pyrrolidinone ***3c***

From **2c** with 60% yield (587 mg). TLC (Hexane/EtOAc 1:1) R_f 0.45. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, tBu) , 2.30 (m, 2H, C_4-H_2), 2.70–2.90 (m, 4H, C_5-H_2 and CH_2β Tyr), 3.70 (m, 2H, C_2-H_2), 4.30–4.55 (m, 1H, CHα Tyr), 4.90 (s, 2H, CH_2 benzyl), 5.25 (t, J = 6.5 Hz, 1H, NH), 6.85–7.00 (m, 4H, ArH Tyr), 7.28–7.35 (m, 5H, ArH benzyl). MS: MH^+ (FAB) *m/z* 439. Anal. $C_{25}H_{30}N_2O_5$ (C, H, N).

5.1.11. 1-[(tert-butoxycarbonyl)-L-Phenylalanyl]-3-pyrrolidinone ***3d***

From **5**, with 90% yield (2 g). TLC (Hexane/EtOAc 3:2) R_f 0.54. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (s, 9H, tBu), 2.25 (m, 2H, C_4-H_2), 2.60–2.80 (m, 4H, C_2-H_2 and CH_2β Phe), 3.65 (m, 2H, C_5-H_2), 4.25–4.55 (m, 1H, CHα Phe), 5.70 (t, 1H, NH), 7.05 (m, 5H, ArH Phe). IR: 2980, 1762, 1760, 1640 cm^{-1}. MS: MH^+ (FAB) *m/z* 333. Anal. $C_{18}H_{24}N_2O_4$ (C, H, N).

5.1.12. 1-[(tert-butoxycarbonyl)-L-Tyrosyl]-3-pyrrolidinone ***3e***

To a stirred solution of **3c** (36 mg, 0.082 mmol) in absolute ethanol (1 mL) was added, under nitrogen atmosphere, 10% Pd–C^{12} (1:1 catalyst/substrate by weight) followed by 1,4-cyclohexadiene (77 μL, 0.82 mmol). The suspension was stirred at room temperature for 2 h. The catalyst was removed by filtration through Celite and was purified by PLC to give the desired compound in 55% yield. TLC (Hexane/EtOAc 1:1) R_f 0.24. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (s, 9H, tBu), 2.38 (m, 2H, C_4-H_2), 2.79–2.90 (m, 4H, C_5-H_2 and CH_2β Tyr), 3.80 (m, 2H, C_2-H_2), 4.30–4.60 (m, 1H, CHα Tyr), 5.33 (t, 1H, NH), 6.65–6.93 (m, 4H, ArH Tyr). MS: MH^+ (FAB) *m/z* 349. Anal. $C_{18}H_{24}N_2O_5$ (C, H, N).

5.1.13. 1-L-Phenylalanyl-3(R, S)-pyrrolidinol trifluoroacetate salt ***4***

The title compound was prepared in a quantitative yield from **6** (2 g, 5.80 mmol) by deprotection with trifluoroacetic acid (TFA). The compound **6** (1.0 equiv.) was treated with a solution of TFA (10.0 equiv.) in CH_2Cl_2 for 2–4 h at room temperature. After evaporation and coevaporation with toluene, the crude product was used in the following step without any further purification. Compounds **8**, **9** were prepared from **7** according to general procedure A.

5.1.14. 1-[N-(Nicotinyl-3-carboxamide)-L-Phenylalanyl]-3-pyrrolidinone ***5***

From nicotinic acid **7** with 64% yield (131 mg). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.36. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (m, 2H, C_4H_2), 2.70–3.05 (m, 4H, C_2H_2 and CH_2β Phe), 3.45–3.75 (m, 2H, C_5H_2), 4.70–4.85 (m, 1H, CHα Phe), 7.05–7.15 (m, 5H, ArH Phe), 7.40–8.75 (m, 4H, ArH Nicotinyl). IR: 1761 cm^{-1} MS: MH^+ (FAB) *m/z* 338. Anal. $C_{19}H_{19}N_3O_3$ (C, H, N).

5.1.15. 1-[N-(2-Quinolinyl-carboxamide)-L-Phenylalanyl]-3-pyrrolidinone ***6***

From Quinaldic acid with 38% yield (110 mg). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.71. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (m, 2H, C_4-H_2), 3.00–3.90 (m, 6H, C_2-H_2 and CH_2β Phe and C_5-H_2), 4.85 (m, 1H, CHα Phe), 7.05–7.20 (m, 5H, ArH Phe), 7.40–8.15 (m, 4H, ArH Quinaldyl). IR: 1761 cm^{-1} MS: MH^+ (FAB) *m/z* 388. Anal. $C_{23}H_{21}N_3O_3$ (C, H, N). Compounds **10**, **11**, **12**, **13**, **14**, were prepared from **7** according to general procedure C.

5.1.16. 1-[N-(benzoyl-carboxamide)-L-Phenylalanyl]-3-pyrrolidinone ***7***

From benzoyl chloride, 57% yield (57 mg). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.64. ^{1}H-NMR (CD_3OD) δ: 2.38 (m, 2H, C_4-H_2), 3.10 (m, 2H, C_2-H_2), 3.65–4.10 (m, 4H, CH_2β Phe and C_5-H_2), 4.90–5.20 (m, 1H, CHα Phe), 7.1–7.75 (m, 10H, ArH Phe and Benzoyl). MS: MH^+ (FAB) *m/z* 337. Anal. $C_{20}H_{20}N_2O_3$ (C, H, N).

5.1.17. 1-[N-(N, N-diphenylcarbamoyl) -L-Phenylalanyl]-3-pyrrolidinone ***8***

From diphenylcarbamoyl chloride, 17% yield (44 mg). TLC (Hexane/EtOAc 3:7) R_f 0.29. ^{1}H-NMR (CD_3OD) δ: 2.42 (m, 2H, C_4-H_2), 2.95 (m, 2H, C_2-H_2), 3.65–3.91 (m, 4H, CH_2β Phe and C_5-H_2), 4.65–4.95 (m, 1H, CHα Phe), 5.30 (t, 1H, NH) 7.20–7.45 (m, 15H, ArH). MS: MH^+ (FAB) *m/z* 428. Anal. $C_{26}H_{25}N_3O_3$ (C, H, N).

5.1.18. 1-[N-(N-methyl, N-phenylcarbamoyl)-L-Phenylalanyl]-3-pyrrolidinone ***9***

From N-methyl, N-phenylcarbamoyl chloride, 31% yield (68 mg). TLC (Hexane/EtOAc 3:7) R_f 0.12. ^{1}H-NMR (CD_3OD) δ: 2.45 (m, 2H, C_4-H_2), 2.88 (m, 2H, C_2-H_2), 3.24 (s, 3H, CH_2 carbamoyl), 3.60–4.10 (m, 4H, $CH_2\beta$ Phe and C_5-H_2), 4.60–4.90 (m, 1H, CHα Phe), 5.10 (t, J = 6.5 Hz, 1H, NH) 7.20–7.41 (m, 10H, ArH). MS: MH^+ (FAB) *m/z* 366. Anal. $C_{21}H_{23}N_3O_3$ (C, H, N).

5.1.19. 1-[N-(octyloxycarbonyl)-L-Phenylalanyl]-3-pyrrolidinone ***10***

From octyl chloroformate, 41% yield (48 mg). TLC (Hexane/EtOAc 6:3) R_f 0.29. ^{1}H-NMR (CD_3OD) δ: 0.91 (t, 3H, CH_3 octyl), 1.30 (m, 12H, CH_2 octyl), 1.60 (m, 2H, O-CH_2 octyl), 2.40 (m, 2H, C_4-H_2), 3.02 (m, 2H, C_2-H_2), 3.70–4.00 (m, 4H, $CH_2\beta$ Phe and C_5-H_2), 4.50–4.75 (m, 1H, CHα Phe), 5.55 (t, 1H, NH) 7.20–7.35 (m, 5H, ArH Phe). MS: MH^+ (FAB) *m/z* 389. Anal. $C_{22}H_{22}N_2O_4$ (C, H, N).

5.1.20. 1-[N-(benzoyloxycarbonyl)-L-Phenylalanyl]-3-pyrrolidinone ***11***

From benzyl chloroformate, 15% yield (16 mg). TLC (CH_2Cl_2/MeOH 9:1) R_f 0.68. ^{1}H-NMR (CD_3OD) δ: 2.55 (m, 2H, C_4-H_2), 2.90 (m, 2H, C_2-H_2), 3.55–3.90 (m, 4H, $CH_2\beta$ Phe and C_5-H_2), 4.35–4.60 (m, 1H, CHα Phe), 4.90 (s, 2H, CH_2 benzyl) 7.15 (m, 10H, ArH Phe and Benzyl). MS: MH^+ (FAB) *m/z* 367. Anal. $C_{21}H_{22}N_2O_4$ (C, H, N).

5.2. Biological methods: in vitro inhibition of HIV-1 replication in MT_4 lymphocytes

The fusogenic effect of HIV in MT_4 cell line was determined as described by Rey et al. [15, 16]. A total of 3 x 10^5 MT_4 cells were infected with 100 µL of diluted virus for 1 h at 37 °C. After three washes, the infected cells were cultured in 24-well cell culture plates in the presence of inhibitor. The appearance of *syncitia* was measured with an inverted optical microscope 5 days after infection. The inhibition concentration was expressed as the concentration of the tested compound which causes 50% inhibition of *syncitia* formation (IC_{50}) but was not toxic for the cells.

For toxicity testing, three replication cultures of each uninfected MT_4 cells (2 x 10^5 cells) were incubated with various concentrations of compounds. Cell viability was determined 6 days from drug addition by trypan blue exclusion.

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