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Pentacycloundecane-based inhibitors of wild-type C-South African HIV-protease

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ABSTRACT

In this study, we present the first account of pentacycloundecane (PCU) peptide based HIV-protease inhibitors. The inhibitor exhibiting the highest activity made use of a natural HIV-protease substrate peptide sequence, that is, attached to the cage (PCU–EAIS). This compound showed nanomolar IC_{50} activity against the resistance-prone wild type C-South African HIV-protease (C-SA) catalytic site via a norstatine type functional group of the PCU hydroxy lactam. NMR was employed to determine a logical correlation between the inhibitory concentration (IC_{50}) results and the 3D structure of the corresponding inhibitors in solution. NMR investigations indicated that the activity is related to the chirality of the PCU moiety and its ability to induce conformations of the coupled peptide side chain. The results from docking experiments coincided with the experimental observed activities. These findings open up useful applications for this family of cage peptide inhibitors, considering the vast number of alternative disease related proteases that exist.

The introduction of HIV-protease inhibitors (PIs) in the mid-1990s dramatically changed the situation for AIDS patients.[1–3] These inhibitors reduced the virus proliferation and subsequent infection and this success made the HIV aspartic protease the prime target for AIDS therapies.[4] Thus inactivation of this machinery results in the production of non-infectious virions.[5] Even though a number of highly effective inhibitors have been produced major obstacles including low oral bioavailability, rapid metabolism and unspecific protein binding[1–3] are still experienced. Increasing numbers of HIV infected patients with severe treatment-associated complications and related deaths make the AIDS pandemic more complex than ever.[6] The increase of drug-resistant proteases as a result of mutations has led to an urgent demand for new drug candidates.[4,5] Mutations associated with PIs have been observed more commonly in the flap and in the active centre, but have also occurred in other sites of the enzyme.[4,5] The HIV-protease subtype C is mainly found in South Africa (C-SA) and shows rapid development of resistance to common drugs.[4]

It was postulated that the incorporation of cage frameworks into biologically active molecules would enhance their activity[7–10] and retard biodegradation.[11,12] Various cage systems have shown biological activity[8,13–15] and enhancement of the transport of the drug across cell membranes.[8,16,17] Adamantane was previously coupled to azidothymidine (AZT).[8,18] Brookes et al.[19] claimed in the early 1990s that the steric bulk of the cage increased the regiospecificity of the drug to particular receptor sites,[20–22] which was confirmed by Geldenhuys et al.[8] This fact was supported by our recent study for various adamantane diamine compounds designed as potential anti-tuberculosis drugs.[23]

Stereochemistry of molecules plays an important role in modulating their biological activity;[24] Kempf et al. reported a 20-fold increase in binding affinity to the recombinant HIV-PR [24] catalytic site of the (*R*,*R*)- and (*R*,*S*)-dihydroxyethylene transition-state isostere compared to the (*S*,*S*)-conformation.[25] Interestingly, until now there is no compelling evidence that enantiomeric cage

Abbreviations: PIs, protease inhibitors; HIV-PR, HIV-protease; AZT, azidothymidine; CNS, central nervous system; PCU, pentacycloundecane.

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Table 1
IC_{50} values for the inhibition of wild type C-SA HIV-1 protease by PCU lactam peptides and control compounds. Structural information from EASY–ROESY NMR and binding energies from docking experiments are also presented

PCU–R1	R2	Yield (%)	Cage–peptide interaction[d] (CP)	Peptide–peptide interaction[e] (PP)	Docked binding energies/kcal mol^{-1}	IC_{50} (μM)
PCU–EAIS[a]	H	47	Yes	—	−10.23[f]	0.078 ± 0.0035
Phenol–EAIS[b]		87	—	—	—	10.0 ± 3.53
PCU–EAIS[a]	Ac	27	—	—	−7.06	>10
PCU–EAI[a]	H	23	Yes	—	—	0.5 ± 0.035
PCU–EVIS[a]	H	33	Yes	—	—	2.0 ± 0.18
PCU–QAIS[a]	H	20	—	—	—	5.0 ± 0.71
PCU–AISa[c]	H	18	Yes	—	−9.41	0.5 ± 0.035
PCU–AISb[c]	H	17	—	Yes	−9.27	10.0 ± 1.06
Atazanavir						0.004 ± 0.00071
Lopinavir						0.025 ± 0.0014

[a] Diastereomeric mixtures.
[b] Enantiopure.
[c] Diastereomer was separated with semi-preparative HPLC.
[d] CP: ROE interaction of the cage protons with a peptide NH in solution was observed.
[e] PP: ROE interaction of the same peptide NH with the peptide side chain in solution was observed.
[f] PCU–EAISa gave the lowest docked energy and was recorded here.

compounds give any difference in selectivity towards receptor sites.[26,27]

With these concepts in mind, we designed PCU lactam-peptide derivatives as novel potential transition-state analogues for HIV-PR inhibition. The PCU lactam contains a hydroxyl carbonyl functional group, resembling a norstatine type transition-state isostere.[28] Furthermore, it consists of a very stable amide bond[29] that can potentially interact with the catalytic residues in the active site. We have coupled various peptide sequences to the racemic PCU–cage (see Table 1 and Fig. 1). The first sequence was selected from a natural HIV-protease substrate, FEAIS[30] where F is replaced with the cage lactam to potentially ensure high specificity of the proposed inhibitor to the active site. This peptide sequence was then systematically varied.

Although the PCU–lactam has a non-conventional bulky structure,[31] it has been reported that the S1 and S1′ sub-binding site of the HIV-PR accommodates bulky hydrophobic substituents.[28] We therefore proposed that the PCU group could potentially occupy these sub-sites. Even though the S2 and S2′ pockets are also of a hydrophobic nature, both hydrophilic and hydrophobic side chains from the *gag* and *gag-pol* polyproteins can occupy these sites.[6] Based on literature and the HIV substrate sequence, we envisaged that either glutamic acid or alanine in the peptide chain would be a preferred binder to the S2 and S2′ sub-sites.

The PCU–peptides were obtained as diastereomers[†] from the coupling of the enantiopure short peptide to the racemic PCU–lactam.[33,34] The sequences of the different inhibitor PCU–peptides are listed in Table 1. The peptides were tested for HIV-PR inhibition activity and the results are also reported in Table 1.

The cage peptide sequence (PCU–EAIS) chosen from the natural HIV-protease substrate (FEAIS[30]) gave the best inhibition activity (IC_{50} = 78 nM). To understand the significance of the role of the cage in the inhibition of the HIV-PR active centre it was replaced with hydroxyl-phenylamine to produce the control peptide (Phenol–EAIS), which had an IC_{50} = 10.0 μM, a 120-fold decrease when compared to PCU–EAIS. Furthermore, it is realised that the hydroxyl-group at position C8 of the PCU–cage is essential, because after acetylation, activity was dramatically decreased (Ac-PCU–EAIS with IC_{50} >10.0 μM).

It is notable that the systematic removal or substitution of the peptide amino acid residues resulted in a decrease in inhibitory activity. Removal of the serine segment resulted in a six-fold decrease (PCU–EAI with IC_{50} = 0.5 μM), the substitution of alanine with valine to PCU–EVIS caused a 25-fold decrease, substitution of the glutamic acid in PCU–EAIS with glutamine resulted in PCU–QAIS with a 64-fold decrease. Removal of the glutamic acid yielded diastereoisomers PCU–AISa (IC_{50} = 0.5 μM) and PCU–AISb (IC_{50} = 10.0 μM), which were separated by preparative HPLC. These diastereomers exhibited a 20-fold difference in activity.

The role of stereochemistry of molecules on their biological activity motivated us to investigate the influence of the cage chirality on the HIV PR inhibition using applied high-resolution heteronuclear NMR techniques. EASY–ROESY[32] experiments record through space ROE proton–proton interactions and enabled us to deduce crucial information about the 3D structures of these short cage peptides. Certain carbon signals of PCU–AISa were split (C-1 and C-10). The splitting was reduced upon heating and disappeared completely at 333 K (60 °C), indicating that different interconvertible conformations exist. These conformations are most certainly induced by the chiral cage structure and are remarkably stable in the light of high temperature (60 °C) required to obtain inter-conversion.

PCU–AISa showed long-range ROE correlations between the NHc amide protons of alanine with the PCU protons H-1, H-10 and H-3 and the side chain methyl protons of alanine (H-3′) also showed an interaction with H-3 of the cage. This first conformation was named CP (long-range cage–peptide interaction). For PCU–AISb a ROE interaction of the alanine-NHc (8.10 ppm) with the serine side chain (H-8′—see Fig. 2) was observed and named PP (peptide–peptide interaction).

We therefore concluded that the chirality of the cage induces different secondary structures on the peptide chains either interacting with the cage (CP) or with the peptide itself (PP). The difference in HIV-PR inhibition activities of these two inhibitors (PCU–AISa is 20 times more active that PCU–AISb) suggests that the peptide conformations induced by the cage enantiomers play an important role in the activity of these inhibitors.

The EASY–ROESY spectrum of the most potent inhibitor, the diastereomeric mixture of PCU–EAIS revealed the same dominant CP conformation as observed for PCU–AISa. We could not detect the PP conformation as present in PCU–AISb, but a second, most likely elongated side chain conformation is present indicated by the splitting of C-1 and C-10 signals. To prove that the CP conformation is biological relevant and present in the conditions of binding to the HIV-PR, NMR spectra of PCU–EAIS were also recorded in an aqueous buffer (D_2O) used for the HIV-PR inhibition assay; similar EASY–ROESY correlations and HSQC spectra were observed as with DMSO (see Table 11 in the Supplementary data).

[†] The chirality of the cage diastereomers were deduced from the computational work. Accordingly the stereochemistry for the cage peptides are 8-(*R*)-11-(*R*)-PCU–AISa and 8-(*S*)-11-(*S*)-PCU–AISb.

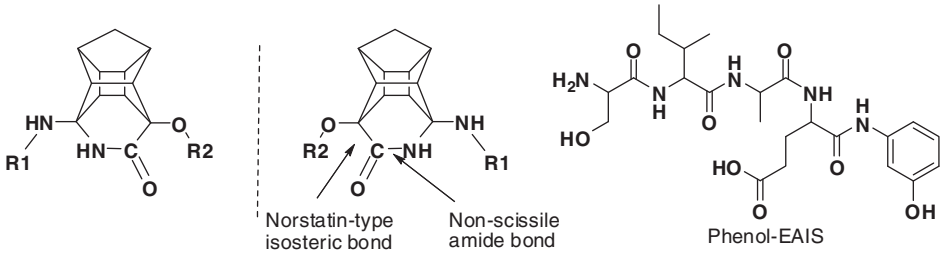

Figure 1. The structures of PCU lactam peptides and control compounds.

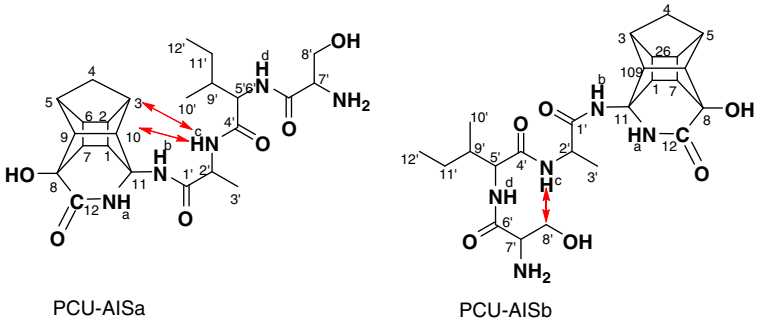

Figure 2. EASY-ROESY long-range correlations observed for the two cage peptide diastereomers.

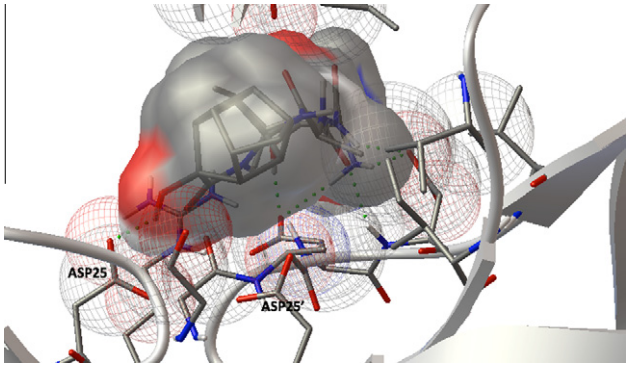

Figure 3. Close up view of the lowest energy docked structure for PCU–AISa (CP) with C-SA HIV-PR. The 3D presentations for all docking results are available as PDB files with the Supplementary data.

The ROESY spectra of compounds PCU–EAI and PCU–EVIS also only revealed the CP conformation, but not the PP conformation. The ROESY spectra of PCU–QAIS and PCU–AISb showed no sign of CP interactions and these peptides were found to be the weakest inhibitors in this group. This suggests that the in solution chiral induced conformation of the cage peptides can be correlated to HIV-PR inhibition activities.

Docking of the most potent inhibitor PCU–EAISa, revealed that the hydroxyl-group of the PCU–lactam (the proposed norstatine moiety) was forming a hydrogen bond with Asp25 of the dimeric catalytic triad residues Asp25-Thr26-Gly27 (A/B chains), most noticeable Asp25 and Asp25′ (see Fig. 3). The docked structures of Ac-PCU–EAISa and PCU–EAISb failed to reveal any interaction between the cage lactam and the two Asp25 segments of the PR. The binding energies of the docked inhibitor enzyme complexes are reported in Table 1.

The crude docked binding energies are largely in accordance with the in vitro HIV-PR inhibition results. Docking of the same inhibitors with C-SA PR where only one of the Asp25 residues was protonated, gave similar docking behaviour to the unprotonated Asp25 residues.

Conclusions: The combination of the PCU–cage with a HIV-PR substrate peptide yields a potential lead compound for HIV-PR inhibition. The cage with the natural HIV-protease substrate (PCU–EAIS) exhibits the best activity (IC_{50} of 78 nM). Variation of this sequence leads to reduced activity. Removal of the cage from the sequence results in a dramatic drop in inhibition activity. Protection of the cage hydroxyl-group which is proposed to be a norstatine type isosteric bond, resulted in a complete loss of inhibitory activity. These observations confirm the vital contribution of the specific PCU–cage. All active inhibitors exhibited the same EASY-ROESY interactions between the cage protons and the peptide side chain. For the first time the effect of PCU cage chirality on bioactivity was demonstrated. The docking results largely agreed with the in vitro observed activities. Employment of advanced computational and NMR techniques, combined with a systematic variation of alternative cage analogues and peptide sequences should lead to the design of more active cage peptide inhibitors in future. The possibility of the cage peptides inhibit some of the many alternative diseases related protease families will also be investigated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.105.

References and notes

1. Rodriguez-Barrios, F.; Gago, F. *Curr. Top. Med. Chem.* **2004**, *4*, 991.
2. Zhang, X. Q.; Schooley, R. T.; Gerber, J. G. *J. Infect. Dis.* **1999**, *180*, 1833.
3. Acosta, E. P.; Kakuda, T. N.; Brundage, R. C.; Anderson, P. L.; Fletcher, C. V. *Clin. Infect. Dis.* **2000**, *30*, S151.

4. Velaquez-Campoy, A.; Vega, S.; Fleming, E.; Bacha, U.; Sayed, Y.; Dirr, H. W.; Freire, E. *AIDS Rev.* **2003**, *5*, 165.
5. Lee, T.; Laco, G. S.; Torbett, B. E.; Fox, H. S.; Lerner, D. L.; Elder, J. H.; Wong, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 939.
6. Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249.
7. James, B.; Viji, S.; Mathew, S.; Nair, M. S.; Lakshmanan, D.; Kumar, R. A. *Tetrahedron Lett.* **2007**, *48*, 6204.
8. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. *Med. Res. Rev.* **2005**, *25*, 21.
9. Oliver, D. W.; Malan, S. F. *Med. Chem. Res.* **2008**, *17*, 137.
10. Oliver, D. W.; Dekker, T. G.; Snyckers, F. O.; Fourie, T. G. *J. Med. Chem.* **1991**, *34*, 851.
11. Bisetty, K.; Corcho, F. J.; Canto, J.; Kruger, H. G.; Perez, J. J. *J. Pept. Sci.* **2006**, *12*, 92.
12. Ito, F. M.; Petroni, J. M.; de Lima, D. P.; Beatriz, A.; Marques, M. R.; de Moraes, M. O.; Costa-Lotufo, L. V.; Montnegro, R. C.; Magalhaes, H. I. F.; Pessoa, C. D. O. *Molecules* **2007**, *12*, 271.
13. Oliver, D. W.; Dekker, T. G.; Snyckers, F. O. *Arzneimittelforschung/Prog. Drug Res.* **1991**, *41–1*, 549.
14. Inamoto, Y.; Aigami, K.; Kadono, T.; Nakayama, H.; Takatsuki, A.; Tamura, G. *J. Med. Chem.* **1977**, *20*, 1371.
15. Aigami, K.; Inamoto, Y.; Takaishi, N.; Fujikura, Y.; Takatsuki, A.; Tamura, G. *J. Med. Chem.* **1976**, *19*, 536.
16. Schwab, R. S.; England, A. C.; Dc, P.; Young, R. R. *J. Am. Med. Assoc.* **1969**, *208*, 1168.
17. Neumeyer, J. L. *Principles of Medicinal Chemistry*; Lea and Febiger: London, Philadelphia, 1989. p 223.
18. Tsuzuki, N.; Hama, T.; Kawada, M.; Hasui, A.; Konishi, R.; Shiwa, S.; Ochi, Y.; Futaki, S.; Kitagawa, K. *J. Pharm. Sci.* **1994**, *83*, 481.
19. Brookes, K. B.; Hickmott, P. W.; Jutle, K. K.; Schreyer, C. A. *S. Afr. J. Chem.* **1992**, *45*, 8.
20. Gerzon, K.; Kau, D. *J. Med. Chem.* **1967**, *10*, 189.
21. Rapala, R. T.; Kraay, R. J.; Gerzon, K. *J. Med. Chem.* **1965**, *8*, 580.
22. Voldeng, A. N.; Bradley, C. A.; Kee, R. D.; King, E. L.; Melder, F. L. *J. Pharm. Sci.* **1968**, *57*, 1053.
23. Onajole, O. K.; Govender, P.; van Heiden, P. D.; Kruger, H. G.; Maguire, G. E. M.; Wiid, I.; Govender, T. *Eur. J. Med. Chem.* **2010**, *45*, 2075.
24. Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A. *J. Med. Chem.* **2000**, *43*, 1271.
25. Kempf, D. J.; Norbeck, D. W.; Codacovi, L. M.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. A.; Knigge, M. F.; Vasavanonda, S.; Craigkennard, A.; Saldivar, A.; Rosenbrook, W.; Clement, J. J.; Plattner, J. J.; Erickson, J. *J. Med. Chem.* **1990**, *33*, 2687.
26. Grobler, E.; Grobler, A.; Van der Schyf, C. J.; Malan, S. F. *Bioorg. Med. Chem.* **2006**, *14*, 1176.
27. Malan, S. F.; Dockendolf, G.; Van der Walt, J. J.; van Rooyen, J. M.; van der Schyf, C. J. *Pharmazie* **1998**, *53*, 859.
28. Brik, A.; Wong, C. H. *Org. Biomol. Chem.* **2003**, *1*, 5.
29. Kruger, H. G.; Martins, F. J. C.; Viljoen, A. M. *J. Org. Chem.* **2004**, *69*, 4863.
30. Lee, J.; Huh, M. S.; Kim, Y. C.; Hattori, M.; Otake, T. *Antiviral Res.* **2010**, *85*, 425.
31. Kruger, H. G.; Mdluli, P.; Power, T. D.; Raasch, T.; Singh, A. *J. Mol. Struct. (Theochem.)* **2006**, *771*, 165.
32. Thiele, C. M.; Petzold, K.; Schleucher, J. *Chem. Eur. J.* **2009**, *15*, 585.
33. Marchand, A. P.; Allen, R. W. *J. Org. Chem.* **1974**, *39*, 1596.
34. Marchand, A. P. In *Advances in Theoretically Interesting Molecules*; JAI Press: Greenwich CT, 1989; Vol. 1, p 357.