Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3614–3617

Dipeptidyl nitriles as human dipeptidyl peptidase I inhibitors

Jon Bondebjerg,[a,*] Henrik Fuglsang,[a] Kirsten Rosendal Valeur,[a] John Pedersen[b] and Lars Nærum[a]

[a]*Arpida A/S, Vesterbrogade 188, DK-1800 Frederiksberg C, Denmark*
[b]*Prozymex A/S, Dr. Neergaards Vej 17, DK-2970 Hørsholm, Denmark*

Received 8 December 2005; revised 16 January 2006; accepted 18 January 2006
Available online 2 May 2006

Abstract—Using a dipeptide nitrile scaffold we have identified a potent and selective inhibitor of human dipeptidyl peptidase I.

Human dipeptidyl peptidase I (hDPPI, cathepsin C, EC 3.4.14.1) is a highly conserved lysosomal cysteine exopeptidase that belongs to the papain family.[1–5] The enzyme is constitutively expressed in many tissues with highest levels in lung, kidney, liver, and spleen. DPPI is a 200 kDa homotetramer consisting of four identical subunits, each composed of three different chains: a heavy chain, a light chain, and an exclusion domain.[6a,b] The S1 pocket is located near the surface, exposed to the solvent. The S2 pocket is deep and hydrophobic, with a chloride ion and two solvent molecules at the bottom, and Asp 1 at the entrance to anchor the substrate via an electrostatic interaction. The exclusion domain blocks extension beyond the S2 pocket, and thus prevents endopeptidase activity. Once activated,[7] DPPI catalyzes sequential removal of dipeptides from the N-termini of peptide and protein substrates with broad specificity.[3,8,9] However, DPPI only cleaves substrates with a free N-terminal amino group and does not cleave substrates with P1 or P1′ Pro, P1 Ile, and P2 ornithine, Lys or Arg.

DPPI is an important enzyme in lysosomal protein degradation and recent studies in DPPI knock-out mice show that DPPI functions as a key enzyme in the activation of granule serine proteases in cytotoxic T lymphocytes and natural killer cells (granzymes A and B),[3,10a] mast cells (chymase and tryptase),[10b] and neutrophils (cathepsin G, proteinase 3, and elastase).[11]

Dominant-negative mutations within the human DPPI genes are linked to the Papillon-Lefèvre syndrome,[12] a disease characterized by early periodontitis, palmoplantar hyperkeratosis, and a predisposition to bacterial infections. This indicates that DPPI plays an important role in the immune system. Recent data[13] show that disruption of DPPI genes is beneficial for the survival from sepsis and indicate that DPPI is potentially a new target for the treatment of sepsis. Thus, in combination with predictive animal models, inhibitors of DPPI will be beneficial for further studies on the physiological role of DPPI.

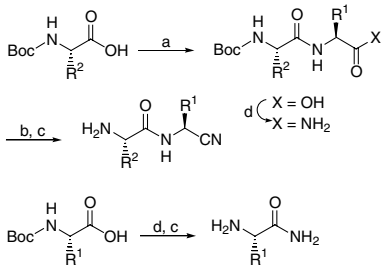
Inhibitors of DPPI include dipeptide diazomethyl ketones,[14a,b] a dipeptide nitrile (**1**),[15] dipeptide vinyl sulfones,[16] dipeptide acyloxymethyl and fluoromethyl ketones,[16] dipeptide *O*-acyl hydroxamic acids,[17] arginine-based peptides,[18] and phosphinic tripeptides.[19] The naturally occurring cysteine protease inhibitor E-64 also inhibits DPPI at high concentrations.[20]

We have recently reported semicarbazide-derived inhibitors of hDPPI.[21] Herein, we report a series of dipeptidyl nitriles as inhibitors of hDPPI. Nitriles have previously been reported as inhibitors of cathepsin B,[22] cathepsin K,[23a,b] cathepsin L,[23b] cathepsin S,[23b,24] papain,[23b] and dipeptidyl peptidase IV (DPPIV).[25a]

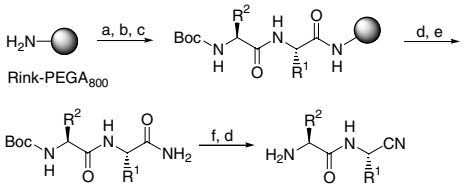
The nitriles were synthesized by dehydration of the corresponding dipeptide amides, using $POCl_3$ and imidazole in pyridine, as previously reported (Schemes 1 and 2).[25a,b] The protected dipeptide amides were either prepared in solution (entries, **2**,**8**–**9**, Scheme 1) or on solid phase using the Rink amide linker (entries **3**–**7**, Scheme 1). Solid phase yields (9–21%) were generally lower than

Keywords: Dipeptidyl nitriles; Cathepsin C; Dipeptidyl peptidase I; DPPI; Inflammation; Sepsis.
* Corresponding author. Tel.: +4533780018; fax: +4570222365; e-mail: jbondebjerg@arpida.dk

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.01.102



Scheme 1. Reagents and conditions: (a) CDI, THF; then $H_2NCH(R_1)COOH$, base or $H_2NCH(R_1)CONH_2{\cdot}HCl$, base; (b) $POCl_3$, imidazole, pyridine, −40 °C to room temperature; (c) TFA; (d) CDI, THF; then NH_3 in 2-propanol.

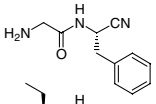
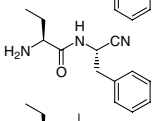
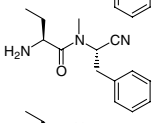
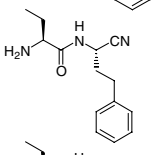
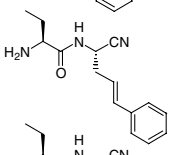
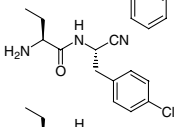
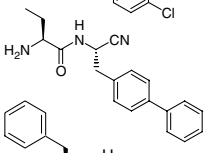
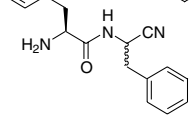
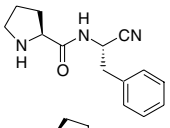
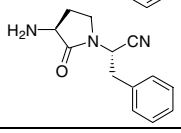


Scheme 2. Reagents and conditions: (a) $FmocHNCH(R_1)COOH$, TBTU, NEM, DMF; (b) 20% piperidine, DMF; (c) $BocHNCH(R_2)$-COOH, TBTU, NEM, DMF; (d) TFA; (e) Boc_2O, NEM; (f) $POCl_3$, imidazole, pyridine, −40 °C to room temperature.

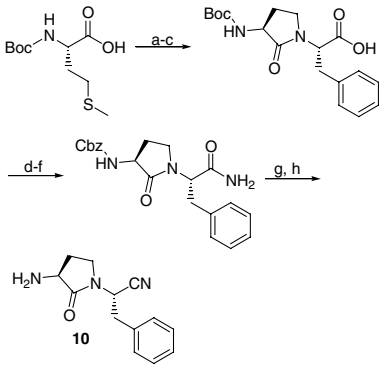
those in solution (21–48%). In the former approach, the amides were formed from the carboxylic acids via activation with CDI, followed by treatment with ammonia in propanol. In the latter approach, it was necessary to re-protect the amino group with Boc_2O after cleavage from the solid phase, prior to performing the dehydration. Entry **3** was prepared according to Scheme 2, starting from Fmoc-*N*-Me-Phe-OH. All final products were purified by preparative HPLC (Gilson HPLC system, Zorbax 300SB RP-18 21.2 mm × 25 cm column, 0.1% TFA in water/acetonitrile eluent system in a linear gradient, flow 15 mL/min., UV/vis-155 detector at 215 and 254 nm) to >95% purity and characterized by LC–MS and ^{1}H NMR. Several of the purified nitriles were obtained as a mixture of isomers, presumably diastereomers arising from racemization. Tripeptide nitriles have been previously shown to be prone to racemization due to the increased acidity of the nitrile α-position.[25b] The A:B isomeric ratio was determined by ^{1}H NMR (Table 1), as distinct isomeric resonances were evident. For entry **8** it was possible to isolate the isomers by preparative HPLC.

Entry **10** was prepared (Scheme 3) via Boc-methionine, using CDI and phenylalanine methyl ester hydrochloride to assemble the crude dipeptide in high yield (96%) and purity (>95 % by HPLC). Methylation with methyl iodide afforded the sulfonium iodide in quantitative yield. Cyclization to form the lactame was achieved with NaH in DMF/DCM 1:1, giving the crude carboxylic acid in 58% yield and >94% HPLC purity directly after workup. Approximately 10% racemization of the Phe α-carbon was observed in ^{1}H NMR, as previously reported.[26] Attempts to form the nitrile in the presence of the Boc-protecting group failed, and this was thus exchanged for the more robust Cbz group in two steps (90% overall, no purification). Activation of the carboxylic acid with CDI and subsequent treatment with ammonia in 2-propanol yielded 29% of the carboxamide after purification. Dehydration with trifluoroacetic anhydride (TFAA) in DCM afforded the protected nitrile in 48% yield after HPLC purification. Finally, the Cbz group was removed by transfer hydrogenation, using cyclohexene and Pd/C in ethanol at 80 °C, to give the desired product **10** in 38% yield after HPLC purification (>99% purity). This compound was a 9:1 mixture of isomers according to ^{1}H NMR.

Table 1. IC_{50} values for **1**–**10**

Entry	Structure	IC_{50} (DPPI) (nM)	Ratio[a] A:B
1		4787 ± 456	N.D.[b]
2		172 ± 13	3:1
3		94 ± 4% remaining enzymatic activity at 10 μM	5:1
4		822 ± 140	N.D.[b]
5		137 ± 27	7:1
6		96 ± 8	6:1
7		13 ± 3	11:1
8		294 ± 16[c] 418 ± 27[d] 6296 ± 123[e]	Isomers separated
9		9637 ± 208	Trace of isomer
10		N.I.[f]	9:1

[a] Determined by ^{1}H NMR.
[b] Not detectable.
[c] Isomer A.
[d] Isomer A + B (44:55 ratio by HPLC).
[e] Isomer B.
[f] No inhibition at 20 μM.



Scheme 3. Reagents and conditions: (a) CDI, H-Phe-OMe·HCl, DIPEA (96%); (b) MeI (100%); (c) NaH, DMF/DCM (58%, crude); (d) TFA/DCM (100%); (e) CbzCl, DIPEA (90%, crude); (f) CDI, then NH_3 in propanol (29%); (g) TFAA/DCM (48%); (h) Pd/C, cyclohexene/EtOH, 80 °C (38%).

Entry **1**, previously reported as an inhibitor of hDPPI with $K_i = 2700$ nM,[15] was found to have $IC_{50} = 4787 \pm 456$ nM in our assay. Using this as a starting point, and having previously identified (*S*)-2-aminobutyric acid as an optimal P2 residue for a series of semicarbazide-based inhibitors of DPPI,[21] led us to prepare **2** with a >25-fold increase in potency ($IC_{50} = 172 \pm 13$ nM). However, N-methylation of the amide resulted in almost complete loss of activity at 10 μM (entry **3**), presumably owing to unfavorable geometry and disruption of the hydrogen bonding network, as previously proposed to explain intolerance toward P1 Pro.[6a] Subsequently, we focused on exploring the P1 substituent with respect to potency. Attention was turned to hydrophobic, aromatic residues, known to be good P1 substrate residues.[8] Homologation of the P1 side chain gave a >4-fold reduction in potency (entry **4**), whereas **5** with a 3-phenyl-2-propenyl P1 side chain was found to be slightly more potent compared to **2**. However, when comparing the results it should be kept in mind that **2** has the lowest A:B isomeric ratio of the series. Interestingly, *p*-chloro substitution is well tolerated (entry **6**), and *p*-phenyl substitution of **2** resulted in the most potent inhibitor in the series (entry **7**, $IC_{50} = 13 \pm 3$ nM). The results are consistent with the S1 pocket being located at the surface of the enzyme, exposed to the solvent, and tolerant to many different residues.[8]

Introduction of P2 Pro (entry **9**) resulted in loss of activity, as did the presence of a P2 lactame ring (entry **10**). Both these modifications presumably affect the interactions with Asp 1 and Gly 277 in the S2 binding site.[6a,b,8] Compound **7** was also found to be selective for DPPI over cathepsins B, H, and L, and did not show significant inhibition of the CYP450 enzymes (Table 2).

In conclusion, we have identified **7**, a potent and selective dipeptide nitrile inhibitor of hDPPI, which shows only weak inhibition of the CYP450 enzymes. The compound was rapidly metabolized in rat liver microsomes. Further exploration of this class of inhibitors can lead to other potent compounds for potential use in treatment of inflammatory diseases (Table 3).

Table 2. Selectivity profile for **7**

Enzyme	IC_{50} (nM)
Cathepsin B	> 10,000[a]
Cathepsin H	> 10,000[a]
Cathepsin L	> 10,000[a]
CYP1A2	14896 ± 556
CYP2C9	> 20,000[a]
CYP2C19	> 20,000[a]
CYP2D6	12605 ± 556
CYP3A4	3604 ± 3410

[a] 10 μM or 20 μM cut-off determination ($n = 2$).

Table 3. Metabolic stability[a]

Entry	(%)[b]
2	80
4	74
6	70
7	3
8	45
9	39

[a] In rat liver microsomes (Cerep, Seattle). All values are means of two determinations.

[b] % Remaining after 15 min. The values for reference compounds Imipramine, Verapamil, and Terfenadine are 11, 63, and 60, respectively.

Acknowledgments

We gratefully acknowledge Anne-Lise R. Gudmundsson and Jannie Rosendahl Christensen for their technical support, and Conni Lauritzen and Gitte Petersen (Prozymex A/S) for supplying purified recombinant human DPPI.

References and notes

1. Gutmann, H. R.; Fruton, J. S. *J. Biol. Chem.* **1948**, *174*, 851.
2. Ishidoh, K.; Muno, D.; Sato, N.; Kominami, E. *J. Biol. Chem.* **1991**, *266*, 16312.
3. McGuire, M. J.; Lipsky, P. E.; Thiele, D. L. *Arch. Biochem. Biophys.* **1992**, *295*, 280.
4. Rao, N. V.; Rao, G. V.; Hoidal, J. R. *J. Biol. Chem.* **1997**, *272*, 10260.
5. Pham, C. T. N.; Armstrong, R.; Zimonjic, D. B.; Popescu, N. C.; Payan, D. G.; Ley, T. J. *J. Biol. Chem.* **1997**, *272*, 10695.
6. (a) Turk, D.; Janjic, V.; Stern, I.; Podobnik, M.; Lamba, D.; Dahl, S. W.; Lauritzen, C.; Pedersen, J.; Turk, V.; Turk, B. *EMBO J.* **2001**, *20*, 6570; (b) Olsen, J. G.; Kadziola, A.; Lauritzen, C.; Pedersen, J.; Larsen, S.; Dahl, S. W. *FEBS Lett.* **2001**, *506*, 201.
7. Dahl, S. W.; Halkier, T.; Lauritzen, C.; Dolenc, I.; Pedersen, J.; Turk, V.; Turk, B. *Biochemistry* **2001**, *40*, 1671.
8. Tran, T. V.; Ellis, K. A.; Kam, C.-M.; Hudig, D.; Powers, J. C. *Arch. Biochem. Biophys.* **2002**, *403*, 160.
9. McDonald, J. N.; Zeitman, B. B.; Reilly, T. J.; Ellis, S. *J. Biol. Chem.* **1969**, *244*, 2693.
10. (a) Pham, C. T. N.; Ley, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8627; (b) Sheth, P. D.; Pedersen, J.; Walls, A. F.; McEuen, A. R. *Biochem. Pharmacol.* **2003**, *66*, 2251; (c) Wolters, P. J.; Pham, C. T. N.; Muilenberg, D. J.; Ley, T. J.; Caughey, G. H. *J. Biol. Chem.* **2001**, *276*, 18551.

11. Adkinson, A. M.; Raptis, S. Z.; Kelley, D. G.; Pham, C. T. N. *J. Clin. Invest.* **2002**, *109*, 363.
12. Hart, P. S.; Zhang, Y.; Firatli, E.; Uygur, C.; Lotfazar, M.; Michalec, M. D.; Marks, J. J.; Lu, X.; Coates, B. J.; Seow, W. K.; Marshall, R.; Williams, D.; Reed, J. B.; Wright, J. T.; Hart, T. C. *J. Med. Genet.* **2000**, *37*, 927.
13. Mallen-St. Clair, J.; Pham, C. T. N.; Villalta, A.; Caughey, G. H.; Wolters, P. J. *J. Clin. Invest.* **2004**, *113*, 628.
14. (a) Thiele, D. L.; Lipsky, P. E.; McGuire, M. J. U.S. Patent 5,602,102. 1997; (b) Green, G. D. J.; Shaw, E. *J. Biol. Chem.* **1981**, *256*, 1923.
15. Thompson, S. A.; Andrews, P. R.; Hanzlik, R. P. *J. Med. Chem.* **1986**, *29*, 104.
16. Kam, C.-M.; Götz, M. G.; Koot, G.; McGuire, M.; Thiele, D.; Hudig, D.; Powers, J. C. *Arch. Biochem. Biophys.* **2004**, *427*, 123.
17. Demuth, H.-U.; Heiser, U.; Niestroj, A. WO 03/022871 A2, 2003.
18. Horn, M.; Pavlik, M.; Doleckova, L.; Baudys, M.; Mares, M. *Eur. J. Biochem.* **2000**, *267*, 3330.
19. Mucha, A.; Pawelczak, M.; Hurek, J.; Kafarski, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3113.
20. Barrett, A. J.; Kembhavi, A. A.; Brown, M. A.; Kirschke, H.; Knight, C. G.; Tamai, M.; Hanada, K. *Biochem. J.* **1982**, *201*, 189.
21. Bondebjerg, J.; Fuglsang, H.; Valeur, K. R.; Kaznelson, D. W.; Hansen, J. A.; Pedersen, R. O.; Krogh, B. O.; Jensen, B. S.; Lauritzen, C.; Petersen, G.; Pedersen, J.; Nærum, L. *Bioorg. Med. Chem.* **2005**, *13*, 4408.
22. Greenspan, P. D.; Clark, K. L.; Tomassi, R. A.; Cowen, S. D.; McQuire, L. W.; Farley, D. L.; van Duzer, J. H.; Goldberg, R. L.; Zhou, H.; Du, Z.; Fitt, J. J.; Coppa, D. E.; Fang, Z.; Macchia, W.; Zhu, L.; Capparelli, M. P.; Goldstein, R.; Wigg, A. M.; Doughty, J. R.; Bohacek, R. S.; Knap, A. K. *J. Med. Chem.* **2001**, *44*, 4524.
23. (a) Robichaud, J.; Oballa, R.; Prasit, P.; Falgueyret, J.-P.; Percival, M. D.; Wesolowski, G.; Rodan, S. B.; Kimmel, D.; Johnson, C.; Bryant, C.; Venkatraman, S.; Setti, E.; Mendonca, R.; Palmer, J. T. *J. Med. Chem.* **2003**, *46*, 3709; (b) Löser, R.; Schilling, K.; Dimmig, E.; Gütschow, M. *J. Med. Chem.* **2005**, *48*, 7688.
24. Ward, Y. D.; Thomson, D. S.; Frye, L. L.; Cywin, C. L.; Morwick, T.; Emmanuel, M. J.; Zindell, R.; McNeil, D.; Bekkali, Y.; Giradot, M.; Hrapchak, M.; DeTuri, M.; Crane, K.; White, D.; Pav, S.; Wang, Y.; Hao, M.-H.; Grygon, C. A.; Labadia, M. E.; Freeman, D. M.; Davidson, W.; Hopkins, J. L.; Brown, M. L.; Spero, D. M. *J. Med. Chem.* **2002**, *45*, 5471.
25. (a) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774; (b) Stüber, W.; Kosina, H.; Heimburger, N. *Int. J. Pept. Protein Res.* **1988**, *31*, 63.
26. Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Med. Chem.* **1982**, *47*, 104.