A Cyclopent-2-enecarbonyl Group Mimics Proline at the P2 Position of Prolyl Oligopeptidase Inhibitors

Elina M. Jarho,*,† Jarkko I. Venäläinen,‡ Juhani Huuskonen,†,# Johannes A. M. Christiaans,†,§ J. Arturo Garcia-Horsman,‡ Markus M. Forsberg,‡ Tomi Järvinen,† Jukka Gynther,† Pekka T. Männistö,‡ and Erik A. A. Wallén†

Department of Pharmaceutical Chemistry and Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland

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Abstract: With the aim to replace the natural amino acid proline by a proline mimetic structure, a cyclopent-2-enecarbonyl moiety was studied at the P2 position of prolyl oligopeptidase (POP) inhibitors. The cyclopent-2-enecarbonyl moiety proved to be an excellent proline mimetic at the P2 position of POP inhibitors. The replacement is particularly useful when increased lipophilicity is needed.

Among the 20 common amino acids, proline has a unique role because of its cyclic structure. It causes constraints on a polypeptide backbone and can protect a peptide from nonspecific hydrolysis by peptidases. It may also serve as a regulatory structure in maturation and degradation of peptides and in protein folding. Thus, it is not surprising that proline is often highly conserved in peptides and specific peptidases have evolved to hydrolyze bonds before and after proline residues.[1−3]

Several protease inhibitors are recently under investigation as potential drugs. Many of them have a peptide-like structure, and they suffer from poor bioavailability and susceptibility to nonspecific degradation. These problems have led to the development of amino acid mimetics that could be used to replace the natural amino acids. The unique structure of proline makes it challenging to replace.[4,5]

Prolyl oligopeptidase (POP, EC 3.4.21.26) is a proline-specific serine peptidase that preferentially hydrolyzes peptides at the carboxyl side of proline. POP has been related to cognitive disorders because several cognition-enhancing neuropeptides are substrates of POP.[6−8] The inhibitors of POP have been shown to increase the levels of these peptides in the brain[9] and to prevent scopolamine-induced amnesia in rats.[10] They have also been shown to enhance cognition in a model of early Parkinsonism in monkeys.[11] Furthermore, expression of the POP gene is increased in the hypothalamus and the cortex of old rats[12] and decreased in an enriched environment.[13] It has also recently been reported that the inhibiton of the POP of *Trypanosoma cruzi* could be a therapeutic target.[14−16]

L-Proline is the preferred amino acid at the P2 position of POP inhibitors. Only a few successful replacements of this proline have been reported.[17−20] The L-prolyl residue has been replaced by groups that contain a five-membered carbon ring, such as a cyclopentanecarbonyl group and a cyclopent-1-enecarbonyl group, but the resulting compounds had a decreased inhibitory activity.[21,22] In the present study, the pyrrolidine ring was successfully replaced by a cyclopent-2-enecarbonyl group. The same replacement has previously been studied at the P2 position of thrombin inhibitors with moderate results.[23]

The synthetic routes are presented in Scheme 1. (±)-2-Formyl-cyclopent-2-enecarboxylic acid (**1**) and (±)-cyclopent-2-ene-1,2-dicarboxylic acid 1-methyl ester (**2**) were synthesized as described in the literature with small modifications.[23,24] DCC and HOBt were used to couple benzylamine with **2**, and the methyl ester of the obtained product was hydrolyzed with LiOH to obtain 2-benzylcarbamoylcyclopent-2-enecarboxylic acid (**3**). Reaction of **1** with (3-phenylpropyl)magnesium bromide (prepared in situ) produced a secondary alcohol, which was oxidized with oxalyl chloride/DMSO to obtain (±)-2-(4-phenylbutyryl)cyclopent-2-enecarboxylic acid (**4**). Compounds **3** and **4** were activated with pivaloyl chloride and reacted with pyrrolidine to yield end products **9** and **8**, respectively. The enantiomers of **9** and **8** were not separated. Compound **3** was activated with pivaloyl chloride and reacted with 2(*S*)-(acetoxyacetyl)-pyrrolidine. The acetyl group was hydrolyzed with K_2CO_3 in water and methanol, yielding end product **5**. The diastereomers of **5** were separated on silica support. The separated compounds were confirmed to be diastereomers by NMR. Compound **4** was activated with pivaloyl chloride and reacted with L-proline methyl ester. The methyl ester group was hydrolyzed with LiOH in water and methanol. Thus, the obtained free carboxylic acid was activated with ethyl chloroformate and treated with 25% aqueous NH_3. The carboxylic acid amide was then dehydrated with trifluoroacetic anhydride to yield end product **7**. The diastereomers of **7** were separated on silica support. The separated compounds were confirmed to be diastereomers by NMR. Compound **10** was prepared from cyclopent-1-ene-1,2-dicarboxylic anhydride and benzylamine. Compound **10** was then activated with pivaloyl chloride and reacted with pyrrolidine to yield reference compound **11**. Reference compounds **6**, SUAM-1221,[25−27] and JTP-4819[28] were prepared as described in the literature.

The partition coefficients (P) were determined in a 1-octanol−phosphate buffer system with the traditional shake-flask method. The $\log P$ values are shown in Table 1.

The inhibitory activities of the novel and reference compounds are shown in Table 1. The tight-binding inhibitors are highly potent compounds with the IC_{50} values in the same range as the enzyme concentration

* To whom correspondence should be addressed. Phone: +358-17-162460. Fax: +358-17-162456. E-mail: Elina.Jarho@uku.fi.
† Department of Pharmaceutical Chemistry.
‡ Department of Pharmacology and Toxicology.
# Present address: Department of Chemistry, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland.
§ Present address: Altana Pharma bv, P.O. Box 31, 2130 AA Hoofddorp, The Netherlands.

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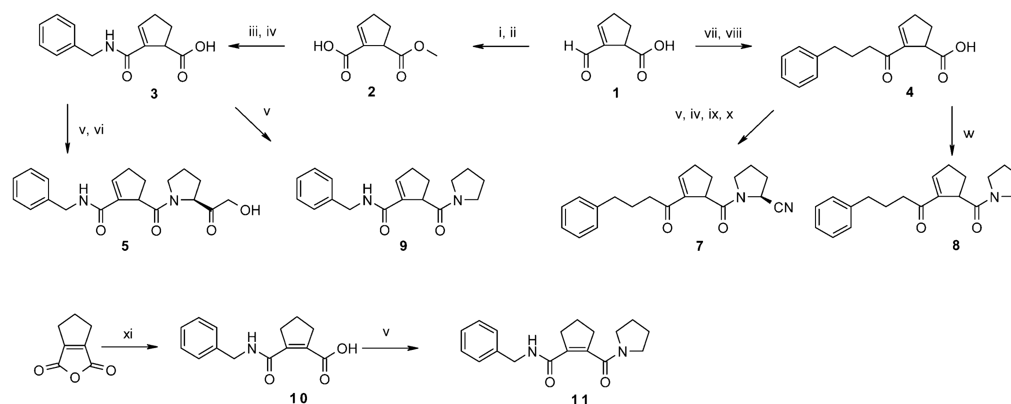
Scheme 1[a]



[a] Reagents and conditions: (i) $CH(OCH_3)_3$, *p*-TsOH; (ii) resorcinol, $NaH_2PO_4 \cdot H_2O$, $NaClO_2$/*t*-BuOH, H_2O; (iii) benzylamine, HOBt, Et_3N, DCC/CH_3CN, 0−20 °C; (iv) LiOH/MeOH, H_2O; (v) (1) Et_3N, $(CH_3)_3CCOCl$/DCM, 0 °C, (2) Et_3N, an amine or a salt of an amine; (vi) K_2CO_3/MeOH, H_2O, 0−20 °C; (vii) (1) Mg, I_2, 1-bromo-3-phenylpropane/Et_2O, (2) compound **1**; (viii) (1) oxalyl chloride, DMSO/DCM, −78 °C, (2) Et_3N; (ix) (1) Et_3N, C_2H_5OCOCl/THF, −10 °C, (2) 25% aqueous NH_3/THF, room temperature; (x) Et_3N, trifluoroacetic anhydride/THF, 0−20 °C; (xi) benzylamine/THF, 0−20 °C.

Table 1. Structures, Inhibitory Activities (95% Confidence Intervals), and log *P* Values of the Reference and Novel Compounds

Compound	Structure	IC_{50} (nM)[a]		K_i (nM)[b]		Log P
JTP-4819		0.2	(0.16 - 0.27)	0.06	(0.023 - 0.087)	0.2
5a[c]		0.3	(0.18 - 0.56)	0.15	(0.080 - 0.220)	0.6
5b[d]		52	(39 – 71)			
6		0.2	(0.17 - 0.29)	0.02	(0.014 - 0.033)	1.6
7a[c]		0.2	(0.12 - 0.36)	0.03	(0.014 - 0.043)	2.4
7b[d]		5.0	(3.9 - 6.5)			
SUAM-1221		2.2	(1.9 - 2.5)			1.8
8		1.3	(0.7 - 2.7)			
9		9.0	(5.5 – 15)			
11		230	(160 - 330)			

[a] The IC_{50} values were determined against POP of porcine brain homogenate. [b] The K_i values were determined against purified porcine POP for those tight-binding inhibitors that had the IC_{50} value in the subnanomolar range. [c] The chiral center marked with the asterisk was assumed to have *R* configuration. The absolute configuration was not verified. [d] The chiral center marked with the asterisk was assumed to have *S* configuration. The absolute configuration was not verified.

in the in vitro assay. The K_i values were determined for tight-binding inhibitors to obtain the accurate inhibitory potencies.

The activities of diastereomers **5a** and **5b** differed considerably from each other. The IC_{50} value of **5a** was 0.3 nM, while the IC_{50} value of **5b** was 52 nM. At the P2 position L-amino acids have repeatedly caused better activities than D-amino acids.[25,29,30] Consequently, the configurations of **5a** and **5b** were assigned assuming that the more active diastereomer **5a** has the L-proline mimetic at the P2 position. The configurations were not verified. Diastereomer **5a** was almost equipotent with reference compound JTP-4819.

Compound **7a** was equipotent with reference compound **6**. It had a K_i value of 0.03 nM and was also assumed to have the L-proline mimetic at the P2 position. The absolute configuration was not verified. The other diastereomer **7b** was less active with an IC_{50} of 5 nM.

The 2(*S*)-hydroxyacetyl derivative **5a** and 2(*S*)-cyano derivative **7a** are tight-binding inhibitors of prolyl oligopeptidase. Normally, these electrophilic groups increase the inhibitory activity over 10-fold compared to an unsubstituted pyrrolidine. Compound **8** has the unsubstituted pyrrolidine at the P1 position. It was tested as a mixture of enantiomers, and despite that, the IC_{50} value was as low as 1.30 nM. On the basis of the activity differences between **5a** and **5b** and between **7a** and **7b**, it can be assumed that the more active enantiomer is even more potent than the reference compound SUAM-1221.

POP inhibitors, which contain a cyclopent-1-enecarbonyl group at the P2 position, have been published in the literature.[22] One such inhibitor (**11**) and the corresponding cyclopent-2-enecarbonyl analogue (**9**) were synthesized. As expected, **11** was less active, with an IC_{50} of 230 nM, than **9** with an IC_{50} of 9 nM. The structure of the cyclopent-1-enecarbonyl group is not a good proline mimetic and is therefore moderately active. The cyclopent-2-enecarbonyl group mimics a proline residue rather well, as observed by the inhibitory activity.

To study the effect of the cyclopent-2-enecarbonyl moiety on the lipophilicity, the log *P* values were determined for a selection of compounds (Table 1). The replacement of the prolyl moiety by the cyclopent-2-enecarbonyl moiety changed the urea group of JTP-4819 to the amide group of **5a**. This change increased the log *P* value by 0.4 units. The change of the amide group of **6** to the ketone group of **7a** increased the log *P* value by 0.8 units. On the basis of these results, it seems that the replacement of a proline with a cyclopent-2-enecarbonyl group increases the log *P* value but the magnitude depends on the substituents.

The replacement of the L-prolyl residue by the cyclopent-2-enecarbonyl group introduced an α,β-unsaturated ketone or amide to the novel compounds. The α,β-unsaturated ketones gave valuable information about the structure−activity relationships, but their stability needs further evaluation because of the activated double bond. However, the α,β-unsaturated amides are not as reactive because of the electron-donating effect of the nitrogen atom.

The L-prolyl moiety was replaced by the cyclopent-2-enecarbonyl structure at the P2 position of prolyl oligopeptidase inhibitors. In all cases, the cyclopent-2-enecarbonyl analogue proved to be equipotent to the reference compound. Furthermore, this replacement increased the lipophilicity of the compounds. This study shows that a cyclopent-2-enecarbonyl structure can be used to replace a prolyl residue at the P2 position of POP inhibitors.

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Supporting Information Available: Experimental details and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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References

(1) Brandts, J. F.; Halvorson, H. R.; Brennan, M. Consideration of the possibility that the slow step in protein denaturation reactions is due to cis−trans isomerism of proline residues. *Biochemistry* **1975**, *14*, 4953−4963.

(2) Vanhoof, G.; Goossens, F.; De Meester, I.; Hendriks, D.; Scharpe, S. Proline motifs in peptides and their biological processing. *FASEB J.* **1995**, *9*, 736−744.

(3) Yaron, A.; Naider, F. Proline-dependent structural and biological properties of peptides and proteins. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31−81.

(4) Leung, D.; Abbenante, G.; Fairlie, D. P. Protease inhibitors: current status and future prospects. *J. Med. Chem.* **2000**, *43*, 305−341.

(5) De Nanteuil, G.; Portevin, B.; Lepagnol, J. Prolyl endopeptidase inhibitors: a new class of memory enhancing drugs. *Drugs Future* **1998**, *23*, 167−179.

(6) Koida, M.; Walter, R. Post-proline cleaving enzyme. Purification of this endopeptidase by affinity chromatography. *J. Biol. Chem.* **1976**, *251*, 7593−7599.

(7) Walter, R. Partial purification and characterization of post-proline cleaving enzyme: enzymatic inactivation of neurohypophyseal hormones by kidney preparations of various species. *Biochim. Biophys. Acta* **1976**, *422*, 138−158.

(8) Wilk, S. Prolyl endopeptidase. *Life Sci.* **1983**, *33*, 2149−2157.

(9) Toide, K.; Fujiwara, T.; Iwamoto, Y.; Shinoda, M.; Okamiya, K.; et al. Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on neuropeptide metabolism in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1996**, *353*, 355−362.

(10) Yoshimoto, T.; Kado, K.; Matsubara, F.; Koriyama, N.; Kaneto, H.; et al. Specific inhibitors for prolyl endopeptidase and their anti-amnesic effect. *J. Pharmacobiodyn.* **1987**, *10*, 730−735.

(11) Schneider, J. S.; Giardiniere, M.; Morain, P. Effects of the prolyl endopeptidase inhibitor S 17092 on cognitive deficits in chronic low dose MPTP-treated monkeys. *Neuropsychopharmacology* **2002**, *26*, 176−182.

(12) Jiang, C. H.; Tsien, J. Z.; Schultz, P. G.; Hu, Y. The effects of aging on gene expression in the hypothalamus and cortex of mice. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1930−1934.

(13) Rampon, C.; Jiang, C. H.; Dong, H.; Tang, Y. P.; Lockhart, D. J.; et al. Effects of environmental enrichment on gene expression in the brain. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12880−12884.

(14) Grellier, P.; Vendeville, S.; Joyeau, R.; Bastos, I. M.; Drobecq, H.; et al. *Trypanosoma cruzi* prolyl oligopeptidase Tc80 is involved in nonphagocytic mammalian cell invasion by trypomastigotes. *J. Biol. Chem.* **2001**, *276*, 47078−47086.

(15) Bal, G.; Van der Veken, P.; Antonov, D.; Lambeir, A. M.; Grellier, P.; et al. Prolylisoxazoles: potent inhibitors of prolyloligopeptidase with antitrypanosomal activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2875−2878.

(16) Vendeville, S.; Goossens, F.; Debreu-Fontaine, M. A.; Landry, V.; Davioud-Charvet, E.; et al. Comparison of the inhibition of human and *Trypanosoma cruzi* prolyl endopeptidases. *Bioorg. Med. Chem.* **2002**, *10*, 1719−1729.

(17) Tsuru, D.; Yoshimoto, T.; Koriyama, N.; Furukawa, S. Thiazolidine derivatives as potent inhibitors specific for prolyl endopeptidase. *J. Biochem. (Tokyo)* **1988**, *104*, 580−586.

(18) Barelli, H.; Petit, A.; Hirsch, E.; Wilk, S.; De Nanteuil, G.; et al. S 17092-1, a highly potent, specific and cell permeant inhibitor of human proline endopeptidase. *Biochem. Biophys. Res. Commun.* **1999**, *257*, 657−661.

(19) De Nanteuil, G. U.S. Patent 5506237, 1996.

(20) Wallén, E. A.; Christiaans, J. A.; Saarinen, T. J.; Jarho, E. M.; Forsberg, M. M.; et al. Conformationally rigid *N*-acyl-5-alkyl-L-prolyl-pyrrolidines as prolyl oligopeptidase inhibitors. *Bioorg. Med. Chem.* **2003**, *11*, 3611−3619.

(21) Ohno, H. European Patent 0268190, 1988.

(22) Faraci, S. World Patent 9118891, 1991.

(23) Nöteberg, D.; Brånalt, J.; Kvarnström, I.; Linschoten, M.; Musil, D.; et al. New proline mimetics: synthesis of thrombin inhibitors incorporating cyclopentane- and cyclopentenedicarboxylic acid templates in the P2 position. Binding conformation investigated by X-ray crystallography. *J. Med. Chem.* **2000**, *43*, 1705−1713.

(24) Stevens, H. C.; Rinehart, J. K.; Lavanish, J. M.; Trenta, G. M. The hydrolysis of 7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one. *J. Org. Chem.* **1971**, *36*, 2780−2784.

(25) Arai, H.; Nishioka, H.; Niwa, S.; Yamanaka, T.; Tanaka, Y.; et al. Synthesis of prolyl endopeptidase inhibitors and evaluation of their structure−activity relationships: in vitro inhibition of prolyl endopeptidase from canine brain. *Chem. Pharm. Bull. (Tokyo)* **1993**, *41*, 1583−1588.

(26) Saito, M.; Hashimoto, M.; Kawaguchi, N.; Shibata, H.; Fukami, H.; et al. Synthesis and inhibitory activity of acyl-peptidyl-pyrrolidine derivatives toward post-proline cleaving enzyme; a study of subsite specificity. *J. Enzyme Inhib.* **1991**, *5*, 51−75.

(27) Houssin, R.; Bernier, J.-L.; Hénichart, J.-P. A convenient and general method for the preparation of *tert*-butoxycarbonylaminoalkanenitriles and their conversion to mono-*tert*-butoxycarbonylalkanediamines. *Synthesis* **1988**, 259−261.

(28) Kobayashi, K. U.S. Patent 5536737, 1996.

(29) Portevin, B.; Benoist, A.; Remond, G.; Herve, Y.; Vincent, M.; et al. New prolyl endopeptidase inhibitors: in vitro and in vivo activities of azabicyclo[2.2.2]octane, azabicyclo[2.2.1]heptane, and perhydroindole derivatives. *J. Med. Chem.* **1996**, *39*, 2379−2391.

(30) Yoshimoto, T.; Tsuru, D.; Yamamoto, N.; Ikezawa, R.; Furukawa, S. Structure activity relationship of inhibitors specific for prolyl endopeptidase. *Agric. Biol. Chem.* **1991**, *55*, 37−43.

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