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An introduction of a pyridine group into the structure of prolyl oligopeptidase inhibitors

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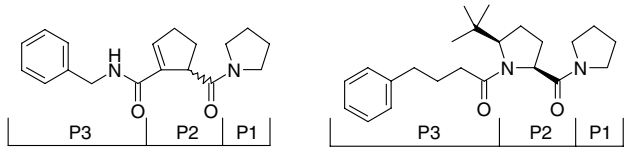
Abstract—A series of ionizable prolyl oligopeptidase inhibitors were developed through the introduction of a pyridyl group to the P3 position of the prolyl oligopeptidase inhibitor structure. The study was performed on previously developed prolyl oligopeptidase inhibitors with proline mimetics at the P2 position. The 3-pyridyl group resulted in equipotent compounds as compared to the parent compounds. It was shown that the pyridyl group improves water solubility and, in combination with a 5(*R*)-*tert*-butyl-L-prolyl group at the P2 position, good lipophilicity can be achieved.

Human prolyl oligopeptidase (POP) is an 80 kDa serine protease that hydrolyzes oligopeptides after prolyl residues. Several studies have shown that specific POP inhibitors can prevent memory impairments[1–3] and have neuroprotective effects[4] in rats, improve cognition in a model of early Parkinsonism in monkeys,[5] and improve performance in verbal memory tests in humans.[6] In aged mice, the expression of the POP gene has been shown to be up-regulated in the hypothalamus[7] and the hippocampus.[8] All these results imply that POP is associated with neurodegeneration and memory deficits but the physiological role of POP is not still fully understood. Initially, POP was suggested to have a function in the maturation and the degradation of neuropeptides, which are involved in memory and learning. This idea has been called into question because POP is mainly described as a cytosolic enzyme and neuropeptides are located in transcellular space. During the last years, some light has been shed on the intracellular functions of POP. Inhibition of POP has been shown to elevate IP_3 levels, which was suggested to explain the memory-enhancing properties of POP inhibitors.[9,10] Recently, POP was also proposed to have a role in protein trafficking and secretion.[11]

Unlike many small-molecule drugs, typical POP inhibitors are unionizable compounds. An ionizable group may improve water solubility and it also allows salt formation. In later stages of drug development, the proper choice of salt can be used to modify stability, solubility, and pharmaceutical processing properties.

Two unionizable POP inhibitors with different proline mimetics at the P2 positions were earlier presented by our group (Fig. 1).[12,13] These compounds were further investigated as to whether an ionizable pyridyl group



IC_{50} = 9.0 nM (compound **4**) IC_{50} = 1.2 nM (compound **12**)

Figure 1. Previously published POP inhibitors, which possess proline mimetics at the P2 site.

Keywords: Prolyl oligopeptidase; Inhibitor; Pyridine.
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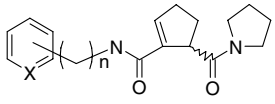
could be introduced into the structure. The pK_a value of pyridine is 5.2 and thus, it is mostly in the unionized form at the physiological pH 7.4. However, a significant fraction is in the ionized form in the slightly acidic environment of the duodenum,[14] which allows dissolution that is a prerequisite for the absorption.

A literature search revealed that a pyridyl moiety has occasionally been included in some series of POP inhibitors, but no systematic study regarding the position and the substitution of the pyridine group has been performed.[15–21] One potent peptide-like POP inhibitor, which possessed a pyridine-2-carboxyl amide moiety at the P3 position, was reported by Tsuda et al.[18] In the present study, the phenyl group at the P3 site was successfully replaced by a pyridyl group in two series of POP inhibitors. The chain length and the substitution pattern were optimized. The effect of the pyridyl moiety on lipophilicity and water solubility was also studied.

The synthetic routes for the novel compounds are presented in Scheme 1. Compounds **1**, **4**,[12] and **12**[13] were synthesized as described earlier. Compound **1** was activated with pivaloyl chloride and reacted with pyrrolidine at 0 °C to obtain (±)-5-(pyrrolidine-1-carbonyl)-cyclopent-1-enecarbaldehyde (**2**). The aldehyde group of compound **2** was oxidized with $NaClO_2$ to obtain (±)-5-(pyrrolidine-1-carbonyl)-cyclopent-1-enecarboxylic acid (**3**). Compound **3** was reacted with DCC, HOBt, and an appropriate amine to yield compounds **5a**–**c**, **6**, and **7a**–**c**. Boc-5(*R*)-*tert*-butyl-L-proline (**8**) was prepared according to published procedures[22,23] with slight modifications as described earlier.[13] Boc-5(*R*)-*tert*-butyl-L-proline (**8**) and Boc-L-proline (**9**) were activated with pivaloyl chloride and reacted with pyrrolidine to yield compounds **10** and **11**, respectively. Compounds **10** and **11** were deprotected in HCl-saturated ethyl acetate to yield 5(*R*)-*tert*-butyl-L-prolyl-pyrrolidine and L-prolyl-pyrrolidine. 4-Pyridin-3-yl-butyric acid and 3-pyridin-3-yl-propionic acid were reacted with EDC, HOBt, and 5(*R*)-*tert*-butyl-L-prolyl-pyrrolidine to yield compounds **13a** and **13b**, respectively. 4-Pyridin-3-yl-butyric acid was prepared according to a published procedure[24] with small modifications.[25] L-Prolyl-pyrrolidine was reacted with 3-pyridin-3-yl-propionyl chloride and nicotinoyl chloride to yield compounds **14a** and **14c**, respectively. EDC and HOBt were used to couple L-prolyl-pyrrolidine with (pyridin-3-yl)acetic acid to obtain compound **14b**.

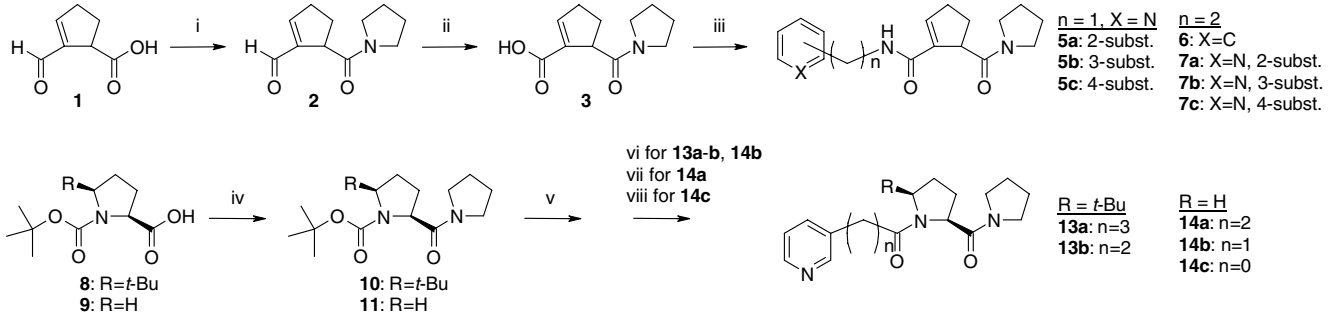
Compound **4**[12] possesses a cyclopentenecarbonyl moiety at the P2 position. The IC_{50} value of compound **4** is 9 nM for the racemic mixture. It was chosen as the parent compound for the first series of compounds presented in Table 1. The replacement of the phenyl group of compound **4** with a 2- or 4-pyridyl group gave compounds **5a** and **5c**, respectively. These substitutions resulted in over 2-fold lower potency. However, the replacement with a 3-pyridyl group gave compound **5b**, which was equipotent with the parent compound **4**. In order to further optimize the position of the pyridyl group, the chain length of compounds **4** and **5a**–**5c** was extended by one methylene group, which resulted in compounds **6** and **7a**–**c**, respectively. The extension of the chain of compound **4** caused a 6-fold increase in potency; the IC_{50} value of compound **6** was 1.5 nM. Again, the replacement of the P3 phenyl group by the 3-pyridyl moiety gave the most potent compound **7b**

Table 1. Inhibitory activities with 95% confidence intervals and log *P* values of the (±)-5-(pyrrolidine-1-carbonyl)-cyclopent-1-enecarboxylic acid amides



Compound	X	Subst	*n*	IC_{50}[a] (nM)		log *P*[b]
4	C	—	1	9.0	(5.5–15)	1.6[c]
5a	N	2	1	24	(22–26)	0.3[d]
5b	N	3	1	9.7	(8.5–11)	0.4[d]
5c	N	4	1	19	(15–26)	0.4[d]
6	C	—	2	1.5	(1.3–1.9)	1.9[c]
7a	N	2	2	5.2	(4.4–6.2)	0.6[d]
7b	N	3	2	2.2	(1.9–2.6)	0.6[d]
7c	N	4	2	4.8	(3.8–6.1)	0.5[d]

[a] The IC_{50} values were determined against POP from porcine brain.[28]
[b] The reported values are for the unionized species.
[c] Determined with the shake-flask method.
[d] Determined with pH metric titration using a Sirius PCA200 automatic titrator.



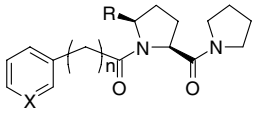
Scheme 1. Synthesis of the novel compounds. Reagents and conditions: (i) 1—Et_3N, $(CH_3)_3CCOCl/CH_2Cl_2$, 0 °C, 2—Et_3N, pyrrolidine/CH_2Cl_2, 0 °C; (ii) resorcinol, $NaH_2PO_4{\cdot}H_2O$, $NaClO_2$/*t*-BuOH, H_2O; (iii) an appropriate amine, Et_3N, HOBt, DCC/CH_3CN, 0–20 °C; (iv) 1—Et_3N, $(CH_3)_3CCOCl/CH_2Cl_2$, 0 °C, 2—Et_3N, pyrrolidine/CH_2Cl_2, room temperature; (v) HCl/EtOAc; (vi) an appropriate carboxylic acid, Et_3N, HOBt, EDC·HCl/CH_2Cl_2, 0–20 °C; (vii) 3-pyridin-3-yl-propionyl chloride HCl, Et_3N/CH_2Cl_2, 0–20 °C; (viii) DMAP, nicotinoyl chloride HCl/pyridine.

(2.2 nM), while the 2- and 4-pyridyl analogs, **7a** and **7c**, were less active. Compound **7b** was almost equipotent with compound **6** and over 4-fold more potent than its shorter counterpart **5b**. These results confirmed that the 3-pyridyl group can be used to replace the phenyl group at the P3 position of POP inhibitors and the optimal chain length is the same as for the phenyl group, despite the difference in their polarities.

High lipid solubility is a prerequisite for the passive brain penetration of small-molecule drugs[26] and the theoretical optimum for the log*P* value is near 2.[27] Although lipophilicity is not the only factor that determines brain penetration and the optimal lipophilicity can vary between compound classes, it should be taken into account when novel CNS targeted compounds are designed. The log*P* values were determined for compounds **4**, **5a**–**c**, **6**, and **7a**–**c** (Table 1). The presented log*P* values for compounds **5a**–**c** and **7a**–**c** are values for the unionized species, which are mainly found at the physiological pH 7.4 (the pK_a values range between 4.4 and 5.5). The log*P* value of compound **4** was 1.6. The replacement of the phenyl moiety by the more polar pyridyl moiety dropped the log*P* values to 0.3–0.4 for compounds **5a**–**5c**. The extension of the chain increased the log*P* value to 1.9 for compound **6**, but again the replacement of the phenyl moiety by the pyridyl moiety dropped the log*P* values to 0.5–0.6 for compounds **7a**–**7c**.

Compound **12**[13] possesses a lipophilic 5(*R*)-*tert*-butyl-L-prolyl moiety at the P2 position. The IC_{50} value of compound **12** is 1.2 nM and the log*P* value is 3.3. It was chosen as the parent compound for the second series of compounds presented in Table 2. In this series, only the 3-pyridyl group was studied because it had given the most potent compounds in the first series. The replacement of the P3 phenyl group of compound **12** by the 3-pyridyl group resulted in compound **13a** having an IC_{50} value of 2.1 nM. It was only slightly less potent than compound **12** and equipotent with compound **7b**, which was the most potent pyridyl derivative in the first series. However, it has to be kept in mind that **13a** possesses the more active configuration of L-proline, while **7b** was tested as a racemic mixture. To study whether the change of the skeleton affects the optimal position of the pyridyl group, the P3 chain of compound **13a** was shortened by one methylene group resulting in compound **13b**. The results were consistent with the first series; a decrease in the chain length decreased the potency and the IC_{50} value of compound **13b** was 13 nM. The effect of even shorter chain lengths was studied with compounds **14a**–**c**, which have an L-prolyl residue at the P2 site. The change to an L-prolyl group at the P2 site was made to avoid steric hindrance that the bulky 5(*R*)-*tert*-butyl group causes when the P3 ring is brought closer to the P2 ring. The removal of the 5(*R*)-*tert*-butyl group of compound **13b** resulted in compound **14a** and in over 2-fold lower potency; the IC_{50} value of **14a** was 30 nM. The P3 chain length of compound **14a** was shortened by one and two methylene groups to obtain compounds **14b** and **14c**, respectively. Both compounds were less potent than compound **14a**, confirming that the optimal position for the pyridyl moiety is three carbon atoms away from the carbonyl group.

Table 2. Inhibitory activities with 95% confidence intervals and log*P* values of the *N*-alkanoyl 5(*R*)-*tert*-butyl-L-prolyl-pyrrolidines and *N*-alkanoyl L-prolyl-pyrrolidines



Compound	X	*n*	R	IC_{50}[a] (nM)		log*P*[b]
12	C	3	*t*-Bu	1.2	(1.0–1.4)	3.3[c]
13a	N	3	*t*-Bu	2.1	(1.9–2.4)	2.0[d]
13b	N	2	*t*-Bu	13	(8.4–20)	1.8[d]
14a	N	2	H	30	(24–39)	0.3[d]
14b	N	1	H	56	(46–70)	−0.2[d]
14c	N	0	H	44	(29–67)	−0.3[d]
15 SUAM-1221	C	3	H	2.2	(1.9–2.5)	1.8[c]

[a] The IC_{50} values were determined against POP from porcine brain.[28]
[b] The reported values are for the unionized species.
[c] Determined with the shake-flask method.
[d] Determined with pH metric titration using a Sirius PCA200 automatic titrator.

The log*P* values of the second series of compounds are presented in Table 2. Again, the reported log*P* values are for the unionized species. The replacement of the phenyl moiety of compound **12** by the polar pyridyl moiety dropped the log*P* value from 3.3 to the theoretical optimum 2.0 for compound **13a**. The drop was of the same order of magnitude as in the first series of the compounds; 20-fold drop in partition coefficient P. Compound **13b** with shorter chain length had a log*P* value 1.8. A comparison between the log*P* values of compounds **13b** and **14a** (log*P* = 0.3) shows that the *tert*-butyl group greatly increases lipophilicity and consequently, the log*P* values of compounds **14a**–**c** were low. The IC_{50} and log*P* values of compound **13a** are comparable to those of the unionizable reference compound **15**, SUAM-1221, which is a potent POP inhibitor that can penetrate into the CNS (Table 2).[29]

The water solubility was determined for the parent compounds of the two series, compounds **4** and **12**, their 3-pyridyl analogs **5b** and **13a**, and for the reference compound **15**, SUAM-1221. The solubilities were determined after 3-day-shaking in 50 mM phosphate buffer (pH 7.4, ionic strength 0.15 M) up to 6 mg/mL, which can be considered adequate even for high-dose compounds with poor permeability.[30] While the solubility of compound **15** was at least 6 mg/mL, compounds **4** and **12** with proline mimetics at the P2 sites had decreased values; 3.0 and 0.9 mg/mL, respectively. However, the change of the phenyl moiety to the 3-pyridyl moiety overcame this decrease and the solubilities of compounds **5b** and **13a** were at least 6 mg/mL.

The present study proved that a pyridyl group can be introduced to the P3 position of the POP inhibitor structure. However, the inhibitory activity was dependent on

the substitution of the pyridyl group and the P3 chain length. The 3-pyridyl group gave the most potent compounds and its optimal position was the same as for the phenyl group; three atoms between the P3 carbonyl group and the aromatic ring. The studied parent compounds had decreased water solubility. The introduction of an ionizable pyridyl group gave excellent water solubility to the novel compounds. In conclusion, the introduction of the 3-pyridyl group at the P3 position in combination with a 5(*R*)-*tert*-butyl-L-prolyl moiety at P2 position was used to optimize the physico-chemical properties while maintaining an excellent inhibitory activity.

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