New Prolyl Oligopeptidase Inhibitors Developed from Dicarboxylic Acid Bis(L-prolyl-pyrrolidine) Amides

Erik A. A. Wallén,*,† Johannes A. M. Christiaans,†,# Elina M. Jarho,† Markus M. Forsberg,‡ Jarkko I. Venäläinen,‡ Pekka T. Männistö,‡,§ and Jukka Gynther†,§

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

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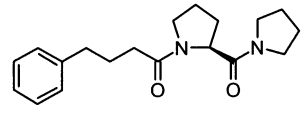
Isophthalic acid bis(L-prolyl-pyrrolidine) amide is a very potent prolyl oligopeptidase inhibitor, but it has a log *P* value of −0.2, which is very low for a compound targeted to the brain. Therefore, these types of compounds were further modified to improve the structure−activity relationships, with the focus on increasing the log *P* value. The inhibitory activity against prolyl oligopeptidase from pig brain was tested in vitro. The most promising compounds resulted from replacing the pyrrolidinyl group at the P5 site by cycloalkyl groups, such as cyclopentyl and cyclohexyl groups, and by a phenyl group. These compounds are slightly more potent, and they have a significantly higher log *P* value. The potency of these compounds was further increased by replacing the pyrrolidinyl group at the P1 site by 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups.

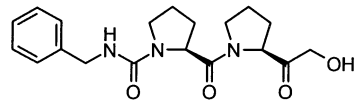
Introduction

The serine protease prolyl oligopeptidase (POP, previously called prolyl endopeptidase or post-proline cleaving enzyme, EC 3.4.21.26) is a large enzyme of 80 kDa that preferentially hydrolyzes proline-containing oligopeptides at the carboxyl side of a prolyl residue.[1] Many peptide hormones and neuropeptides have one or more proline residues, and the processing and degradation of such peptides often require the use of proline-specific enzymes.[2−4] Several substrates of POP, such as substance P, vasopressin, neurotensin, and thyroliberin, are implicated in learning and memory.[5] In addition, a low level of substance P is characteristic in the brain of Alzheimer's patients, and administration of substance P is able to block β amyloid-induced neurotoxicity.[6] There is no firm evidence of increased POP activity in Alzheimer's patients, because rather low enzyme activities are correlated with the severity of Alzheimer's disease.[7] On the other hand, this result is thought to reflect the degree of neuronal damage. It was recently reported that in rat the expression of POP gene was decreased when the animals were exposed to an enriched environment and the expression was increased manyfold in the hypothalamus and the cortex in aged rats.[8,9] Therefore, it has been postulated that centrally acting POP inhibitors that increase neuropeptide concentrations[10] might be beneficial in patients with cognitive disturbances. Indeed, POP inhibitors have been shown to reverse scopolamine-induced amnesia in rats and to improve cognition in MPTP-treated monkeys.[11−14]

Most described low molecular weight POP inhibitors have an acyl-L-prolyl-pyrrolidine backbone, wherein a lipophilic acyl end group has been reported to be important for high inhibitory activity.[15] Two well-known reference compounds are 4-phenylbutanoyl-L-prolyl-pyrrolidine (SUAM-1221) and benzylcarbamoyl-L-prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine (JTP-4819).



SUAM-1221



JTP-4819

A series of dicarboxylic acid bis(L-prolyl-pyrrolidine) amides [the compounds consist of two L-prolyl-pyrrolidines connected by a dicarboxylic acid unit; the naming of the compounds is based on the peptide type of naming; alternatively they could be called dicarboxylic acid bis(L-proline pyrrolidide) amide], which do not have the lipophilic acyl end group, were recently described as new potent POP inhibitors.[16] The most active compounds in this series were the isophthalic acid bis(L-prolyl-pyrrolidine) amide type of compounds **1a**−**d** and 3,3-dimethylglutaric acid bis(L-prolyl-pyrrolidine) amide **1e**. The postulated P1−P5 site assignation presented in Chart 1 is based on the structure−activity relationships at the P1 site, which show that the formyl, cyano, and hydroxyacetyl groups of compounds **1b**−**d** interact with the serine residue (Ser554) at the active site of the enzyme.

The IC_{50} values for compounds **1a**−**e** were 26, 1.3, 1.5, 0.39, and 13 nM, respectively, against POP from pig brain. Although compounds **1a**−**e** were potent inhibitors, the log *P* value of compound **1a** was only −0.2. This is considerably lower than the generally acceptable log *P* value between 2 and 3 for compounds that have to

* Corresponding author (e-mail wallen@uku.fi).
† Department of Pharmaceutical Chemistry.
# Present address: Altana Pharma, P.O. Box 31, 2130 AA Hoofddorp, The Netherlands.
‡ Department of Pharmacology and Toxicology.
§ Finncovery, Ltd.

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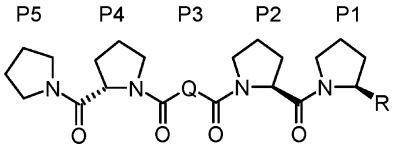
Chart 1



1a: Q = *m*-phenylene, R = H
1b: Q = *m*-phenylene, R = CHO
1c: Q = *m*-phenylene, R = CN
1d: Q = *m*-phenylene, R = $COCH_2OH$
1e: Q = 2,2-dimethylpropylene, R = H

penetrate the blood−brain barrier in order to reach the brain. Therefore, further investigations were carried out with the focus on improving the log *P* value of this type of compound.

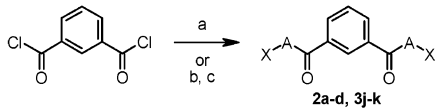
In the same in vitro assay SUAM-1221 and JTP-4819 had IC_{50} values of 2.2 and 0.2 nM, respectively. The log *P* values for SUAM-1221 and JTP-4819 were 1.8 and 0.2, respectively. In vivo, these two compounds have been reported to improve performance in memory- and learning-related tests, such as the passive avoidance test for rats with scopolamine-induced amnesia.[17,18] For a compound that penetrates the blood−brain barrier, JTP-4819 has a remarkably low log *P* value. Therefore, the log *P* value should be used only as a first prediction of the blood−brain barrier penetration.

The starting point for the development of new POP inhibitors was compound **1a**. Although this compound was slightly less potent and to some extent also less druglike than SUAM-1221, it represented a new type of POP inhibitor that had the potential to be developed further. To study the structure−activity relationships of compound **1a** further, some modifications of the molecular structure were made. The L-prolyl groups at the P2 and P4 sites were replaced by acyclic α-amino acid residues. The pyrrolidinyl group at the P5 site was replaced by different alkylamino, cycloalkyl, alkyl, and phenyl groups. In combination with the replacements at the P5 site the pyrrolidinyl group at the P1 site was also replaced symmetrically by some of these groups. All compounds were tested in vitro against POP from pig brain, and the log *P* values for a selection of them were determined.

Synthetic Chemistry. The synthetic procedures for the compounds are presented in Scheme 1. The earlier presented synthetic route for compound **1a** could be used only for its symmetrical glycyl analogue,[16] because this route may cause racemization for other α-amino acids other than glycine and L-proline. The other symmetrical compounds were synthesized by reacting 2 equiv of the α-aminoacyl derivative AX with isophthaloyl dichloride.

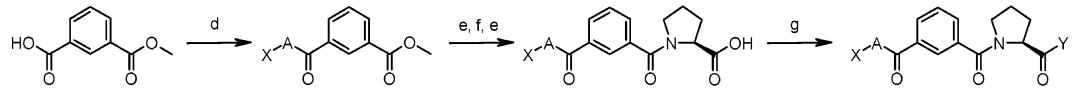
The synthesis of the unsymmetrical compounds with a central isophthaloyl group was started from iso-

Scheme 1[a]



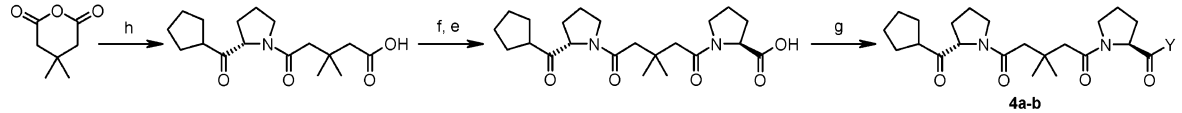
2a-d, 3j-k

2a: AX = Gly-pyrrolidine
2b: AX = L-Ala-pyrrolidine
2c: AX = L-Phe-pyrrolidine
2d: AX = L-Met-pyrrolidine
3j: AX = 2(*S*)-(cyclopentanecarbonyl)pyrrolidinyl
3k: AX = 2(*S*)-acetylpyrrolidinyl



2e, 3a-c, 3e-g, 3l-r

2e: AX = L-Ala-pyrrolidine; Y = pyrrolidinyl
3a: AX = L-Pro-pyrrolidine; Y = benzylamino
3b: AX = L-Pro-pyrrolidine; Y = azepanyl
3c: AX = L-Pro-pyrrolidine; Y = piperazinyl
3e: AX = 2(*S*)-(cyclopentanecarbonyl)pyrrolidinyl ; Y = pyrrolidinyl
3f: AX = 2(*S*)-acetylpyrrolidinyl ; Y = pyrrolidinyl
3g: AX = 2(*S*)-benzoylpyrrolidinyl ; Y = pyrrolidinyl
3l: AX = 2(*S*)-(cyclopentanecarbonyl)pyrrolidinyl ; Y = 2(*S*)-cyanopyrrolidinyl
3m:AX = 2(*S*)-(cyclohexanecarbonyl)pyrrolidinyl ; Y = 2(*S*)-cyanopyrrolidinyl
3n: AX = 2(*S*)-acetylpyrrolidinyl ; Y = 2(*S*)-cyanopyrrolidinyl
3o: AX = 2(*S*)-isobutanoylpyrrolidinyl ; Y = 2(*S*)-cyanopyrrolidinyl
3p: AX = 2(*S*)-benzoylpyrrolidinyl ; Y = 2(*S*)-cyanopyrrolidinyl
3q: AX = 2(*S*)-(cyclopentanecarbonyl)pyrrolidinyl ; Y = 2(*S*)-(hydroxyacetyl)pyrrolidinyl
3r: AX = 2(*S*)-acetylpyrrolidinyl ; Y = 2(*S*)-(hydroxyacetyl)pyrrolidinyl



4a-b

4a: Y = 2(*S*)-cyanopyrrolidinyl
4b: Y = 2(*S*)-(hydroxyacetyl)pyrrolidinyl

[a] (a) AX, NaOH/diethyl ether (dichloromethane), water; (b) glycine, NaOH/diethyl ether, water; (c) (1) pivaloyl chloride, Et_3N/dichloromethane, (2) pyrrolidine, Et_3N/dichloromethane; (d) (1) pivaloyl chloride, Et_3N/dichloromethane, (2) AX, Et_3N/dichloromethane; (e) LiOH/MeOH, water; (f) (1) pivaloyl chloride, Et_3N/dichloromethane, (2) L-proline methyl ester, Et_3N/dichloromethane; (g) (1) pivaloyl chloride, Et_3N/dichloromethane, (2) Y (mono-Boc-piperazine and 2(*S*)-(acetoxyacetyl)pyrrolidine were used in the coupling reaction when Y was piperazinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl; the protecting groups had to be removed to obtain the end product), Et_3N/dichloromethane; (h) 2(*S*)-(cyclopentanecarbonyl)pyrrolidine, Et_3N/tetrahydrofuran.

Table 1. Inhibitory Activity of the Compounds of Series **2** against POP from Pig Brain (95% Confidence Intervals Given in Parentheses)

compd	R, R′ (α-amino acid)		R″, R‴ (α-amino acid)		IC_{50}, nM
1a	$-(CH_2)_3-$	(L-Pro)	$-(CH_2)_3-$	(L-Pro)	26 (21−32)
2a	H, H	(Gly)	H, H	(Gly)	640 (490−820)
2b	methyl, H	(L-Ala)	methyl, H	(L-Ala)	87 (78−97)
2c	benzyl, H	(L-Phe)	benzyl, H	(L-Phe)	81 (61−107)
2d	$-(CH_2)_2SCH_3$, H	(L-Met)	$-(CH_2)_2SCH_3$, H	(L-Met)	110 (65−180)
2e	methyl, H	(L-Ala)	$-(CH_2)_3-$	(L-Pro)	39 (33−46)

Table 2. Inhibitory Activity of the Compounds of Series **3** against POP from Pig Brain (95% Confidence Intervals Given in Parentheses; log *P* Values Are Presented If Determined)

compd	X	Y	IC_{50}, nM	log *P*
1a	pyrrolidin-1-yl	pyrrolidin-1-yl	26 (21−32)	−0.2
1b	pyrrolidin-1-yl	2(*S*)-formylpyrrolidin-1-yl	1.3 (1.1−1.6)	
1c	pyrrolidin-1-yl	2(*S*)-cyanopyrrolidin-1-yl	1.5 (1.1−2.2)	
1d	pyrrolidin-1-yl	2(*S*)-(hydroxyacetyl)pyrrolidin-1-yl	0.39 (0.24−0.62)	
3a	benzylamino	pyrrolidin-1-yl	31 (27−35)	
3b	azepan-1-yl	pyrrolidin-1-yl	23 (18−28)	
3c	piperazin-1-yl	pyrrolidin-1-yl	33 (28−39)	
3d	4-Boc-piperazin-1-yl	pyrrolidin-1-yl	170 (150−200)	
3e	cyclopentyl	pyrrolidin-1-yl	14 (10−20)	1.1
3f	methyl	pyrrolidin-1-yl	65 (58−74)	
3g	phenyl	pyrrolidin-1-yl	18 (15−22)	
3h	methoxy	cyclopentyl	640 (550−750)	
3i	methoxy	pyrrolidin-1-yl	54 (35−81)	
3j	cyclopentyl	cyclopentyl	78 (73−83)	2.7
3k	methyl	methyl	54% inhib at 0.1 mM	
3l	cyclopentyl	2(*S*)-cyanopyrrolidin-1-yl	1.1 (0.90−1.4)	0.8
3m	cyclohexyl	2(*S*)-cyanopyrrolidin-1-yl	0.72 (0.56−0.93)	
3n	methyl	2(*S*)-cyanopyrrolidin-1-yl	4.2 (2.5−7.3)	−0.6
3o	isopropyl	2(*S*)-cyanopyrrolidin-1-yl	1.6 (1.0−2.5)	0.3
3p	phenyl	2(*S*)-cyanopyrrolidin-1-yl	1.3 (1.0−1.8)	1.1
3q	cyclopentyl	2(*S*)-(hydroxyacetyl)pyrrolidin-1-yl	0.61 (0.46−0.80)	0.2
3r	methyl	2(*S*)-(hydroxyacetyl)pyrrolidin-1-yl	1.2 (0.88−1.7)	

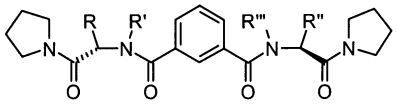
phthalic acid monomethyl ester, which was activated with pivaloyl chloride and reacted with the α-aminoacyl derivative AX. The methyl ester group was hydrolyzed. The resulting carboxylic acid was activated with pivaloyl chloride and reacted with L-proline methyl ester. The methyl ester group was hydrolyzed. The resulting carboxylic acid was activated with pivaloyl chloride and reacted with the amine Y.

The synthesis of the unsymmetrical compounds with a central 3,3-dimethylglutaroyl group was started by reacting 3,3-dimethylglutaric anhydride with 2(*S*)-(cyclopentanecarbonyl)pyrrolidine. The resulting carboxylic acid was activated with pivaloyl chloride and reacted with L-proline methyl ester. The methyl ester group was hydrolyzed. The resulting carboxylic acid was activated with pivaloyl chloride and reacted with the amine Y.

In Vitro Assay for POP Activity. The inhibitory effect of the novel compounds on POP activity of pig brain was determined according to a method described earlier.[19] Suc-Gly-Pro-7-amino-4-methylcoumarin was used as substrate, and the formation of 7-amino-4-methylcoumarin was determined fluorometrically with a microplate fluorescence reader.

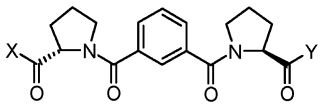
Results and Discussion

The importance of the L-prolyl groups at the P2 and P4 sites in compound **1a** was studied by synthesizing a series of compounds **2a**−**e** (Table 1). In compounds **2a**−**d** both L-prolyl groups were replaced symmetrically by glycyl, L-alanyl, L-phenylalanyl, and L-methionyl groups, respectively. All four compounds inhibited the enzyme, the L-alanyl derivative **2b** and the L-phenylalanyl derivative **2c** being the most potent ones having IC_{50} values of 87 and 81 nM, respectively. However, these two compounds were still less potent than the corresponding L-prolyl derivative **1a**. To study the relevance of symmetry in this series, an unsymmetrical compound was also made. The unsymmetrical mixed L-prolyl and L-alanyl derivative **2e** had an IC_{50} value of 39 nM. Although it was more potent than the symmetrical L-alanyl derivative **2b**, it was still slightly less potent than the symmetrical L-prolyl derivative **1a**. This series of compounds shows that replacing either one or both L-prolyl groups at the P2 and P4 sites by acyclic α-amino acid residues lowers the potency.



2

The second strategy was to make modifications on the pyrrolidinyl groups at the P1 and P5 sites of compound **1a**, resulting in a series of compounds **3** (Table 2). In compounds **3a**−**c** the P5 pyrrolidinyl group was replaced by other alkylamino groups such as the benzylamino, azepanyl, and piperazinyl groups, respectively, whereas the P1 pyrrolidinyl group was not modified. Interestingly, all three compounds were equipotent as compared



3

to compound **1a**, having IC_{50} values in the range of 23−33 nM. The Boc-protected piperazinyl derivative **3d**, which was an intermediate in the synthesis of **3c**, was less active with an IC_{50} value of 170 nM. Compounds **3a**−**d** give strong evidence for great freedom at the P5 site. This is in agreement with an earlier study in which the P5 site was not contributing to the binding of the substrate to the enzyme.[20]

Focusing on increasing the log *P* value of compound **1a**, a replacement of the nitrogen atom of the pyrrolidinyl group at the P5 site by a carbon atom was made, resulting in the cyclopentyl derivative **3e**. The pyrrolidinyl group at the P5 site was also replaced by methyl and phenyl groups, resulting in compounds **3f** and **3g**, respectively. As compared to compound **1a**, the cyclopentyl derivative **3e** and the phenyl derivative **3g** were more potent, having IC_{50} values of 14 and 18 nM, respectively, whereas the methyl derivative **3f** was less potent, having an IC_{50} value of 65 nM. This result shows that although the P5 site does not contribute to the binding of the substrate to the enzyme, it does have a small effect on the in vitro potencies of the inhibitors **3e** and **3f**, where the only difference is the alkyl substituent at the P5 site.

Because the cyclopentyl and phenyl groups can also act as the P1 site, as reported in an earlier study [4-phenylbutanoyl-2(*S*)-(cyclopentanecarbonyl)pyrrolidine and 4-phenylbutanoyl-2(*S*)-benzoylpyrrolidine had IC_{50} values of 30 and 23 nM, respectively, in the same in vitro assay],[21] the high potency of compounds **3e** and **3g** could result from their binding in the opposite direction with the P5 site as the P1 site to the active site of the enzyme. To study this hypothesis the two intermediates **3h** and **3i** were also tested. Compound **3h** has a cyclopentyl group and compound **3i** has a pyrrolidinyl group at the P1 site. Both compounds have an L-proline methyl ester group at the P4−P5 sites, which is not a preferred group at the P1−P2 sites. The L-proline methyl ester group might be hydrolyzed in the in vitro assay. However, if this happens, the resulting free L-proline group is also not a preferred group at the P1−P2 sites. The cyclopentyl derivative **3h** had an IC_{50} value of only 640 nM, whereas the pyrrolidinyl derivative **3i** had an IC_{50} value of 54 nM. To study the direction of binding further, both pyrrolidinyl groups at the P1 and P5 sites of compound **1a** were replaced symmetrically by cyclopentyl and methyl groups, resulting in compounds **3j** and **3k**, respectively. The cyclopentyl derivative **3j** was moderately potent, having an IC_{50} value of 78 nM, whereas the methyl derivative **3k** was a poor inhibitor, having only 54% inhibition at 0.1 mM.

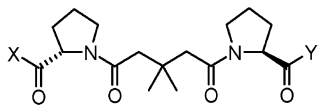
On the basis of the results from compounds **3h**−**j**, it is obvious that a cyclopentyl group can act as a P1 group. However, replacing the pyrrolidinyl group at the P1 site by a cyclopentyl group always decreased the potency. Therefore, it is unlikely that compound **3e** would bind in the opposite direction with the P5 site as the P1 site to the active site of the enzyme. This conclusion applies most probably also to compound **3g**, on the basis of the result from the earlier study in which the cyclopentyl and the phenyl groups at the P1 site were reported to give equipotent compounds.[21] From the result from compound **3k**, it is apparent that a methyl group cannot act as a P1 group.

Table 3. Inhibitory Activity of the Compounds of Series **4** against POP from Pig Brain (95% Confidence Intervals Given in Parentheses; log *P* Values Are Presented If Determined)

compd	X	Y	IC_{50}, nM	log *P*
1e	pyrrolidin-1-yl	pyrrolidin-1-yl	13 (12−14)	
4a	cyclopentyl	2(*S*)-cyanopyrrolidin-1-yl	0.57 (0.38−0.84)	
4b	cyclopentyl	2(*S*)-(hydroxyacetyl)-pyrrolidin-1-yl	0.32 (0.23−0.45)	0.7

Analogues of compounds **3e**−**g** in which the pyrrolidinyl group at the P1 site was replaced by 2(*S*)-cyanopyrrolidine and 2(*S*)-(hydroxyacetyl)pyrrolidine groups were synthesized in order to increase the potency. In this group of compounds the P5 site was varied further, including compounds with cyclohexyl and isopropyl groups at the P5 site. The resulting compounds **3l**−**r** were highly potent, having IC_{50} values in the range of 0.6−4.2 nM. The 2(*S*)-(hydroxyacetyl)pyrrolidine group at the P1 site increased the potency slightly more than the 2(*S*)-cyanopyrrolidine group. The methyl group at the P5 site resulted again in the least potent compounds with both 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups at the P1 site.

As reported earlier for compound **1e**, the central isophthaloyl group at the P3 site could be replaced by a 3,3-dimethylglutaroyl group.[16] The 3,3-dimethylglutaroyl analogues of compounds **3l** and **3q** were made, resulting in compounds **4a** and **4b**, respectively (Table 3). Both compounds were highly potent, having IC_{50} values in the range of 0.3−0.6 nM.



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The increased potency of compounds **1b**−**d**, **3l**−**r**, and **4a**,**b** is a result of the interaction between the formyl, cyano, and hydroxyacetyl substituents of the pyrrolidinyl group at the P1 site and the serine residue (Ser554) at the active site of the enzyme. The formyl group has been reported to form a reversible hemiacetal adduct with the serine residue at the active site of the enzyme,[22] and the hydroxyacetyl group has been reported to give a tight binding type of inhibition.[19] Most probably the cyano group interacts analogously. Therefore, the compounds **1b**−**d**, **3l**−**r**, and **4a**,**b** are transition state analogue inhibitors, whereas the other compounds are substrate analogue inhibitors.

This study resulted in very potent compounds, and the log *P* values for a selection of them were determined (Tables 2 and 3). The log *P* values for compounds **1a**, **3e**, and **3j** were −0.2, 1.1, and 2.7, respectively, indicating that each replacement of a nitrogen atom of the pyrrolidine groups at the P1 and P5 sites by a carbon atom resulted in an increase of the log *P* value by >1 log unit. The effect of different P5 groups on the log *P* value was studied by comparing compounds **3l**, **3n**, **3o**, and **3p**, having log *P* values of 0.8, −0.6, 0.3, and 1.1, respectively. A replacement of the cyclopentyl group at the P5 site by a methyl or a isopropyl group lowered the log *P* value, whereas a replacement by a phenyl

group slightly raised the log *P* value. A comparison between compounds **3e**, **3l**, and **3q**, having log *P* values of 1.1, 0.8, and 0.2, respectively, showed that a replacement of the pyrrolidinyl group at the P1 site by a 2(*S*)-cyanopyrrolidine group lowered the log *P* only slightly, whereas a replacement by a 2(*S*)-(hydroxyacetyl)pyrrolidine group lowered the log *P* value by almost 1 log unit. The log *P* value for compound **4b** was also determined to establish the effect of the replacement of the central isophthaloyl group at the P3 site by a 3,3-dimethylglutaroyl group. Compound **4b** had a log *P* value of 0.7, which was slightly higher than the log *P* value of 0.2 for compound **3q**.

The trends of the measured log *P* values combined with the data of the in vitro potency clearly point out compounds **3e**, **3g**, **3l**, **3m**, **3p**, **4a**, and **4b** as good candidates for further pharmacological studies. The log *P* values for these compounds are in the same range as for SUAM-1221 and JTP-4819, which were reported to be active in vivo. Compound **3j** has the highest log *P* value, and it might also have to be considered as a candidate for further pharmacological studies. However, the log *P* value is a first prediction of the blood−brain barrier penetrability, and no selection of compounds for further pharmacological studies should be made on the basis of the log *P* value alone.

Conclusions

The structure−activity relationship for compound **1a** was studied with the focus on increasing the log *P* value. Replacing one or both L-prolyl groups at the P2 and the P4 sites with acyclic α-amino acid residues, such as glycyl, L-alanyl, L-phenylalanyl, and L-methionyl, decreased the potency. Replacing the pyrrolidinyl group at the P5 site by other alkylamino groups, such as benzylamino, azepanyl, and piperazinyl groups, resulted in equipotent compounds, indicating that there is a great freedom at the P5 site. Replacing the nitrogen atom of the pyrrolidinyl group at the P5 site by a carbon atom slightly increased the potency. On the other hand, replacing the nitrogen atoms of both pyrrolidinyl groups at the P1 and P5 sites by carbon atoms slightly decreased the potency. However, each replacement of a nitrogen atom by a carbon atom increased the log *P* by >1 log unit. Furthermore, replacing the pyrrolidinyl group at the P5 site by methyl, isopropyl, cyclohexyl, and phenyl groups was also allowed, whereas replacing the pyrrolidinyl group at the P1 site by a methyl group was not allowed. The most potent compounds resulted from replacing the pyrrolidinyl group at the P5 site with cyclopentyl, cyclohexyl, or phenyl combined with replacing the pyrrolidinyl group at the P1 site by 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups. The 2(*S*)-cyanopyrrolidinyl group decreased the log *P* value only slightly, whereas the 2(*S*)-(hydroxyacetyl)pyrrolidine group decreased the log *P* value by almost 1 log unit. By replacing the central isophthaloyl group at the P3 site by a 3,3-dimethylglutaroyl group, the log *P* value of the 2(*S*)-(hydroxyacetyl)pyrrolidinyl derivative could be raised slightly. In all, this study resulted in very potent POP inhibitors with improved log *P* values for penetrating the blood−brain barrier. Further pharmacological studies for these compounds have to be carried out next.

Experimental Section

Analytical. NMR spectra were recorded on a Bruker Avance 500 spectrometer (500.1 MHz for ^{1}H and 125.8 MHz for ^{13}C) or a Bruker AM 400 spectrometer (400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C), $CDCl_3$ was used as solvent (if not otherwise noted), and chemical shifts are expressed in parts per million relative to tetramethylsilane as internal standard. For compounds **3c**, **3l**−**r**, and **4a**,**b** the ^{13}C NMR spectra are not presented, due to difficulties in assigning the spectra. Amides N-terminal to prolyl groups have energetically similar cis and trans isomers (also sometimes referred to as rotamers), which complicates the NMR spectra. For the intermediates **3h** and **3i** no ^{13}C NMR spectra were measured. Positive ion mass spectra were acquired with ESI-MS, using a Finnegan MAT LCQ quadropole ion trap mass spectrometer equipped with an ESI source. Combustion analysis for CHN was measured on an EA1110 ThermoQuest CE Instruments elemental analyzer. No melting points were determined as the products were mostly hygroscopic amorphous compounds.

Synthesis. All chemicals and solvents were of commercial quality and were purified if necessary following standard procedures. Boc-L-prolyl-pyrrolidine, Boc-2(*S*)-(cyclopentanecarbonyl)pyrrolidine, Boc-2(*S*)-(cyclohexanecarbonyl)pyrrolidine, Boc-2(*S*)-isobutanoylpyrrolidine, Boc-2(*S*)-acetylpyrrolidine, Boc-2(*S*)-benzoylpyrrolidine, Boc-2(*S*)-(acetoxyacetyl)pyrrolidine, and Boc-2(*S*)-cyanopyrrolidine were synthesized according to reported procedures.[21,23,24]

Procedure A: Amide Formation Using Pivaloyl Chloride Activation. A solution of pivaloyl chloride (1.0 mmol) in dichloromethane was added to a solution of carboxylic acid (1.0 mmol) and triethylamine (1.1 mmol) in dichloromethane at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. A solution of triethylamine (1.1 mmol) and the amine (1.0−1.1 mmol) in dichloromethane was slowly added at 0 °C (if the amine was in the form of a trifluoroacetic acid salt or HCl salt, then 3.3 mmol of triethylamine was used and the triethylamine was added separately before the addition of the amine). The reaction mixture was stirred for 2−3 h or overnight at room temperature. The dichloromethane solution was washed with 30% citric acid, saturated NaCl, and saturated $NaHCO_3$. The dichloromethane phase was dried with anhydrous Na_2SO_4 and evaporated.

Procedure B: Removal of Boc-Protecting Group. Trifluoroacetic acid (2−4 mL) was added to the Boc-protected amine (1.0 mmol) in dichloromethane (5−10 mL) at 0 °C, and the reaction was stirred at 0 °C for 2 h. The solvent was removed, and the product was evaporated in vacuo, yielding the corresponding amine trifluoroacetic acid salt. Alternatively, an excess of ethyl acetate saturated with HCl was added to the Boc-protected amine at room temperature, and the reaction mixture was stirred at room temperature for 30 min; afterward, the solvent was removed and the product was evaporated in vacuo, yielding the corresponding amine HCl salt.

Procedure C: Bis-amide Formation with Isophthaloyl Dichloride. A solution of isophthaloyl dichloride (1.0 mmol) in diethyl ether or dichloromethane was mixed with a solution of amine (2.0 mmol) in diethyl ether or dichloromethane in the presence of an excess of 4 M NaOH at 0 °C with vigorous stirring. The reaction was stirred vigorously for 1−2 h at room temperature. Dichloromethane was added, and the phases were separated. The organic phase was washed with saturated NaCl, dried with anhydrous Na_2SO_4, and evaporated.

Procedure D: Hydrolysis of a Carboxylic Acid Methyl Ester. LiOH (1.0−1.5 mmol) was added to a solution of the carboxylic acid methyl ester (1.0 mmol) in a small volume of 25% water in methanol. The reaction was stirred overnight or longer at room temperature. The methanol was evaporated, and the residue was dissolved in water. The aqueous phase was washed with dichloromethane. The aqueous phase was made acidic with 2−3 M HCl, and the product was extracted with dichloromethane. The dichloromethane phase was dried with anhydrous Na_2SO_4 and evaporated, yielding the product.

Procedure E: Hydrolysis of an *O*-Acetyl Group. K_2CO_3 (1.1 mmol) was added very slowly to a solution of the acetyl-

protected compound (1.0 mmol) in 50% methanol in water (6 mL) at 0 °C. The reaction was stirred 1 h at room temperature. The methanol was evaporated. Dichloromethane was added, and the dichloromethane phase was washed with saturated NaCl, dried with anhydrous Na_2SO_4, and evaporated.

Isophthalic acid bis(glycyl-pyrrolidine) amide (2a) was prepared according to the method described for **1a**.[16] The product was crystallized from dichloromethane−hexane. MS (ESI), *m/z* 387 ($[MH]^+$); ^{1}H NMR δ 1.89 (qui, 4H, J = 6.8 Hz), 2.01 (qui, 4H, J = 6.8 Hz), 3.46 (t, 4H, J = 6.8 Hz), 3.54 (t, 4H, J = 6.8 Hz), 4.19 (d, 4H, J = 4.3 Hz), 7.45−8.33 (m, 4H) 7.62 (br t, 2H, J = 4.3 Hz); ^{13}C NMR δ 24.11, 25.92, 42.55, 45.53, 46.00, 125.89, 128.71, 130.15, 134.33, 166.44, 166.54. Anal. ($C_{20}H_{26}N_4O_4 \cdot 0.5CH_2Cl_2$) C, H, N.

Boc-L-alanyl-pyrrolidine. A solution of *N*,*N*′-dicyclohexylcarbodiimide (10.9 g, 53 mmol) in acetonitrile (100 mL) was added to a solution of Boc-L-alanine (10.0 g, 53 mmol) and *N*-hydroxysuccinimide (6.2 g, 53 mmol) in acetonitrile (100 mL) at −20 °C. The reaction was stirred at −20 °C for 2 h, and then it was left without stirring at −20 °C overnight. The formed *N*,*N*′-dicyclohexylurea was filtered off and the filtrate evaporated. The residue was scraped with hexane, and the last traces of hexane were evaporated in vacuo, yielding the activated ester (15.0 g, 52 mmol). Pyrrolidine (8.7 mL, 104 mmol) was added to a solution of the activated ester in tetrahydrofuran (200 mL) at room temperature. The reaction mixture was stirred overnight. The solvent was evaporated, and the residue was dissolved in dichloromethane. The dichloromethane solution was washed with 30% citric acid, saturated NaCl, and saturated $NaHCO_3$. The dichloromethane phase was dried with anhydrous Na_2SO_4 and evaporated. Purification was by flash chromatography (yield 9.1 g, 37 mmol, 72%). Boc-L-phenylalanyl-pyrrolidine and Boc-L-methionyl-pyrrolidine were prepared according to the same method.

Isophthalic Acid Bis(L-alanyl-pyrrolidine) Amide (2b). Boc-L-alanyl-pyrrolidine (1.2 g, 5.0 mmol) was deprotected according to procedure B. The L-alanyl-pyrrolidine trifluoroacetic acid salt was reacted according to procedure C. Purification was by flash chromatography (yield 0.98 g, 2.4 mmol, 95%). MS (ESI), *m/z* 415 ($[MH]^+$); ^{1}H NMR δ 1.45 (d, 6H, J = 6.9 Hz), 1.86−2.05 (m, 8H), 3.42−3.75 (m, 8H), 4.95 (br qui, 2H, $J \approx 7$ Hz), 7.46−8.31 (m, 4H), 7.53 (br d, 2H, J = 7.4 Hz); ^{13}C NMR δ 18.26, 24.16, 26.10, 46.15, 46.51, 47.41, 125.62, 128.80, 130.33, 134.46, 165.74, 171.00. Anal. ($C_{22}H_{30}N_4O_4 \cdot 0.2H_2O$) C, H, N.

Isophthalic acid bis(L-phenylalanyl-pyrrolidine) amide (2c) was prepared according to the method described for **2b**. MS (ESI), *m/z* 567 ($[MH]^+$); ^{1}H NMR δ 1.52−1.82 (m, 8H), 2.61−2.68 (m, 2H), 3.08−3.21 (m, 4H), 3.30−3.36 (m, 2H), 3.43−3.52 (m, 4H), 5.11−5.15 (m, 2H), 7.21−7.30 (m, 10H), 7.43−8.29 (m, 4H), 7.60 (br d, 2H, J = 8.0 Hz); ^{13}C NMR δ 24.04, 25.78, 39.55, 45.87, 46.47, 53.09, 125.58, 127.03, 128.46, 128.81, 129.50, 130.54, 134.20, 136.46, 165.89, 169.86. Anal. ($C_{34}H_{38}N_4O_4 \cdot 1.5H_2O$) C, H, N.

Isophthalic acid bis(L-methionyl-pyrrolidine) amide (2d) was prepared according to the method described for **2b**. MS (ESI), *m/z* 535 ($[MH]^+$); ^{1}H NMR δ 1.85−2.14 (m, 6H), 2.12 (s, 3H), 2.58−2.68 (m, 2H), 3.41−3.48 (m, 1H), 3.56−3.65 (m, 2H), 3.79−3.85 (m, 1H), 5.08−5.14 (m, 2H), 7.33−8.22 (m, 4H), 7.80 (br d, J = 8.1 Hz); ^{13}C NMR δ 15.75, 24.21, 26.14, 30.56, 32.11, 46.19, 46.64, 50.67, 125.83, 128.56, 130.27, 133.93, 166.10, 170.25. Anal. ($C_{26}H_{38}N_4O_4S_2 \cdot 0.4H_2O$) C, H, N.

Isophthalic Acid Mono(L-alanyl-pyrrolidine) Amide. Boc-L-alanyl-pyrrolidine (2.4 g, 10 mmol) was deprotected according to procedure B. Isophthalic acid monomethyl ester (1.8 g, 10 mmol) and the L-alanyl-pyrrolidine trifluoroacetic acid salt were coupled according to procedure A. The methyl ester group was hydrolyzed according to procedure D. Yield 1.9 g (6.6 mmol, 66%).

Isophthalic Acid L-Alanyl-pyrrolidine L-Proline Amide. Isophthalic acid mono(L-alanyl-pyrrolidine) amide (1.9 g, 6.6 mmol) and L-proline methyl ester HCl salt (1.1 g, 6.6 mmol) were coupled according to procedure A. Purification was by flash chromatography. The methyl ester group was hydrolyzed according to procedure D. Yield 0.76 g (2.0 mmol, 30%).

Isophthalic Acid L-Alanyl-pyrrolidine L-Prolyl-pyrrolidine Amide (2e). Isophthalic acid L-alanyl-pyrrolidine L-proline amide (0.76 g, 2.0 mmol) and pyrrolidine (0.18 mL, 2.2 mmol) were coupled according to procedure A. Purification was by flash chromatography. Yield 0.68 g (1.54 mmol, 79%); MS (ESI), *m/z* 441 ($[MH]^+$); ^{1}H NMR δ 1.44 (d, J = 6.9 Hz, 3H), 1.50−2.28 (m, 12H), 2.55−3.99 (m, 10H), 4.25−4.95 (m, 2H), 7.28−8.07 (m, 4H); ^{13}C NMR δ 18.40, 24.15, 24.20, 25.57, 26.09, 26.25, 29.03, 46.08, 46.13, 46.46, 47.42, 50.31, 58.24, 125.95, 128.58, 128.78, 130.45, 134.31, 136.94, 165.75, 168.55, 170.41, 170.83. Anal. ($C_{24}H_{32}N_4O_4 \cdot 0.3H_2O$) C, H, N.

Isophthalic Acid Mono(L-prolyl-pyrrolidine) Amide. Boc-L-prolyl-pyrrolidine (8.3 g, 30.9 mmol) was deprotected using HCl-saturated ethyl acetate (50 mL) according to procedure B. Isophthalic acid monomethyl ester (5.6 g, 30.9 mmol) and the L-prolyl-pyrrolidine HCl salt were coupled according to procedure A. Purification was by flash chromatography. The methyl ester group was hydrolyzed according to procedure D. Yield 5.1 g (16.0 mmol, 52%).

Isophthalic Acid (L-Proline Methyl Ester) L-Prolyl-pyrrolidine Amide (3i). Isophthalic acid mono(L-prolyl-pyrrolidine) amide (5.1 g, 16.0 mmol) and L-proline methyl ester HCl salt (2.6 g, 16.0 mmol) were coupled according to procedure A. Purification was by flash chromatography. Yield 4.2 g (9.8 mmol, 61%); MS (ESI), *m/z* 428 ($[MH]^+$); ^{1}H NMR δ 1.30 (m, 12H), 2.67−3.98 (m, 8H), 3.78 (s, 3H), 4.27−4.85 (m, 2H), 7.38−7.77 (m, 4H). Anal. ($C_{23}H_{29}N_3O_5 \cdot 0.4H_2O$) C, H, N.

Isophthalic Acid L-Proline L-Prolyl-pyrrolidine Amide. The methyl ester group of **3i** was hydrolyzed according to procedure D. Yield 3.2 g (7.9 mmol, 81%).

Isophthalic Acid L-Prolylbenzylamine L-Prolyl-pyrrolidine Amide (3a). Isophthalic acid L-proline L-prolyl-pyrrolidine amide (0.65 g, 1.6 mmol) and benzylamine (0.17 mL, 1.6 mmol) were coupled according to procedure A. Purification was by flash chromatography. Yield 0.48 g (0.95 mmol, 60%); MS (ESI), *m/z* 503 ($[MH]^+$); ^{1}H NMR δ 1.30−2.71 (m, 12H), 2.97−3.74 (m, 8H), 3.74−4.85 (m, 4H), 7.06−7.76 (m, 9H); ^{13}C NMR δ 24.19, 25.43, 25.58, 26.23, 27.41, 28.97, 43.53, 46.07, 46.43, 50.30, 50.49, 58.28, 60.12, 125.98, 127.26, 127.50, 128.46, 128.64, 128.73, 129.20, 136.35, 136.80, 138.38, 168.40, 170.31, 170.49, 170.89. Anal. ($C_{29}H_{34}N_4O_4 \cdot 0.3H_2O$) C, H, N.

Isophthalic acid L-prolylazepane L-prolyl-pyrrolidine amide (3b) was prepared according to the method described for **3a**. MS (ESI), *m/z* 495 ($[MH]^+$); ^{1}H NMR δ 1.30−2.32 (m, 20H), 2.70−4.00 (m, 12H), 4.27−4.52 (m, 2H), 7.35−7.81 (m, 4H); ^{13}C NMR δ 24.20, 25.56, 26.25, 26.77, 26.93, 26.95, 27.58, 29.01, 29.13, 29.58, 46.03, 46.42, 46.53, 47.95, 50.29, 50.33, 56.83, 58.21, 126.04, 128.20, 129.02, 129.07, 136.68, 136.78, 168.54, 168.67, 170.36, 171.59. Anal. ($C_{28}H_{38}N_4O_4 \cdot 0.9H_2O$) C, H, N.

Mono-Boc-piperazine. A solution of di-*tert*-butyl dicarbonate (2.2 g, 10 mmol) in dichloromethane (20 mL) was added slowly to a solution of piperazine (1.7 g, 20 mmol) in dichloromethane at 0 °C. The reaction mixture was stirred at room temperature overnight. The dichloromethane solution was washed with saturated $NaHCO_3$ and water. Diethyl ether (60 mL) was added, and the organic phase was washed with water. The organic phase was dried with anhydrous Na_2SO_4 and evaporated. Purification was by flash chromatography. Yield 1.0 g (5.4 mmol, 54%).

Isophthalic acid L-prolyl-(Boc-piperazine) L-prolyl-pyrrolidine amide (3d) was prepared according to the method described for **3a**. MS (ESI), *m/z* 582 ($[MH]^+$); ^{1}H NMR δ 1.44 (s, 1.4H), 1.45 (s, 0.7H), 1.47 (s, 4.8H), 1.48 (s, 1.9H), 1.60−2.30 (m, 12H), 2.66−4.98 (m, 16H), 4.23−5.06 (m, 2H), 7.31−7.79 (m, 4H); ^{13}C NMR δ 24.19, 25.57, 26.24, 28.39, 28.98, 29.39, 30.80, 42.17, 45.72, 46.03, 46.40, 47.14, 50.18, 50.30, 56.35, 58.23, 60.34, 80.21, 126.06, 128.27, 128.99, 129.15, 136.46, 136.70, 154.62, 168.54, 168.65, 170.30, 170.58. Anal. ($C_{31}H_{43}N_5O_6 \cdot 1.2H_2O$) C, H, N.

Isophthalic Acid 2(*S*)-L-Prolyl-piperazine L-Prolyl-pyrrolidine Amide (3c). Compound **3d** (0.21 g, 0.36 mmol)

was deprotected using ethyl acetate saturated with HCl according to procedure B (methanol had to added to increase the solubility, and it was reacted overnight). Purification was by flash chromatography. The product was crystallized from dichloromethane and ethyl acetate saturated with HCl. Yield 0.15 g (0.29 mmol, 80%); MS (ESI), *m/z* 482 ($[MH]^+$); ^{1}H NMR (DMSO-d_6) δ 1.44−2.32 (m, 12H), 2.58−4.01 (m, 16H), 4.34−5.02 (m, 2H), 7.14−7.62 (m, 4H), 9.10−9.80 (m, 2H). Anal. ($C_{26}H_{35}N_5O_4$·HCl·1.8H_2O) C, H, N.

Isophthalic Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine L-Prolyl-pyrrolidine Amide (3e). Boc-2(*S*)-(cyclopentanecarbonyl)pyrrolidine (0.40 g, 1.5 mmol) was deprotected according to procedure B. Isophthalic acid mono(L-prolyl-pyrrolidine) amide (0.49 g, 1.5 mmol) and the 2(*S*)-(cyclopentanecarbonyl)pyrrolidine trifluoroacetic acid salt were coupled according to procedure A. Purification was by flash chromatography. Yield 0.46 g (0.99 mmol, 66%); MS (ESI), *m/z* 466 ($[MH]^+$); ^{1}H NMR δ 1.29−2.31 (m, 20H), 2.52−3.98 (m, 8H), 4.28−4.87 (m, 2H), 7.35−7.78 (m, 4H); ^{13}C NMR δ 24.19, 25.48, 25.58, 26.06, 26.18, 26.24, 28.61, 28.70, 29.00, 29.60, 46.06, 46.42, 49.19, 50.30, 50.33, 58.27, 64.46, 125.99, 128.36, 129.00, 129.15, 136.44, 136.65, 168.60, 168.64, 170.36, 210.92. Anal. ($C_{27}H_{35}N_3O_4$·0.2H_2O) C, H, N.

Isophthalic acid 2(*S*)-acetylpyrrolidine L-prolyl-pyrrolidine amide (3f) was prepared according to the method described for **3e**. MS (ESI), *m/z* 412 ($[MH]^+$); ^{1}H NMR δ 1.60−2.32 (m, 12 H), 2.29 (s, 3H), 2.67−3.99 (m, 8H), 4.29−4.87 (m, 2H), 7.36−7.80 (m, 4H); ^{13}C NMR δ 24.18, 25.39, 25.60, 26.23, 27.06, 28.21, 28.98, 46.04, 46.40, 50.24, 50.33, 58.26, 65.43, 125.99, 128.42, 129.01, 129.25, 136.21, 136.63, 168.46, 168.93, 170.28, 206.35. Anal. ($C_{23}H_{29}N_3O_4$·0.7H_2O) C, H, N.

Isophthalic acid 2(*S*)-benzoylpyrrolidine L-prolyl-pyrrolidine amide (3g) was prepared according to the method described for **3e**. MS (ESI), *m/z* 474 ($[MH]^+$); ^{1}H NMR δ 1.50−2.44 (12H), 2.16−3.98 (m, 8H), 4.27−4.86 (m, 1H), 5.16−5.71 (m, 1H), 7.15−8.08 (m, 9H); ^{13}C NMR δ 24.20, 25.39, 25.61, 26.25, 29.00, 29.50, 46.05, 46.43, 50.15, 50.34, 58.28, 61.51, 126.04, 128.11, 128.37, 128.58, 128.69, 129.09, 129.16, 133.30, 135.36, 136.51, 168.55, 168.60, 170.37, 197.41. Anal. ($C_{28}H_{31}N_3O_4$·0.7H_2O) C, H, N.

Isophthalic Acid Bis-2(*S*)-(cyclopentanecarbonyl)pyrrolidine Amide (3j). Boc-2(*S*)-(cyclopentanecarbonyl)pyrrolidine (0.61 g, 2.28 mmol) was deprotected according to procedure B. The 2(*S*)-(cyclopentanecarbonyl)pyrrolidine trifluoroacetic acid salt in dichloromethane was reacted according to procedure C. Purification was by flash chromatography. Yield 0.45 g (0.98 mmol, 86%); MS (ESI), *m/z* 465 ($[MH]^+$); ^{1}H NMR δ 1.29−2.05 (m, 22H), 2.20−2.32 (m, 2H), 2.53−2.63 (m, 0.4H), 3.07−3.15 (m, 1.6H), 3.43−3.83 (m, 4H), 4.36−4.57 (m, 0.4H), 4.81−4.88 (m, 1.6H), 7.23−7.75 (m, 4H); ^{13}C NMR δ 26.05, 26.05, 26.19, 28.63, 28.65, 29.59, 49.27, 50.31, 64.42, 125.90, 128.43, 129.02, 136.54, 168.57, 210.86. Anal. ($C_{28}H_{36}N_2O_4$·0.2H_2O) C, H, N.

Isophthalic acid bis-2(*S*)-acetylpyrrolidine amide (3k) was prepared according to the method described for **3j**. MS (ESI), *m/z* 357 ($[MH]^+$); ^{1}H NMR δ 1.84−2.28 (m, 8H), 2.29 (bs, 6H), 3.45−3.81 (m, 4H), 4.40−4.47 (m, 0.4H), 4.71−4.77 (m, 1.6H), 7.14−7.78 (m, 4H); ^{13}C NMR δ 25.38, 27.20, 28.20, 50.22, 65.38, 126.05, 128.49, 128.56, 129.07, 136.29, 136.35, 168.75, 206.25. Anal. ($C_{20}H_{24}N_2O_4$·0.4H_2O) C, H, N: calcd, 7.70; found, 7.16.

Isophthalic Acid Mono-2(*S*)-(cyclopentanecarbonyl)-pyrrolidine Amide. Boc-2(*S*)-(cyclopentanecarbonyl)pyrrolidine (1.3 g, 4.9 mmol) was deprotected according to procedure B. Isophthalic acid monomethyl ester (0.88 g, 4.9 mmol) and the 2(*S*)-(cyclopentanecarbonyl)pyrrolidine trifluoroacetic acid salt were coupled according to procedure A. The methyl ester group was hydrolyzed according to procedure D. Yield 1.28 g (4.1 mmol, 84%).

Isophthalic Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine (L-Proline Methyl Ester) Amide (3h). Isophthalic acid mono-2(*S*)-(cyclopentanecarbonyl)pyrrolidine amide (1.28 g, 4.1 mmol) and L-proline methyl ester HCl salt (0.75 g, 4.5 mmol) were coupled according to procedure A. Purification was by flash chromatography. Yield 1.19 g (2.8 mmol, 68%); MS (ESI), *m/z* 427 ($[MH]^+$); ^{1}H NMR δ 1.30−2.36 (m, 16H), 2.60 (qui, 0.1H, J = 8.0 Hz), 3.12 (qui, 0.9H, J = 8.0 Hz), 3.47−3.67 (m, 4H), 3.78 (br s, 3H), 4.29−4.88 (m, 2H), 7.37−7.77 (m, 4H). Anal. ($C_{24}H_{30}N_2O_5$) C, H, N.

Isophthalic Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine L-Proline Amide. The methyl ester group of **3h** (0.89 g, 2.1 mmol) was hydrolyzed according to procedure D. Yield 0.87 g (2.1 mmol, 100%). Isophthalic acid 2(*S*)-(cyclohexanecarbonyl)pyrrolidine L-proline amide, isophthalic acid 2(*S*)-isobutanoylpyrrolidine L-proline amide, isophthalic acid 2(*S*)-acetylpyrrolidine L-proline amide, and isophthalic acid 2(*S*)-benzoylpyrrolidine L-proline amide were prepared according to the same method.

Isophthalic Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine L-Prolyl-2(*S*)-cyanopyrrolidine Amide (3l). Boc-2(*S*)-cyanopyrrolidine (0.42 g, 2.1 mmol) was deprotected according to procedure B. Isophthalic acid 2(*S*)-(cyclopentanecarbonyl)-pyrrolidine L-proline amide (0.87 g, 2.1 mmol) and the 2(*S*)-cyanopyrrolidine trifluoroacetic acid salt were coupled according to procedure A. Purification was by flash chromatography. Yield 0.23 g (0.47 mmol, 22%); MS (ESI), *m/z* 491 ($[MH]^+$); ^{1}H NMR δ 1.33−2.32 (m, 20H), 2.55−4.03 (m, 7H), 4.15−4.88 (m, 3H), 7.35−7.75 (m, 4H). Anal. ($C_{28}H_{34}N_4O_4$·0.1H_2O) C, H, N.

Isophthalic acid 2(*S*)-(cyclohexanecarbonyl)pyrrolidine L-prolyl-2(*S*)-cyanopyrrolidine amide (3m) was prepared according to the method described for **3l**. MS (ESI), *m/z* 505 ($[MH]^+$); ^{1}H NMR δ 1.18−2.32 (m, 22H), 2.56−2.73 (m, 1H), 3.16−4.03 (m, 6H), 4.16−4.90 (m, 3H), 7.31−7.75 (m, 4H). Anal. ($C_{29}H_{36}N_4O_4$·0.3H_2O) C, H, N.

Isophthalic acid 2(*S*)-acetylpyrrolidine L-prolyl-2(*S*)-cyanopyrrolidine amide (3n) was prepared according to the method described for **3l**. MS (ESI), *m/z* 437 ($[MH]^+$); ^{1}H NMR δ 1.71−2.33 (m, 12H), 2.29 (s, 3H), 2.67−4.02 (m, 6H), 4.20−4.88 (m, 3H), 7.38−7.76 (m, 4H). Anal. ($C_{24}H_{28}N_4O_4$·0.2H_2O) C, H, N.

Isophthalic acid 2(*S*)-isobutanoylpyrrolidine L-prolyl-2(*S*)-cyanopyrrolidine amide (3o) was prepared according to the method described for **3l**. MS (ESI), *m/z* 465 ($[MH]^+$); ^{1}H NMR δ 1.17 (d, 3H, J = 6.9 Hz), 1.24 (d, 3H, J = 6.9 Hz), 1.76−2.32 (m, 12H), 2.62−4.03 (m, 7H), 4.15−4.91 (m, 3H), 7.32−7.75 (m, 4H). Anal. ($C_{26}H_{32}N_4O_4$·0.5H_2O) C, H, N.

Isophthalic acid 2(*S*)-benzoylpyrrolidine L-prolyl-2(*S*)-cyanopyrrolidine amide (3p) was prepared according to the method described for **3l**. MS (ESI), *m/z* 499 ($[MH]^+$); ^{1}H NMR δ 1.70−2.46 (m, 12H), 2.60−4.02 (m, 6H), 4.14−4.91 (m, 2H), 5.10−5.71 (m, 1H), 7.12−8.06 (m, 9H). Anal. ($C_{29}H_{30}N_4O_4$·0.7H_2O) C, H, N.

Isophthalic Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine L-Prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine Amide (3q). Boc-2(*S*)-(acetoxyacetyl)pyrrolidine (0.63 g, 2.3 mmol) was deprotected according to procedure B. Isophthalic acid 2(*S*)-(cyclopentanecarbonyl)pyrrolidine L-proline amide (0.90 g, 2.2 mmol) and the 2(*S*)-(acetoxyacetyl)pyrrolidine trifluoroacetic acid salt were coupled according to procedure A. Purification was by flash chromatography. The *O*-acetyl group was hydrolyzed according to procedure E. Purification was by flash chromatography. Yield 0.23 g (0.44 mmol, 19%); MS (ESI), *m/z* 524 ($[MH]^+$); ^{1}H NMR δ 1.30−2.33 (m, 20H), 2.54−4.06 (m, 7H), 4.16−4.87 (m, 5H), 7.34−7.75 (m, 4H). Anal. ($C_{29}H_{37}N_3O_6$·0.4H_2O) C, H, N.

Isophthalic acid 2(*S*)-acetylpyrrolidine L-prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine amide (3r) was prepared according to the method described for **3q**. MS (ESI), *m/z* 470 ($[MH]^+$); ^{1}H NMR δ 1.34−2.34 (m, 12H), 2.29 (s, 3H), 2.68−4.06 (m, 6H), 4.22−4.84 (m, 5H), 7.37−7.77 (m, 4H). Anal. ($C_{25}H_{31}N_3O_6$·0.2H_2O) C, H, N.

3,3-Dimethyl Glutaric Acid Mono-2(*S*)-(cyclopentanecarbonyl)pyrrolidine Amide. Boc-2(*S*)-(cyclopentanecarbonyl)pyrrolidine (1.3 g, 5.0 mmol) was deprotected according to procedure B. The 2(*S*)-(cyclopentanecarbonyl)pyrrolidine trifluoroacetic acid salt and triethylamine (2.3 mL, 16.5 mmol) were dissolved in tetrahydrofuran (50 mL). A solution of 3,3-dimethyl glutaric acid anhydride (0.71 g, 5.0 mmol) in tet-

rahydrofuran (20 mL) was added at 0 °C. The reaction was stirred overnight at room temperature. The solvent was evaporated, and the residue was dissolved in water and ethyl acetate. The phases were separated, and the ethyl acetate phase was washed with 0.1 M HCl. The ethyl acetate phase was dried with anhydrous Na_2SO_4 and evaporated. Yield 1.8 g.

3,3-Dimethyl Glutaric Acid 2(*S*)-(Cyclopentanecarbonyl)pyrrolidine L-Proline Amide. 3,3-Dimethyl glutaric acid mono-2(*S*)-(cyclopentanecarbonyl)pyrrolidine amide (0.93 g, 3.0 mmol) and proline methyl ester HCl salt (0.50 g, 3.0 mmol) were coupled according to procedure A. The methyl ester group was hydrolyzed according to procedure D. Yield 1.2 g (3.0 mmol, 100%).

3,3-Dimethylglutaric 2(*S*)-(cyclopentanecarbonyl)pyrrolidine L-prolyl-2(*S*)-cyanopyrrolidine amide (4a) was prepared according to the method described for **3l**. MS (ESI), *m*/*z* 485 ($[MH]^+$); ^{1}H NMR δ 1.17−1.21 (m, 6H), 1.52−2.61 (m, 24H), 2.95−3.09 (m, 1H), 3.47−3.88 (m, 6H), 4.16−4.82 (m, 3H). Anal. ($C_{27}H_{40}N_4O_4 \cdot 0.4H_2O$) C, H, N.

3,3-Dimethylglutaric acid 2(*S*)-(cyclopentanecarbonyl)pyrrolidine L-prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine amide (4b) was prepared according to the method described for **3q**. MS (ESI), *m*/*z* 518 ($[MH]^+$); ^{1}H NMR δ 1.17−1.20 (m, 6H), 1.52−2.59 (m, 24H), 2.95−3.08 (m, 1H), 3.16 (br s, 1H), 3.52−3.68 (m, 5H), 3.87−3.94 (m, 1H), 4.28−4.43 (m, 2H), 4.63−4.70 (m, 3H). Anal. ($C_{28}H_{43}N_3O_6 \cdot 0.2H_2O$) C, H, N.

In Vitro Assay for POP Activity. The whole pig brains, excluding cerebellum and most of the brain stem, of three pigs were frozen in liquid nitrogen within 30 min from slaughtering and stored at −80 °C until homogenized. The brains were homogenized in 3 volumes (w/v) of ice-cold 0.1 M sodium−potassium phosphate buffer (pH 7.0), and the homogenates were centrifuged for 20 min at 4 °C at 10000*g*. The supernatants were pooled and stored in small aliquots at −80 °C until used. The supernatant was thawed in ice and diluted (1:2) with homogenization buffer. In the microplate assay procedure, 10 μL of the enzyme preparation (protein concentration = 4.3 mg/mL) was preincubated with 460 μL of 0.1 M sodium−potassium phosphate buffer (pH 7.0) and 5 μL of a solution of the compound dissolved in DMSO and diluted with 0.1 M sodium−potassium phosphate buffer at 30 °C for 30 min (final DMSO concentration was <0.1%). The controls contained 10 μL of enzyme preparation and 465 μL of 0.1 M sodium−potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 25 μL of 4 mM Suc-Gly-Pro-7-amino-4-methylcoumarin dissolved in 0.1 M sodium−potassium phosphate buffer (pH 7.0), and the mixture was incubated at 30 °C for 60 min. The reaction was terminated by adding 500 μL of 1 M sodium acetate buffer (pH 4.2). Formation of 7-amino-4-methylcoumarin was determined fluorometrically with microplate fluorescence reader (excitation at 360 nm and emission at 460 nm). In the uninhibited reaction, the specific POP activity was 3.8 nmol/min/mg of protein. The final concentration of the compounds in the assay mixture varied from 10^{-12} to 10^{-5} M. The inhibitory activities (percent of control) were plotted against the log concentration of the compound, and the IC_{50} value was determined by nonlinear regression utilizing GraphPad Prism 3.02 software.

Determination of log *P* Values. A known concentration of an inhibitor in phosphate buffer (saturated with 1-octanol, pH 7.4) was shaken with a suitable volume of 1-octanol for 60 min at room temperature. The phases were separated by centrifugation for 5 min at 2000 rpm, and the aqueous phase was analyzed. The partition coefficient was calculated in relation to a control that was treated in the same way as the samples but did not contain 1-octanol. Each partition coefficient was determined at least in triplicate. For each HPLC method 20 mM KH_2PO_4 (pH 7) was used as the aqueous phase. As the organic phase, methanol was used for compound **4b** and 90% acetonitrile for the other compounds. The HPLC methods were tested for linearity and repeatability. The Merck Hitachi HPLC system consisted of a UV detector (L-7400), an interface module (D-7000), a pump (L-7100), an autosampler (L-7250), and a Purospher RP-C18e column (125 × 4 mm, 5 μm).

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