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Design and Synthesis of Novel Prolyl Oligopeptidase Inhibitors

Doctoral dissertation

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ABSTRACT

The serine protease prolyl oligopeptidase (POP) is widely distributed in organisms and within the human body. POP hydrolyzes L-proline containing oligopeptides at the carboxyl side of L-proline. Several substrates of POP, such as substance P, vasopressin, neurotensin and thyroliberin, are implicated in learning and memory formation. It has therefore been postulated, that centrally acting POP inhibitors that increase the concentration of these oligopeptides could be beneficial in patients with cognitive disturbances. The starting point for the design and synthesis of new POP inhibitors were the N-acyl-L-prolyl-pyrrolidine type of inhibitors, where the pyrrolidinyl, L-prolyl and N-acyl groups constitute the P1, P2 and P3 sites, respectively. The inhibitory activities of the new compounds were tested *in vitro* against POP from pig brain. The log P values were also determined for a selection of the new compounds in order to make a first prediction of their ability to penetrate the blood-brain barrier.

In a series of 4-phenylbutanoyl-2(*S*)-acylpyrrolidines the cyclopentyl and phenyl groups were successfully used to replace the pyrrolidinyl group at the P1 site. In a series of dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amides a L-prolyl-pyrrolidine moiety which was connected symmetrically with a short dicarboxylic acid linker, such as glutaric acid, 3,3-dimethylglutaric acid and isophthalic acid, was successfully used to replace the typical lipophilic N-acyl group at the P3 site. The low log P value of this type of compounds was raised by replacing one of the pyrrolidinyl end groups by cyclopentyl, cyclohexyl and phenyl groups. In a series of N-acyl-5-alkyl-L-prolyl-pyrrolidines 5-alkyl-L-prolyl groups were successfully used to replace the L-prolyl group at the P2 site. The N-amides of 5-alkyl-L-prolyl groups are conformationally more rigid than those of unsubstituted L-prolyl groups. A combination of a 5(*R*)-*tert*-butyl-L-prolyl group at the P2 site and Boc group at the P3 site gave the greatest increase of the *in vitro* potency. As an additional effect, the 5-*tert*-butyl-L-prolyl group improved the lipophilicity of the compounds. In combination with the replacements of the P2 and the P3 sites, the pyrrolidinyl group at the P1 site was also replaced by 2(*S*)-formylpyrrolidinyl, 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups, which increased the *in vitro* potency.

The present study gave valuable information over the structure-activity relationship of POP inhibitors and resulted in many successful replacements in the N-acyl-L-prolyl-pyrrolidine structure. However, further pharmacological studies are needed to select a lead compound.

ACKNOWLEDGEMENTS

ABBREVIATIONS

Ac	acetyl
APP	aminopeptidase P
Boc	*tert*-butoxycarbonyl
Bu	butyl
CNS	central nervous system
CPP	carboxypeptidase P
DCM	dichloromethane
DMAP	4-*N*,*N*-dimethylaminopyridine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DPPII	dipeptidyl dipeptidase II
DPPIV	dipeptidyl dipeptidase IV
ESI	electrospray ionization (in mass spetrometry)
Et	ethyl
FID	free-induction decay
HIV1P	human immunodeficiency viron-1 protease
HPLC	high performance liquid chromatography
IC_{50}	50% inhibition concentration
K_i	inhibition constant
log P	logarithm of the partition coefficient
M	molecular mass (in mass spectrometry)
Me	methyl
MS	mass spectrometry
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMR	nuclear magnetic resonance
P1...Pn	amino acid sites counting from the hydrolyzed bond towards the N-terminal end in a peptide substrate (also used for the corresponding binding sites for an inhibitor when it binds to the active site similar to the substrate).
P1'...Pn'	amino acid sites counting from the hydrolyzed bond towards the C-terminal end in a peptide substrate (also used for the corresponding binding sites for an inhibitor when it binds to the active site similar to the substrate).
PCP	prolyl carboxypeptidase
PEP	prolyl endopeptidase (also known as prolyl oligopeptidase)
Ph	phenyl
PhF	phenylfluorenyl
pK_a	negative logarithm of the ionization constant
POP	prolyl oligopeptidase
PPCE	post proline cleaving enzyme (also known as prolyl oligopeptidase)
rt	room temperature

S1...Sn	binding sites of the enzyme for the P1...Pn sites of the substrate/inhibitor.
S1'...Sn'	binding sites of the enzyme for the P1'...Pn' sites of the substrate/inhibitor.
Suc	succinyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tr	trityl, triphenylmethyl
Ts	toluenesulfonyl
UV	ultra violet
Xaa	an unspecified amino acid residue
Yaa	an unspecified amino acid residue
Z	benzyloxycarbonyl
ZIP	Z-L-prolyl-L-prolinal insensitive Z-Gly-Pro-7-amino-4-methyl coumarine hydrolyzing peptidase

LIST OF ORIGINAL PUBLICATIONS

The present doctoral dissertation is based on the following papers I-V:

I   Wallén E A A, Christiaans J A M, Saario S M, Forsberg M M, Venäläinen J I, Paso H M, Männistö P T, Gynther J:
4-Phenylbutanoyl-2(*S*)-acylpyrrolidines and 4-phenylbutanoyl-L-prolyl-2(*S*)-acylpyrrolidines as prolyl oligopeptidase inhibitors.
Bioorg Med Chem 10: 2199-2206, 2002.

II   Wallén E A A, Christiaans J A M, Forsberg M M, Venäläinen J I, Männistö P T, Gynther J:
Dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amides as prolyl oligopeptidase inhibitors.
J Med Chem 45: 4581-4584, 2002.

III   Wallén E A A, Christiaans J A M, Jarho E M, Forsberg M M, Venäläinen J I, Männistö P T, Gynther J:
New prolyl oligopeptidase inhibitors developed from dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amides.
J Med Chem (in press).

IV   Wallén E A A, Christiaans J A M, Gynther J, Vepsäläinen J:
Addition of *tert*-butylcuprate to *N*-acyl-Δ^5-dehydroprolinates as a diastereoselective synthetic procedure for obtaining (2*S*,5*S*)-5-*tert*-butylproline.
Tetrahedron Lett 44: 2081-2082, 2003.

V   Wallén E A A, Christiaans J A M, Saarinen T J, Jarho E M, Forsberg M M, Venäläinen J I, Männistö P T, Gynther J:
Conformationally rigid *N*-acyl-5-alkyl-L-prolyl-pyrrolidines as prolyl oligopeptidase inhibitors.
Bioorg Med Chem 11: 3611-3619, 2003.

CONTENTS

1

GENERAL INTRODUCTION

1. INTRODUCTION

Increasing life expectancy and the occurrence of neurodegenerative pathologies have increased the search for new biological targets implicated in neurodegenerative processes and memory loss. One of these new targets is the inhibition of the serine peptidase prolyl oligopeptidase (POP).

POP is widely distributed in organisms and within the human body. POP hydrolyzes L-proline containing oligopeptides at the carboxyl side of L-proline. Several substrates of POP, such as substance P, vasopressin, neurotensin and thyroliberin, are implicated in learning and memory formation. It has therefore been postulated, that centrally acting POP inhibitors that increase the concentration of these oligopeptides could be beneficial in patients with cognitive disturbances.

One of the first specific POP inhibitors Z-L-prolyl-L-prolinal **1** was reported in 1983. Subsequent reports that this compound protected rats from scopolamine-induced amnesia generated a large interest in the development of new POP inhibitors.
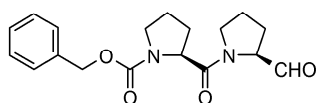
1

During the last two decades a large number of POP inhibitors have been reported in articles and patents. Nevertheless, the molecular diversity of the POP inhibitors has remained relatively low, since most POP inhibitors are based on an *N*-acyl-L-prolyl-pyrrolidine structure. Therefore, the most important objective in the present study was to develop new POP inhibitors with an increased molecular diversity.

2. PROLYL OLIGOPEPTIDASE

POP (*E.C. 3.4.21.26*) belongs to the prolyl oligopeptidase family S9 of the clan SC of serine peptidases (Barrett and Rawlings 1995, Polgár 2002). This family is unrelated to the classical trypsin and subtilisin families of serine peptidases. Other members of the prolyl oligopeptidase family are dipeptidyl peptidase IV (DPPIV), acylaminoacyl peptidase and oligopeptidase B. The amino acid sequence homology of the four peptidases is rather low, but they share a similar three-dimensional structure. The enzymes have the catalytic residues (Ser, Asp and His) concentrated in the C-terminal regions, where the amino acid sequence homology is more significant than in other parts of the enzymes. Regarding their size, they are much larger (about 80 kDa) than the classical serine peptidases trypsin and subtilisin (about 25-30 kDa). The most notable property of the family members is their selectivity, which is restricted to oligopeptides comprising not more than 30 amino acid residues.

POP was recommended its present name in 1992 emphasizing the special characteristics of the enzyme (Webb 1992). Alongside this name the earlier recommended name prolyl endopeptidase (PEP) is still in use by many research groups. In older literature also the name post proline cleaving enzyme (PPCE) is commonly used to refer to this enzyme.

POP is widely distributed in organisms. POP has been isolated from several organs, such as kidney, brain, liver and muscle, of different mammalian species, such as rat, rabbit, lamb, cow and pig, as well as from bacteria, fungi and plants (Polgar 1994). In the human body it has been found in all tissues (Kato *et al.* 1980).

POP is reported to be cytosolic, but also a membrane bound form has been identified and characterized (O'Leary and O'Connor 1995). The presence of the enzyme in serum and seminal plasma suggests that a secreted form also exists (Soeda *et al.* 1984, Siviter and Cockle 1995).

2.1. Substrate specificity

POP activity was first reported in 1971 following the observation that homogenates of human uterus released leucylglycineamide from oxytocin (Figure 1) (Walter *et al.* 1971).

Cys-Tyr-Ile-Gln-Asn-Cys-**Pro-Leu**-Gly-NH$_2$
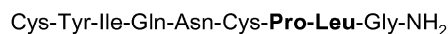
Figure 1. The amino acid sequence of oxytocin (the scissile bond is indicated in bold type).

It has later been established that POP hydrolyzes oligopeptides at the carboxyl side of L-proline, including the amide group of an L-prolineamide at the C-terminus of an oligopeptide (Figure 2) (Wilk 1983, Nomura 1986). The main restrictions are that: a) it does not hydrolyze a peptide bond between two L-proline residues, b) it does not hydrolyze the two first peptide bonds from the N-terminus and c) the largest size of the substrate is about 30 amino acid residues.

peptide-Xaa-Yaa-**Pro-NH**-peptide

Figure 2. A general structure of substrates of POP, where "Xaa" and "Yaa" are unspecified amino acid residues and "peptide" is part of an oligopeptide or simply an H (the scissile bond is indicated in bold type).

In oligopeptides conforming to these restrictions, POP can also hydrolyze a peptide bond at the carboxyl side of L-alanine, L-*N*-methylalanine and sarcosine, although the hydrolytic activity is significantly lower (Nomura 1986, Goossens *et al.* 1996).

2.2. The enzyme structure

Human, pig and bovine POP consist of a single chain 710-residue amino acid sequence (Vanhoof *et al.* 1994, Shirasawa *et al.* 1994, Rennex *et al.* 1991, Yoshimoto *et al.* 1997). The molecular masses for the human lymphocyte POP and human T cell POP are 80,745 and 80,750 Da, respectively. The amino acid sequences of POP from different mammalian sources are highly conserved. As compared to the human sequence the pig brain and bovine brain sequences showed a homology of 97% and 95%, respectively. As compared to each other, the pig brain sequence and the bovine brain sequence were 94% homologous. However, as compared to the sequence of *Flavobacterium meningosepticum* the pig brain and bovine brain sequences showed only a homology of 38% and 48%, respectively (Yoshimoto *et al.* 1991a, Yoshimoto *et al.* 1997). In all cases, the region around the catalytic residues (Ser, Asp and His) and the rest of the C-terminal region was highly conserved.

The pig enzyme serves as a good model for the human enzyme, since all of the 19 differences in the human lymphocyte and pig brain sequences are far from the active site. The crystal structures of pig muscle POP and its complex with the inhibitor Z-L-prolyl-L-prolinal **1** have been published (Fülöp *et al.* 1998). The crystal structure did not reveal any differences between the pig brain and pig muscle sequences. The following description of the structure of POP is based on the crystal structure of pig muscle POP (Figure 3).

POP has a cylindrical shape of an approximate height of 60 Å and a diameter of 50 Å. It consists of two domains, a peptidase domain and a seven-bladed β-propeller domain. The active site, containing the catalytic triad Ser554, Asp641 and His680, is located in a large cavity at the interface of these two domains.

The hydrolysis reaction catalyzed by POP follows a general serine peptidase reaction mechanism, where the first event is the nucleophilic attack by the hydroxyl group of the catalytic Ser554 on the carbonyl group of the scissile bond. The negatively charged oxyanion generated from the carbonyl oxygen of the scissile bond is stabilized by two hydrogen bonds provided by the main chain NH group of Asn555 and the hydroxyl group of Tyr473.

The S1 specificity pocket ensures a hydrophobic environment and a snug fit for the L-proline residue. The pocket is lined by the side chains of Trp595, Phe476, Val644, Val580 and Tyr599 and the side chain carbon atoms of Asn555. The specificity is enhanced by ring stacking between the indole ring of Trp595 and the pyrrolidine ring of the substrate L-proline residue.

The S2 site is less specific for substrate side chains and at this site there is an open cavity which can accommodate the different side chains. A hydrogen bond is formed between the carbonyl oxygen of the residue at the P2 site and the guanidinium group of Arg643.

The S3 binding pocket is lined by the side chains of several non-polar residues, including Phe173, Met235, Cys255, Ile591 and Ala594. This site prefers hydrophobic residues. A hydrogen bond is generated between the carbonyl oxygen of the residue at the P3 site and the side chain of Trp595.

The peptidase domain is built up of residues 1-72 and 428-710, and it has a α/β hydrolase fold. Although the peptidase domain of POP shows low sequence similarity to other α/β hydrolase enzymes, the core of their three-dimensional structures are closely superimposable.

← peptidase domain

- - - - Z-L-prolyl-L-prolinal **1**
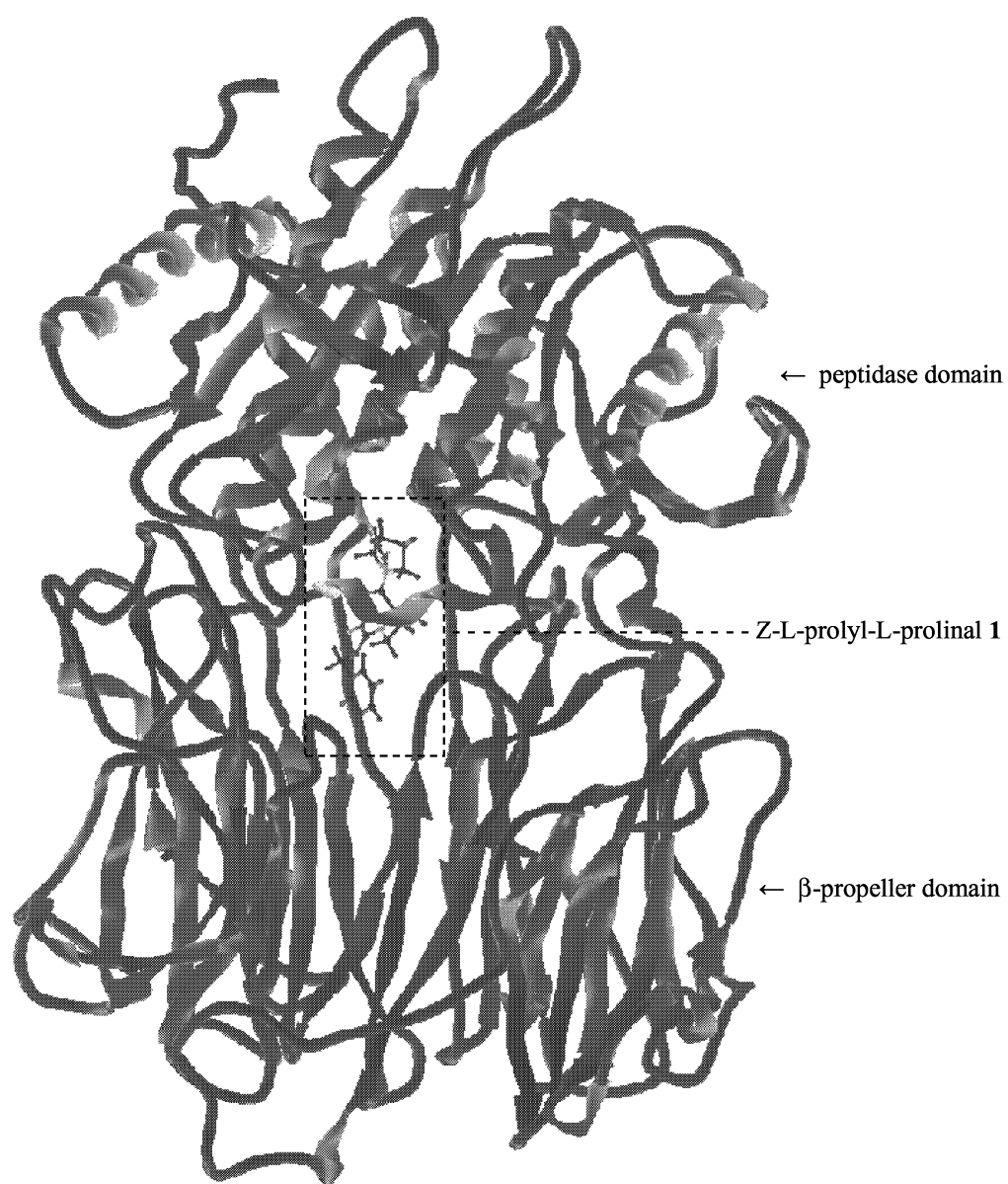
← β-propeller domain

Figure 3. The three-dimensional structure of pig muscle POP with Z-L-prolyl-L-prolinal **1** bound to the active site (the picture was kindly provided by Dr. Antti Poso).

The β-propeller domain is built up of residues 73-427 and it is based on a sevenfold repeat of four-stranded antiparallel β-sheets. The sheets are twisted and radially arranged around their central tunnel. They pack face-to-face, and the predominantly hydrophobic interaction provides most of the required structural stability. The first and last blades are not closed and there are only hydrophobic interactions between them. The narrow entrance of the β-propeller opposite to the active site is much smaller than the diameter of an average peptide, but it can be enlarged by partial separation of the unclosed first and last blades. In this manner, the β-propeller excludes larger peptides and proteins from the central cavity, thus protecting them from proteolysis. This mechanism has been confirmed by introducing a disulphide bond between the first and last blades, which inactivated the enzyme (Fülöp *et al.* 2000).

2.3. Biological relevance

Many biologically important peptides contain proline. Proline is important for peptides both in determining their molecular conformation and in conferring resistance to the action of peptidases. Therefore, the processing and degradation of such peptides often requires the use of proline-specific peptidases (Figure 4) (Vanhoof *et al.* 1995, Cunningham and O'Connor 1997a).

POP activity has been found in all human tissues (Kato *et al.* 1980). The highest activities were found in muscle, testes, kidney and submandibular gland. In the brain, the highest activity was found in the cortex, which suggests that it could play a role in the functions of this brain area, such as learning and memory formation (Irazusta *et al.* 2002).

Several substrates of POP, such as substance P, vasopressin, neurotensin and thyroliberin (Figure 5), are implicated in learning and memory formation (Huston and Hasenöhrl 1995). Furthermore, low levels of substance P are characteristic in the brains of Alzheimer patients and administration of substance P is able to block β amyloid-induced neurotoxicity (Kowall *et al.* 1991).

There is no firm evidence of increased POP activity in Alzheimer patients, since rather low POP activities have been correlated with the severity of Alzheimer's disease, which is thought to reflect the degree of neuronal damage (Laitinen *et al.* 2001). However, it was recently reported that the expression of the POP gene was increased many-fold in the hypothalamus and the cortex in aged rats (Jiang *et al.* 2001). Furthermore, the expression of the POP gene was decreased in rats in an enriched environment that enhances learning and memory formation (Rampon *et al.* 2000).
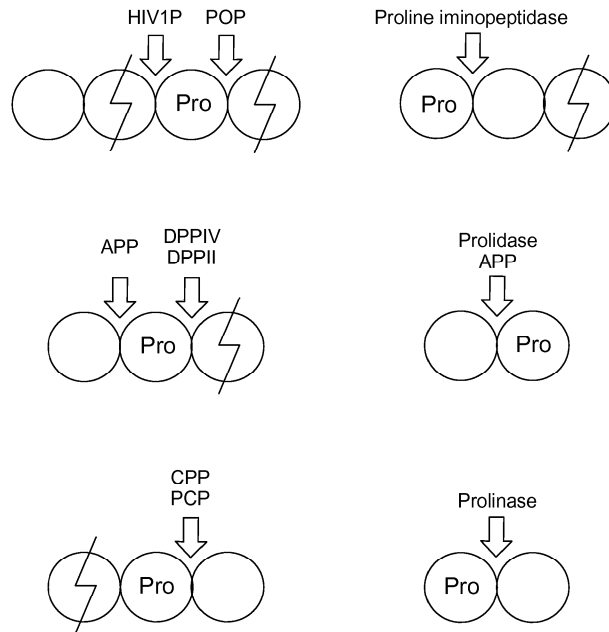
Figure 4. The selectivity of proline-specific peptidases (APP: aminopeptidase P; CPP: carboxypeptidase P; DPPII: dipeptidylpeptidase II; DPPIV: dipeptidylpeptidase IV; HIV1P: human immunodeficiency viron-1 protease; PCP: prolyl carboxypeptidase; POP: prolyl oligopeptidase). A circle represents a single amino acid residue and a circle with a twisted line represents an unspecified number of amino acid residues.

(a) Arg-Pro-Lys-**Pro-Gln**-Gln-Phe-Phe-Gly-Leu-Met

(b) Cys-Tyr-Phe-Gln-Asn-Cys-**Pro-Arg**-Gly

(c) pGlu-Leu-Tyr-Glu-Asn-Lys-**Pro-Arg**-Arg-**Pro-Tyr**-Ile-Leu

(d) pGlu-His-**Pro-NH₂**

Figure 5. The amino acid sequences of memory-related neuropeptide substrates of POP: (a) substance P, (b) vasopressin, (c) neurotensin and (d) thyroliberin (the scissile bonds are indicated in bold type).

POP inhibitors have been reported to increase the concentrations of substance P, vasopressin and thyroliberin in the brain (Miura *et al.* 1995, Toide *et al.* 1996), which is suggested to 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 (MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Yoshimoto *et al.* 1987, Atack *et al.* 1991, Saito *et al.* 1991, Schneider *et al.* 2002).

POP has been implicated in both affective and eating disorders. A decreased serum POP activity has been reported in patients suffering from depression, anorexia and bulimia, whereas an increased serum POP activity has been reported in patients suffering from mania and schizophrenia (Maes *et al.* 1994, Maes *et al.* 1995, Maes *et al.* 2001). However, the use of Z-Gly-Pro-7-amino-4-methyl coumarine as a specific substrate for the determination of POP activity in the serum has been questioned by the discovery of a Z-L-prolyl-L-prolinal insensitive Z-Gly-Pro-7-amino-4-methyl coumarine hydrolyzing peptidase (ZIP) from bovine serum (Cunningham and O'Connor 1997b, Birney and O'Connor 2001). Nevertheless, in a study of the mechanism for the effect of lithium in the treatment of depression, specific POP inhibitors abolished the effects of lithium and elevated the inositol (1,4,5)-triphosphate levels (Williams *et al.* 1999, Williams and Harwood 2000, Williams *et al.* 2002). In addition, the influence of POP on (1,4,5)-triphosphate metabolism has recently been confirmed (Schultz *et al.* 2002).

A parasitic POP has also recently been implicated as a therapeutic target. *Trypanosoma cruzi*, the causative agent of Chagas disease, contains a POP which exhibits the unusual property of cleaving collagens of the extracellular matrix (Santana *et al.* 1997). Inhibitors developed for the POP of *Trypanosoma cruzi* might prevent the invasion phase in Chagas disease (Vendeville *et al.* 2002).

3. PROLYL OLIGOPEPTIDASE INHIBITORS

The development of low molecular weight POP inhibitors during the last two decades has resulted in a large number of compounds (De Nanteuil *et al.* 1998). A search through the literature reveals several hundred POP inhibitors. In addition, many POP inhibitors have only been reported in patents, which makes the total number of compounds even larger.

A detailed comparison of the *in vitro* data for all reported POP inhibitors is not useful as different research groups have used different *in vitro* assays. Therefore, the main objective of this part of the chapter is not to cover all known POP inhibitors in detail, but rather to give a brief overview of the most potent compounds and their general structure-activity relationships. *In vitro* data are presented in the end of this chapter for the numbered compounds (Table 1).

Up till now, only a few POP inhibitors have been reported to have entered clinical studies. The first ones were ONO-1603 **2**, JTP-4819 **3**, S-17092 **4** and Z-321 **5** (De Nanteuil *et al.* 1998, Umemura *et al.* 1999).

3.1. Substrate-like inhibitors

The substrate-like inhibitors are by far the largest group of POP inhibitors. Most of the inhibitors of this type are based on an *N*-acyl-L-prolyl-pyrrolidine structure. The substrate-like inhibitors bind to the active site of the enzyme and they have distinctive P1, P2 and P3 sites, which interact with the corresponding S1, S2 and S3 binding sites of the enzyme. The structural similarities between substrate-like inhibitors and the substrates are apparent (Figure 6).

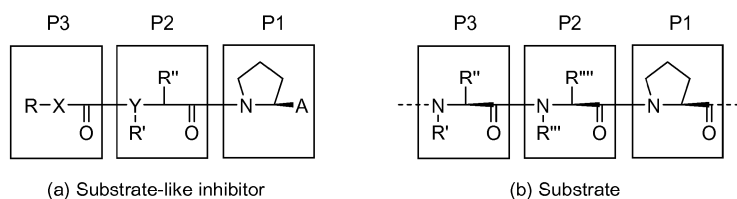(a) Substrate-like inhibitor   (b) Substrate

Figure 6. (a) The general structure for substrate-like inhibitors, wherein R is a lipophilic group (preferably an aralkyl group); X is CH_2, NH or O; Y is N or CH; R' and R" are side groups (usually of an amino acid); A is H, CHO, CN or COR"" (R"" is specified in Section 3.1.1 of this chapter). (b) The general structure for substrates of POP wherein R', R", R"' and R"" are side groups of amino acids.

The most important features of substrate-like POP inhibitors can be seen from Figure 6. The pyrrolidinyl group at the P1 site, the two carbonyl groups at the P2 and P3 sites and the distance of two atoms between these two carbonyl groups are essential for the inhibitory activity. The lipophilic R group has also been recognized to be important.

3.1.1. The P1 site

The S1 binding site of the enzyme is very specific for L-proline. Therefore, almost all potent substrate-like inhibitors have either an unsubstituted or a substituted pyrrolidinyl group at the P1 site. A well known inhibitor with an unsubstituted pyrrolidinyl group at the P1 site is SUAM-1221 **6** (Saito *et al.* 1991).

6
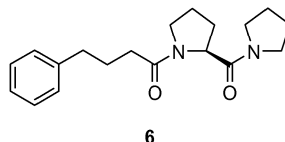
The only successful reported replacements of the pyrrolidine ring structure were pyrrole, 2,3-dihydropyrrole, 2,5-dihydropyrrole, thiazolidine and isoxazoline. These replacements were made based on a very potent compound **7** (Portevin *et al.* 1996). The resulting compound S-17092 **4** and compounds **8-11** were equipotent as compared to compound **7** against rat brain POP. This result is likely to be wide-ranging, since the replacements of the pyrrolidine ring by 2,5-dihydropyrrole and thiazolidine were earlier introduced successfully also for SUAM-1221 **6** (Arai *et al.* 1993). The resulting compounds **12** and **13** were equipotent as compared to SUAM-1221 **6** against canine brain POP.
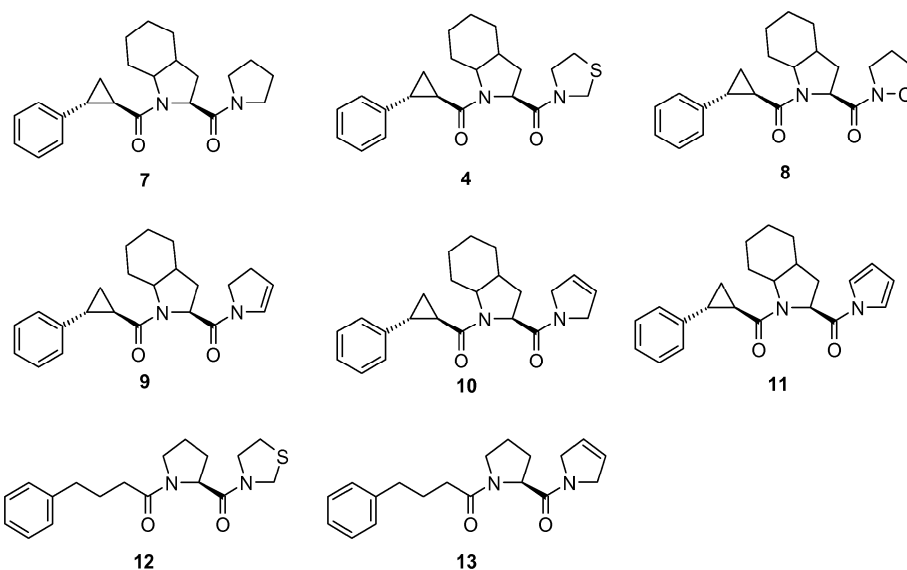
One of the first inhibitors with a 2(*S*)-formylpyrrolidinyl group at the P1 site was Z-L-prolyl-L-prolinal **1** (Wilk and Orlowski 1983). In the same *in vitro* assay Z-L-prolyl-L-prolinal **1** was approximately 40 times more potent against canine brain POP than SUAM-1221 **6** (Tanaka *et al.* 1994). Replacing the 2(*S*)-formyl substituent of the pyrrolidinyl group by either a 2(*S*)-carboxylic acid, a 2(*S*)-hydroxymethyl or a 2-oxo substituent abolished the inhibitory activity (Wilk and Orlowski 1983, Arai *et al.* 1993, Augustyns *et al.* 1995).
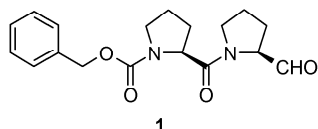
Since the reactivity of the aldehyde function in Z-L-prolyl-L-prolinal **1** might limit its use in biological systems, various acetals and thioacetals of it were made (Augustyns *et al.* 1995). The dimethyl acetal derivative **14** was the most potent one against human peripheral blood mononuclear cell POP, with an intermediate potency between the compounds with a 2(*S*)-formylpyrrolidinyl group and the compounds with an unsubstituted pyrrolidinyl group at the P1 site. Compound **14** was reported to act as the inhibitor and not as a prodrug of Z-L-prolyl-L-prolinal **1**.
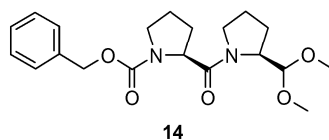
The cyano group has also been used as the 2(*S*)-substituent of the pyrrolidinyl group at the P1 site (Uchida *et al.* 1992, Tanaka *et al.* 1994, Li *et al.* 1996). Compound **15** was reported to be equipotent with Z-L-prolyl-L-prolinal **1** against canine brain POP (Tanaka *et al.* 1994).

15
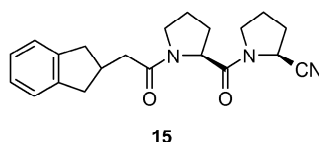
In the enzyme catalyzed hydrolysis reaction, the nucleophilic hydroxyl group of the active serine residue of the enzyme attacks the carbonyl group of the amide bond between the P1 and P1' sites of the substrate, resulting in a tetrahedral transition state. An inhibitor with a 2(*S*)-formylpyrrolidinyl group at the P1 site, such as L-prolyl-L-prolinal **1**, forms an hemiacetal adduct that mimics this tetrahedral transition state (Scheme 1) (Wilk and Orlowski 1983). The formation of the hemiacetal adduct with the inhibitor Z-L-prolyl-L-prolinal **1** has later also been confirmed by crystallography (Fülöp *et al.* 1998). Moreover, Z-L-prolyl-L-prolinal **1** has been reported to be a competitive, slow and tight binding inhibitor, which also supports the formation of a hemiacetal adduct (Bakker *et al.* 1990). The increase of the inhibitory activity by a 2(*S*)-cyanopyrrolidinyl group at the P1 site is comparable to that of the 2(*S*)-formylpyrrolidinyl group. Therefore, it is likely that it acts analogously by forming an iminoether adduct with the active serine residue.
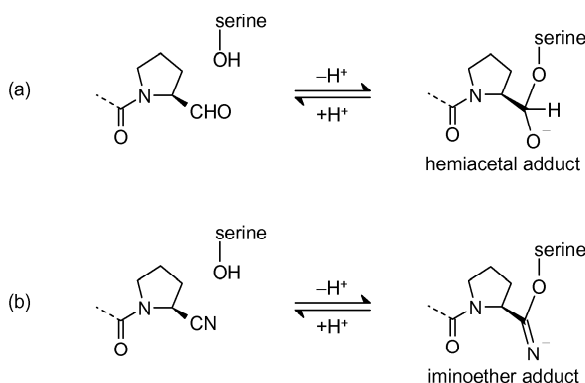
Scheme 1. (a) The reported hemiacetal adduct between an inhibitor with a 2(*S*)-formylpyrrolidinyl group (L-prolinal) at the P1 site and the active serine residue. (b) The proposed iminoether adduct between an inhibitor with a 2(*S*)-cyanopyrrolidinyl group at the P1 site and the active serine residue.

Simple 2(*S*)-acylpyrrolidinyl groups at the P1 site have not been reported to give potent POP inhibitors. However, when the acyl group of the 2(*S*)-acylpyrrolidinyl group includes a certain type of heteroatom at the β-position to the keto function, a comparable increase of the potency as for the 2(*S*)-formylpyrrolidinyl and 2(*S*)-cyanopyrrolidinyl groups has been observed. These inhibitors with an activated 2(*S*)-acylpyrrolidinyl group at the P1 site have often been reported to have similar slow and tight binding inhibitor properties as Z-L-prolyl-L-prolinal **1**.

JTP-4819 **3** is comparable in potency to Z-L-prolyl-L-prolinal **1** (Toide *et al.* 1995). The OH substituent with the oxygen atom at the β-position to the keto function provides the activation for the acyl group. JTP-4819 **3** has been confirmed to be a competitive, tight binding inhibitor (Venäläinen *et al.* 2002).

3
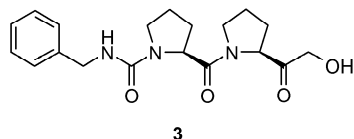
Compound **16** has an heterocyclic ring which activates the acyl group. (Tsutsumi *et al.* 1994) The *sp*^{2} hybridized nitrogen atom in the heterocyclic ring at the β-position to the keto function is essential for the high potency of this compound. Also other heterocyclic rings that fulfilled this requirement were reported to activate the acyl group. In compound **17** the pyridinium group with a *sp*^{2} hybridized nitrogen atom at the β-position to the keto function also activates the acyl group (Steinmetzer *et al.* 1993). Compound **17** was reported to be a competitive, slow binding inhibitor.

16   **17**
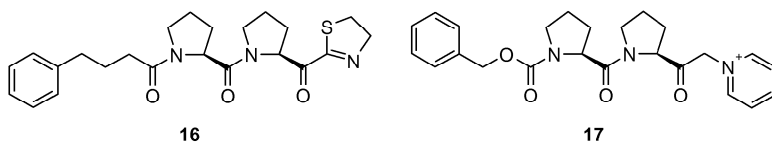
In compound **18** an α-diazo group activates the acyl group. Compound **18** was reported to be a competitive, slow binding inhibitor (Stone *et al.* 1992).

18
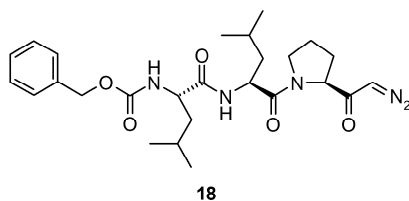
The potent compound **19** has a cyclohexylamino group that at first sight looks to be the P1 site (Tsuda *et al.* 1996a). However, changing the ring size of the cyclohexylamino group did not have any effect on the inhibitory activity. Furthermore, replacing the whole cyclohexylamino group by a pyrrolidinyl group reduced the inhibitory activity strongly. This indicates that the actual P1 site is the L-proline residue, and the amide group with an NH proton next to the keto function provides the activation. Compound **20** was also very potent (Tsutsumi *et al.* 1994). In this compound the ester group next to the keto function provides the activation.

19   **20**
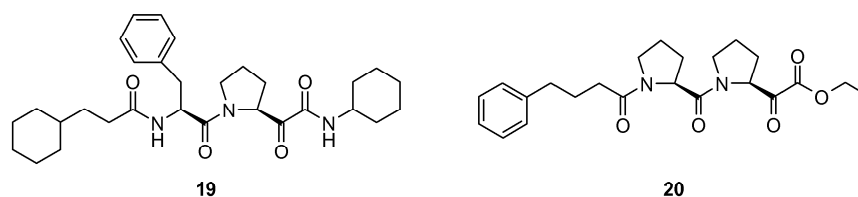
Various ketones with α-fluorine substituents have also been described. Compound **21**, with a 2(*S*)-(2,2,3,3,3-pentafluoropropanoyl)pyrrolidinyl group at the P1 site was reported to be very potent against bovine brain POP (De Nanteuil *et al.* 1998).

21
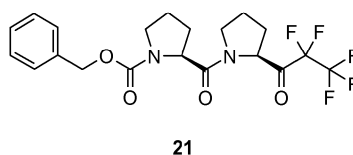
Z-glycyl-2(*S*)-(chloroacetyl)pyrrolidine **22** and other compounds with a 2(*S*)-(chloroacetyl)pyrrolidinyl group at the P1 site were reported to be irreversible inhibitors of POP (Yoshimoto *et al.* 1977, Yoshimoto *et al.* 1987). Irreversible inhibitors are not very useful for inhibiting human POP, because POP is an important enzyme in peptide processing and an irreversible inhibitor is likely to block the enzyme completely for a longer time. Nevertheless, irreversible inhibitors might be useful in inhibiting a parasitic POP. In the search for inhibitors of POP of *Trypanosoma cruzi*, compound **23** was reported to be very potent (Vendeville *et al.* 2002). However, it was not very selective against the parasitic POP, since it was equipotent against human platelet POP.

22   **23**
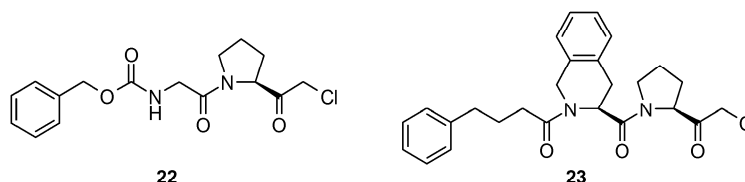
3.1.2. The P2 site

The L-proline residue is the preferred natural α-amino acid residue at the P2 site in the inhibitor structure. Analogues of SUAM-1221 **6** with other natural α-amino acid residues at the P2 site had a reduced inhibitory activity against bovine brain POP (Saito *et al.* 1991). The L-configuration of the α-amino acid was important for the inhibitory activity and it is worth mentioning in this context that the achiral glycine gave the lowest inhibitory activity of the natural α-amino acids.

Replacement of the L-proline residue at the P2 site of SUAM-1221 **6** by an L-thioproline residue resulted in compound **24**, which was approximately three times more active against bovine brain POP than SUAM-1221 **6** (Saito *et al.* 1991).
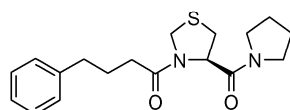
24

Incorporating L-proline analogues with oxygen substituents in the side chain, such as 5-oxo-L-proline, 4-oxo-L-proline, 4(*R*)- and 4(*S*)-hydroxy-L-proline, and *S*-oxo-L-thioproline, at the P2 site of the inhibitor was allowed, but it always reduced the potency slightly (Yoshimoto *et al.* 1987, Saito *et al.* 1991, Yoshimoto *et al.* 1991b).

Since the P2 site is less specific for the L-proline residue than the P1 site, the pyrrolidine ring at the P2 site of SUAM-1221 **6** could be enlarged to a piperidine ring resulting in compound **25**. The replacement decreased the potency to one fifth as compared to SUAM-1221 **1** against bovine brain POP (Saito *et al.* 1991).
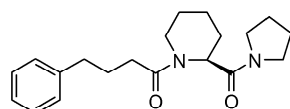
25

Compound **7** has an L-prolyl analogue with a fused ring and compounds **26** and **27** have L-prolyl analogues with bridged rings at the P2 site (Portevin *et al.* 1996). All three compounds were very potent against rat brain POP. As compared to the L-thioprolyl group at the P2 site these bicyclic L-prolyl analogues were more potent against POP of *Flavobacterium meningosepticum*. This result was also supported by the inhibitory activities of the corresponding compounds with a 4-phenylbutanoyl group at the P3 site against POP of *Flavobacterium meningosepticum*.
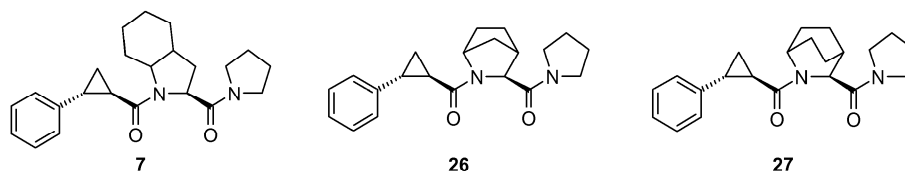
The P2 site is also a useful modification site for introducing selectivity for parasitic POP. Compound **28** was 78 times more potent against POP of *Trypanosoma cruzi* than against human platelet POP (Vendeville *et al.* 2002).
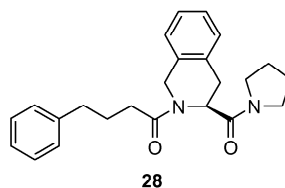
The nitrogen atom of the amide bond between the P2 and the P3 sites is not very important for the inhibitory activity. Two very potent compounds, ONO-1630 **2** and compound **29**, have only an ethylene chain between the two essential carbonyl groups. The chain length of the P2 site is the same as in *N*-acyl-L-prolyl-pyrrolidine type of inhibitors.
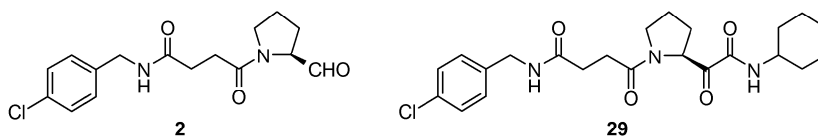
A linking carbocyclic ring has also been applied at the P2 site. Both the *trans* and the *cis* diastereomers **30** and **31**, respectively, were very potent (Bakker *et al.* 1991).

30 (*trans*)
31 (*cis*)
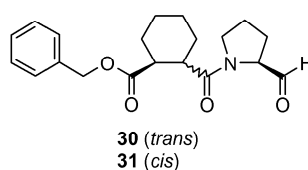
The importance of the carbonyl group at the P2 site has been studied thoroughly. Reducing this carbonyl group to a methylene group abolished the inhibitory activity (Yoshimoto *et al.* 1991b). Also replacement of the carbonyl oxygen atom by a sulphur atom reduced the inhibitory activity strongly (Stöckel-Maschek *et al.* 2000).

3.1.3. The P3 site

The P3 site is usually a lipophilic acyl group, which binds to the S3 site of the enzyme by hydrophobic interactions. The above presented compounds **1-31** all have lipophilic

acyl groups at the P3 site. The following comparison between the different acyl groups at the P3 site is based on compounds with an unsubstituted pyrrolidinyl group at the P1 site, because otherwise the different groups at the P1 site might affect the inhibitory activity more than the different groups at the P3 site.

Many compounds have a aralkanoyl group at the P3 site and the 4-phenylbutanoyl group has been reported to have the optimal chain length (Arai *et al.* 1993). Substitution of the *para*-position of the phenyl group by chloro, hydroxy and methyl groups did not have a significant effect on the inhibitory activity, whereas substitution by methoxy, nitro and amino groups resulted in a moderate decrease of the inhibitory activity.

Other preferred acyl groups are the Z and the benzylcarbamoyl groups. However, the carbamate oxygen and the carbamoyl nitrogen does not seem to be important for the inhibitory activity.

An unbranched alkanoyl group at the P3 site gave also potent compounds, the optimal chain length was reported to be in the range 3-10 methylene groups in the alkanoyl chain (Saito *et al.* 1991, Kánai *et al.* 1997).

Some more rigid acyl groups have also proved to be useful groups at the P3 site. Z-321 **5** is a very potent compound with an indan-2-yl-acetyl group at the P3 site (Tanaka *et al.* 1994). As compared to SUAM-1221 **6** the indan-2-yl-acetyl derivative **32** was three times more potent against canine brain POP. Increasing or decreasing the length of the alkylene chain between the indan-2-yl group and the carbonyl group reduced the potency. The (2*S*)-tetralin-2-yl-acetyl derivative **33** was nine times more active than SUAM-1221 **6**. On the other hand, the (2*R*)-tetralin-2-yl-acetyl derivative **34** was equipotent with SUAM-1221 **6**. It is noteworthy that the compounds **5** and **32-34** have the aromatic ring at the distance of three carbon atoms from the carbonyl group, which is the same distance as in SUAM-1221 **6**.

5

32

33

34
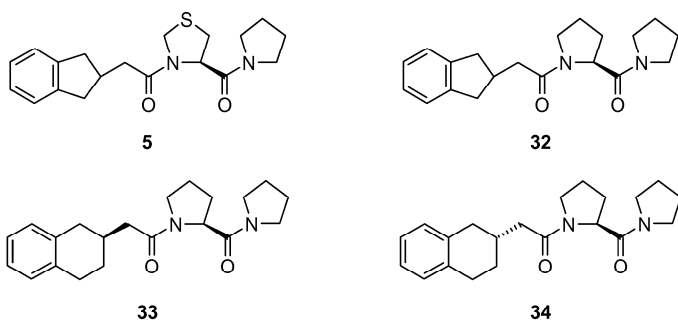
A more rigid acyl group resulted also from incorporating a cyclopropane ring into the flexible aralkanoyl chain. The (1*R*,2*R*)-2-phenylcyclopropylcarbonyl derivative **7** was 30 times more active than the (1*S*,2*S*)-2-phenylcyclopropylcarbonyl derivative **35** against rat brain POP (Portevin *et al.* 1996).
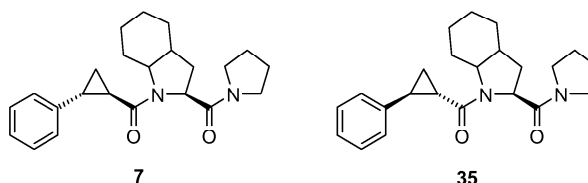
Also more complex aryl moieties have been applied at the P3 site. Compound **36** was slightly more potent than SUAM-1221 **6** against bovine brain POP (Saito *et al.* 1991). The aromatic moiety of a phenyl group was also replaced by a thiophene group, showing that heteroaromatic rings were also allowed (Arai *et al.* 1993). The resulting compound **37** was two times more active than SUAM-1221 against canine brain POP.
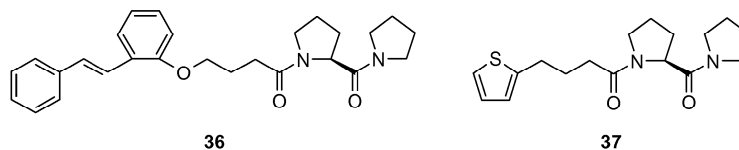
The carbonyl group at the P3 site is indirectly confirmed to be important. There is no reported substrate-like POP inhibitor lacking the P3 carbonyl group. Furthermore, the crystal structure of the enzyme with Z-L-prolyl-L-prolinal **1** showed that there is a hydrogen bond between this carbonyl oxygen and the side chain of Trp595 (Fülöp *et al.* 1998).

3.2. Other types of inhibitors

The POP inhibitors **38-40** have the L-proline, the pyroglutamic acid and the L-thioprolyl residues in the centre of the molecule in the opposite direction as compared to the substrate-like inhibitors (Ajisawa 1991a, Ajisawa 1991b, Ajisawa 1991c, De Nanteuil *et al.* 1998). Compound **40** was the most potent compound in this series of compounds.
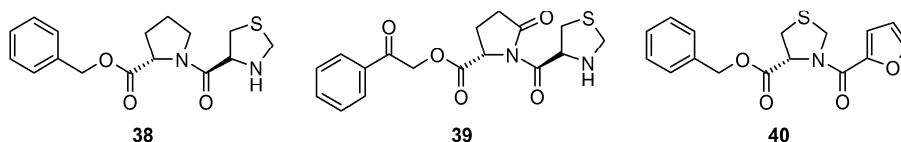
Another type of POP inhibitor different from the typical substrate-like compounds is postatin **41**, which was isolated from *Streptomyces viridochromogenes* (Aoyagi *et al.* 1991, Tsuda *et al.* 1996a). However, postatin has an α-ketoamide group, which increased the potency for the substrate-like compounds **17** and **28**. Therefore it is very likely that the two N-terminal L-valine residues are the P2 and P3 sites, and the 3-amino-2-oxo-valeroyl group is the P1 site.
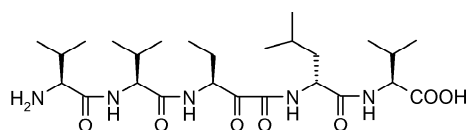
41

The potent compounds **42-45** are somewhat dissimilar to the substrate-like POP inhibitors (Tsuda *et al.* 1996b). These compounds have an α-ketoamide group, which increased the potency for the substrate-like compounds **17** and **28**. Provided that compounds **42-45** bind to the active site in the same way as was suggested for compounds **17** and **28**, they seem to lack the P3 site completely. Furthermore, the P2 site is also rather unusual as compared to substrate-like POP inhibitors.
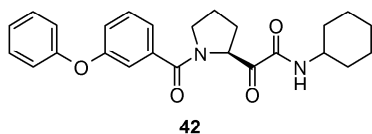
42
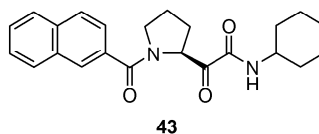
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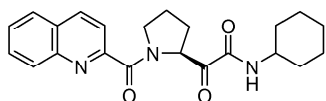
44
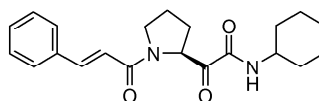
45

The only potent POP inhibitor without any resemblance to the substrate-like compounds is Y-29794 **46** (Nakajima *et al.* 1998). This compound was described to selectively and competitively inhibit rat brain POP in a reversible manner.
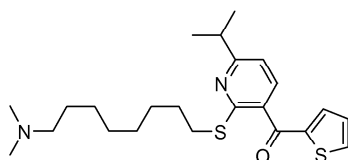
46

Table 1. Detailed *in vitro* data with references for the presented POP inhibitors.

Compound	POP	K_i [nM]	IC_{50} [nM]	Reference
1	rabbit	14		(Wilk and Orlowski 1983)
1	bovine	5.0		(Yoshimoto *et al.* 1987)
1	bovine	3.4	0.74	(Tsuru *et al.* 1988)
1	mouse	0.35		(Bakker *et al.* 1990)
1	human	0.5		(Bakker *et al.* 1990)
1	canine		1.1	(Tanaka *et al.* 1994)
1	rat		54	(Portevin *et al.* 1996)
1	human		1.3	(Kimura *et al.* 1997)
2	pig		84	(Tsuda *et al.* 1996a)
2	rat		57	(Portevin *et al.* 1996)
2	rat	12		(Katsube *et al.* 1996)
3	rat		0.83	(Toide *et al.* 1995)
4	rat		1.3	(Portevin *et al.* 1996)
4	human	1.5		(Barelli *et al.* 1999)
5	canine		10	(Tanaka *et al.* 1994)
6	bovine		190	(Saito *et al.* 1991)
6	bovine		2100	(Yoshimoto *et al.* 1991b)
6	canine		76	(Arai *et al.* 1993)
6	canine		49	(Tanaka *et al.* 1994)
7	rat		1.2	(Portevin *et al.* 1996)
8	rat		2	(Portevin *et al.* 1996)
9	rat		1.3	(Portevin *et al.* 1996)
10	rat		2.4	(Portevin *et al.* 1996)
11	rat		2.3	(Portevin *et al.* 1996)
12	canine		41	(Arai *et al.* 1993)
13	canine		57	(Arai *et al.* 1993)
14	human		120	(Augustyns *et al.* 1995)
15	canine		1.2	(Tanaka *et al.* 1994)
16	pig		3.8	(Tsutsumi *et al.* 1994)
17	*F. meningosepticum*	9.3		(Steinmetzer *et al.* 1993)
18	pig	73		(Stone *et al.* 1992)

Table 1. *Continued.*

Compound	POP	K_i [nM]	IC_{50} [nM]	Reference
19	pig		0.98	(Tsuda *et al.* 1996a)
20	pig		10.3	(Tsutsumi *et al.* 1994)
21	bovine		1	(De Nanteuil *et al.* 1998)
22	bovine	70000		(Yoshimoto *et al.* 1987)
23	*T. cruzi*		2.6	(Vendeville *et al.* 2002)
23	human		1.5	(Vendeville *et al.* 2002)
24	bovine		67	(Saito *et al.* 1991)
24	canine		31	(Arai *et al.* 1993)
25	bovine		890	(Saito *et al.* 1991)
26	rat		1.4	(Portevin *et al.* 1996)
27	rat		5	(Portevin *et al.* 1996)
28	*T. cruzi*		7	(Vendeville *et al.* 2002)
28	human		550	(Vendeville *et al.* 2002)
29	pig		4.9	(Tsuda *et al.* 1996a)
30	mouse	3		(Bakker *et al.* 1991)
31	mouse	3		(Bakker *et al.* 1991)
32	canine		17	(Tanaka *et al.* 1994)
33	canine		5.4	(Tanaka *et al.* 1994)
34	canine		48	(Tanaka *et al.* 1994)
35	rat		33	(Portevin *et al.* 1996)
36	bovine		140	(Saito *et al.* 1991)
37	canine		34	(Arai *et al.* 1993)
38	(data in Japanese)		720000	(Ajisawa *et al.* 1991a)
39	(data in Japanese)		790000	(Ajisawa *et al.* 1991b)
40	(data in Japanese)		2800	(Ajisawa *et al.* 1991c)
41	pig		55	(Tsuda *et al.* 1996b)
42	pig		14	(Tsuda *et al.* 1996b)
43	pig		22	(Tsuda *et al.* 1996b)
44	pig		26	(Tsuda *et al.* 1996b)
45	pig		48	(Tsuda *et al.* 1996b)
46	rat	0.95		(Nakajima *et al.* 1998)

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2

SCOPE OF THE THESIS

This thesis titled "Design and synthesis of novel prolyl oligopeptidase inhibitors" is a part of a larger POP project at the University of Kuopio which was started in 1998. An ultimate objective for the POP project was the discovery of a new lead compound. However, since a true lead compound cannot be recognized from the molecular structure, the physico-chemical properties and the *in vitro* data, the identification of such a compound was not included in the scope of this thesis.

The primary objective for the present study was to develop new potent small molecular weight POP inhibitors, which should have the characteristics of CNS agents. It covers the development of novel POP inhibitors with design, synthesis and characterization of the new compounds. It also includes the *in vitro* evaluation, although the development of the *in vitro* assay is not included in this thesis.

The starting point for the development of new POP inhibitors was the large number of reported substrate-like POP inhibitors, such as Z-L-prolyl-L-prolinal **1**, and the modifications were targeted to the specific sites of the molecular structure (Figure 1).
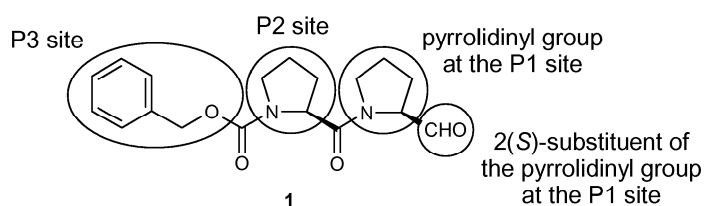

Figure 1. The main modification sites of substrate-like POP inhibitors, exemplified by Z-L-prolyl-L-prolinal **1**.

The main objectives for the development of new POP inhibitors starting from substrate-like compounds were:

1. Developing new 2(*S*)-substituents to the pyrrolidinyl group at the P1 site, which would result in an increase of the potency comparable to that of the 2(*S*)-formyl, 2(*S*)-cyano or 2(*S*)-hydroxyacetyl groups.

2. Replacement of the pyrrolidine moiety at the P1 site by another type of structure. The S1 specificity pocket is very selective for the pyrrolidine ring, and very few attempts have been made to replace it.

3. Replacement of the L-prolyl group at the P2 site. Introduction of a more rigid structure at the central P2 site would make the entire POP inhibitor structure more rigid. (This objective included the development of L-proline analogues in general.)

4. Replacement the typical lipophilic groups at the P3 site. The lipophilic groups at the P3 site have been reported to be important for the inhibitory activity, and very few attempts have been made to replace them more polar groups.

5. Development of a completely new type of POP inhibitor. This could either result from the modifications described in the main objectives 1-4 above or from testing a variety of pyrrolidine containing compounds, based on the fact that the pyrrolidine moiety is found both at the P1 and the P2 sites of many POP inhibitors.

6. Optimization of the physico-chemical properties of the compounds to be suitable as CNS agents. The primary criteria were: a) keeping the molecular mass below 500, b) having a log P value between 2 and 3, and c) trying to increase the water solubility if possible (no quantitative limits were set for the water solubility). These properties should be beneficial for the penetration of the blood-brain barrier.

3

GENERAL EXPERIMENTAL PROCEDURES

1. SYNTHESIS

All chemicals and solvents were of a commercial high purity quality, suitable for organic synthesis, and normally they did not require any purification before use. Only a few solvents, such as tetrahydrofuran, diethyl ether and dimethyl sulfoxide, were dried from traces of water according to common procedures if anhydrous solvents were needed.

Reactions sensitive to moisture were carried out in oven dried (over 140 °C) and desiccator-cooled glassware, using anhydrous solvents and performing the reactions under an inert atmosphere (argon or nitrogen). Reactions in low temperatures (under -20 °C) were also always performed under an inert atmosphere.

The proceeding of the reactions and the success of the purification procedures were monitored initially by thin layer chromatography (Silica Gel 60 F_{254}, Aluminium Sheets, Merck 1.05554.0001) with a suitable eluent and visualized by UV light (254 nm), iodine, heating and/or ninhydrine.

The products were predominantly amorphous hygroscopic materials which could not be crystallized. Therefore, the main purification procedure was flash chromatography (30-60 µm Silica Gel for Flash Chromatography, J. T. Baker 7024-02). An alternative purification procedure to flash chromatography was chromatotron chromatography (Chromatotron model 7924T, Silica Gel 60 PF_{254}, Merck 1.07749.1000).

Remaining solvents and moisture were always removed from isolated products by keeping the compounds *in vacuo* at approximately 0.1 mbar, provided that the boiling point of the compounds allowed it.

2. ANALYTICAL

2.1. Nuclear magnetic resonance (NMR) spectroscopy

The standard ^{1}H and broad band decoupled ^{13}C NMR experiments were used to characterize the synthesized compounds before the use of other analytical procedures. The 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 tetramethylsilane was added to the sample as an internal standard.

The spectra were processed from the recorded FID files with MestRe-C (version 2.3a) software (Departemento Química Orgánica, Universidade de Santiago de Compostela, Spain). Chemical shifts were reported in ppm relative to tetramethylsilane.

2.2. Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS spectra were acquired using a LCQ ion trap mass spectrometer equipped with an electrospray ionization source (Finnigan MAT, San Jose, CA, USA). Full scan mass spectra for positive ions were recorded.

2.3. Elemental analysis

Elemental analyses for C, H, and N were performed on a ThermoQuest CE Instruments EA1110-CHNS-O elemental analysator (ThermoQuest, Italy). Before this equipment was acquired to the University of Kuopio the elemental analyses were also carried out at the University of Joensuu and at the University of Oulu using comparable equipment.

3. DETERMINATION OF THE 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 rt. The phases were separated by centrifugation for 5 min at 2000 rpm and the aqueous phase was analysed. 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$_2$PO$_4$ pH 7 was used as the aqueous phase. Either methanol or 90% acetonitrile was used as the organic phase. The HPLC methods were tested for linearity and repeatability. The Merck Hitachi HPLC system consisted of an 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).

4. *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 dimethyl sulfoxide and diluted with 0.1 M sodium-potassium phosphate buffer at 30 °C for 30 min (final dimethyl sulfoxide concentration was less than 0.1%). The controls contained 10 μl 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 protein. 5-7 inhibitor concentrations were used to determine the IC_{50} values and the final concentrations of the compounds in the assay mixture varied from 10^{-12} M up to 10^{-5} M. 2-4 independent measurements were made for each inhibitor. The inhibitory activities (percent of control) were plotted against the log concentration of the compound, and the IC_{50} value was determined by non-linear regression utilizing GraphPad Prism 3.02 software.

4

4-PHENYLBUTANOYL-2(*S*)-ACYLPYRROLIDINES AND 4-PHENYLBUTANOYL-L-PROLYL-2(*S*)-ACYLPYRROLIDINES AS PROLYL OLIGOPEPTIDASE INHIBITORS

Erik A. A. Wallén, Johannes A. M. Christiaans, Susanna M. Saario, Markus M. Forsberg, Jarkko I. Venäläinen, Hanna M. Paso, Pekka T. Männistö, Jukka Gynther

Abstract. New 4-phenylbutanoyl-2(*S*)-acylpyrrolidines and 4-phenylbutanoyl-L-prolyl-2(*S*)-acylpyrrolidines were synthesized. Their inhibitory activity against prolyl oligopeptidase from pig brain was tested *in vitro*. In the series of 4-phenylbutanoyl-2(*S*)-acylpyrrolidines, the cyclopentanecarbonyl and benzoyl derivatives were the best inhibitors having IC_{50} values of 30 and 23 nM, respectively. This series of compounds shows that the P1 pyrrolidine ring, which is common in most POP inhibitors, can be replaced by either a cyclopentyl ring or a phenyl ring, causing only a slight decrease in the inhibitory activity. In the series of 4-phenylbutanoyl-L-prolyl-2(*S*)-acylpyrrolidines the cyclopentanecarbonyl and benzoyl derivatives were not as active as in the series of 4-phenylbutanoyl-2(*S*)-acylpyrrolidines. The hydroxyacetyl derivative did however show high inhibitory activity. This compound is structurally similar to JTP-4819, which is one of the most potent prolyl oligopeptidase inhibitors. The acyl group in the two series of new compounds seems to bind to different sites of the enzyme, since the second series of new compounds did not show the same cyclopentanecarbonyl or benzoyl specificity as the first series.

5

DICARBOXYLIC ACID *BIS*(L-PROLYL-PYRROLIDINE) AMIDES AS PROLYL OLIGOPEPTIDASE INHIBITORS

Erik A. A. Wallén, Johannes A. M. Christiaans, Markus M. Forsberg, Jarkko I. Venäläinen, Pekka T. Männistö, Jukka Gynther

Abstract. New dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amides were synthesized and their inhibitory activity against prolyl oligopeptidase from pig brain was tested *in vitro*. As compared with earlier described prolyl oligopeptidase inhibitors, these new compounds have in common an L-prolyl-pyrrolidine moiety, but the typical lipophilic acyl end group is replaced by another L-prolyl-pyrrolidine moiety connected symmetrically with a short dicarboxylic acid linker. These compounds are a new type of peptidomimetic prolyl oligopeptidase inhibitors.

6

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, Jukka Gynther

Abstract. Isophthalic acid *bis*(L-prolyl-pyrrolidine) amide was a very potent prolyl oligopeptidase inhibitor, but it had a log P value of -0.2, which was 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.

7

ADDITION OF *TERT*-BUTYLCUPRATE TO (2*S*)-*N*-ACYL-Δ⁵-DEHYDROPROLINATES AS A DIASTEREOSELECTIVE SYNTHETIC PROCEDURE FOR OBTAINING (2*S*,5*S*)-5-*TERT*-BUTYLPROLINE

Erik A. A. Wallén, Johannes A. M. Christiaans, Jukka Gynther, Jouko Vepsäläinen

Abstract. The synthesis of (2*S*,5*S*)-Boc-5-*tert*-butylproline ethyl ester via the addition of *tert*-butylcuprate to (2*S*)-Boc-Δ^5-dehydroproline ethyl ester, formed from (2*S*)-Boc-5-methoxyproline ethyl ester, gives an excellent yield of 94% and a high diastereoselectivity (2*S*,5*S*):(2*S*,5*R*) 78:22. This synthesis opens up a new synthetic route to (2*S*,5*S*)-5-*tert*-butylproline, which is a useful, conformationally rigid, analogue of L-proline.

8

CONFORMATIONALLY RIGID *N*-ACYL-5-ALKYL-L-PROLYL-PYRROLIDINES AS PROLYL OLIGOPEPTIDASE INHIBITORS

Erik A. A. Wallén, Johannes A. M. Christiaans, Taija J. Saarinen, Elina M. Jarho, Markus M. Forsberg, Jarkko I. Venäläinen, Pekka T. Männistö, Jukka Gynther

Abstract. In the *N*-acyl-L-prolyl-pyrrolidine type of prolyl oligopeptidase inhibitors the L-prolyl group was replaced by different 5-alkyl-L-prolyl groups, resulting in a series of *N*-acyl-5-alkyl-L-prolyl-pyrrolidines. Since *N*-amides of 5-alkyl-L-prolines are conformationally more rigid than those of L-proline, the main objective was to make more rigid prolyl oligopeptidase inhibitors. In the series of compounds where the *N*-acyl group was a Boc group, the 5(*R*)-*tert*-butyl group increased the potency strongly. A similar effect was not observed for the 5(*S*)-*tert*-butyl group. In the series of compounds where the *N*-acyl group was a 4-phenylbutanoyl group, the 5(*R*)-*tert*-butyl, 5(*R*)-methyl and 5(*S*)-methyl groups did not have an effect on the potency (the 5(*S*)-*tert*-butyl group was not tested in this series). As an additional effect, the 5-*tert*-butyl groups increased the log P of the compounds 1.5 log units, which might be beneficial when targeting the compounds to the brain.

9

GENERAL DISCUSSION

1. SUMMARY OF THE DEVELOPMENT OF NEW POP INHIBITORS

In this chapter the results from Chapters 4-8 are discussed based on the main objectives in the scope of the thesis in Chapter 2. Some unpublished results are also included, which only serve the function of assisting the general discussion. For this reason, the unpublished results should not be regarded as an integrated part of the thesis.

1.1. Development of new 2(S)-substituents of the pyrrolidinyl group at the P1 site

The effect of the 2(S)-formylpyrrolidinyl, 2(S)-cyanopyrrolidinyl and 2(S)-(hydroxyacetyl)pyrrolidinyl groups at the P1 site was studied by synthesizing a series of typical substrate-like POP inhibitors **1**, also including some reported compounds such as Z-L-prolyl-L-prolinal **1a** and JTP-4819 **1e**. The inhibitory activities of the compounds with 2(S)-formylpyrrolidinyl, 2(S)-cyanopyrrolidinyl and 2(S)-(hydroxyacetyl)pyrrolidinyl groups at the P1 site were in the range of 0.20-0.35 nM against pig brain POP (Table 1). As compared to SUAM-1221 **2**, which has a non-substituted pyrrolidinyl group at the P1 site, the compounds **1** were approximately ten times more potent. The 2(S)-cyanopyrrolidinyl and the 2(S)-(hydroxyacetyl)pyrrolidinyl groups were assumed to interact analogously with the 2(S)-formylpyrrolidinyl group at the P1 site. Compounds with these three groups at the P1 site has later been confirmed to be slow and tight binding POP inhibitors.


1
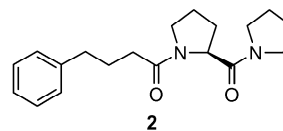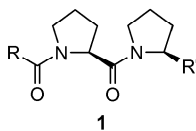
RCO = 4-phenylbutanoyl, Z, benzylcarbamoyl
R' = CHO, CN, $COCH_2OH$

2

Table 1. Compounds of series **1** and their inhibitory activities against pig brain POP.

Compound	RCO	R'	IC_{50} [nM]
1a (Z-L-prolyl-L-prolinal)	Z	CHO	0.35
1b	Z	CN	0.34
1c	4-Phenylbutanoyl	CN	0.22
1d	Z	$COCH_2OH$	0.28
1e (JTP-4819)	Benzylcarbamoyl	$COCH_2OH$	0.22
1f	4-Phenylbutanoyl	$COCH_2OH$	0.20
2 (SUAM-1221)	4-Phenylbutanoyl	H	2.2

In the search for new 2(*S*)-substituents, the tetrazole derivative **3** was made. It had an IC_{50} value of 14 nM against pig brain POP. Although compound **3** was a very interesting new POP inhibitor, the development of it was not continued due to its low inhibitory activity as compared to compounds of series **1** and SUAM-1221 **2**.

3
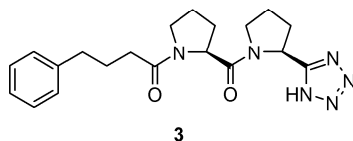
The pyrrolidinyl group at the P1 site was substituted with different 2(*S*)-acyl groups in Chapter 4, resulting in a series of compounds **4**. To our knowledge, simple 2(*S*)-acylpyrrolidinyl groups at the P1 site had not been studied. The compounds of series **4** where R was methyl, cyclopentyl and phenyl were made. These compounds had IC_{50} values in the range 170-1010 nM against pig brain POP. It was evident that a simple 2(*S*)-acylpyrrolidinyl group would not give a very potent POP inhibitor.

4
R = Me, cyclopentyl, Ph
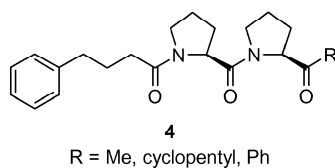
1.2. Replacement of the pyrrolidine moiety at the P1 site

The pyrrolidinyl group at the P1 site was replaced by alkyl, aryl and aralkyl groups in Chapter 4, resulting in a series of compounds **5**. Replacement of the pyrrolidinyl group by cyclopentyl and phenyl groups resulted in the potent compounds **5a** and **5b**, with IC_{50} values of 30 and 23 nM, respectively, against pig brain POP. These new POP inhibitors represent a new type of potent POP inhibitors lacking the pyrrolidinyl group at the P1 site.

5
**5a**: R = cyclopentyl
5b: R = Ph
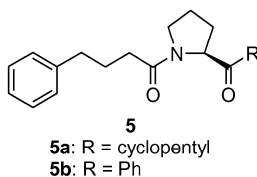
The replacement of the pyrrolidinyl group at the P1 site by a cyclopentyl group was also applied successfully in Chapter 6 for another type of POP inhibitor **6** (see also Section 1.3 in this chapter). Compound **6** had an IC_{50} value of 78 nM against pig brain POP.

6
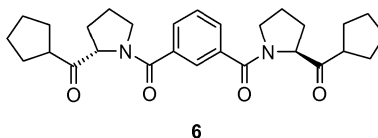
1.3. Dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amide type of compounds

The development of the compounds described in Chapters 5 and 6 was started from a series of relatively simple compounds **7**, with n ranging from 0 to 4. In this series of compounds the one with two methylene groups in the alkylene chain (n = 2) had some inhibitory activity against POP, with an IC_{50} value of 13 μM against rat brain POP (rat brain POP was used in the beginning of the study instead of pig brain POP). This result is not very surprising, since the compound with two methylene groups in the alkylene chain (n = 2) fulfils the criteria for the P1 and P2 sites in substrate-like POP inhibitors presented in Chapter 1.

7
n = 0-4
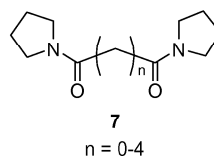
The compounds were developed further by prolonging the structure with an L-prolyl group, resulting in compound **8**. This compound had an IC_{50} value of 110 nM against pig brain POP and 180 nM against rat brain POP.

8
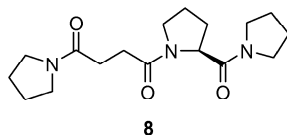
Encouraged by this result, the structure was prolonged further by another L-prolyl group in a symmetrical manner, resulting in a series of compounds **9**, with n ranging from 2 to 4. The compound with two methylene groups in the alkylene chain (n = 2) had an IC_{50} value of 77 nM against pig brain POP. The compound with three methylene groups in the alkylene chain (n = 3) was the most potent one in the series, with an IC_{50} value of 48 nM against pig brain POP.
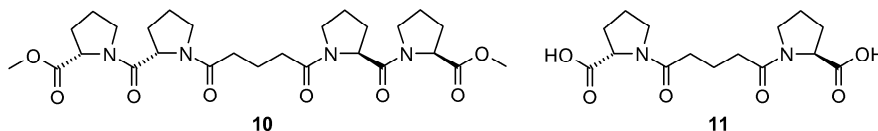

9
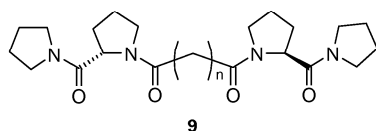
Some slightly different compounds with the same central core structure were also made. Compound **10** had IC_{50} value of 11 μM and compound **11** gave 12% inhibition at a concentration of 100 μM against pig brain POP.

10   **11**
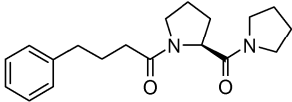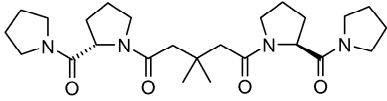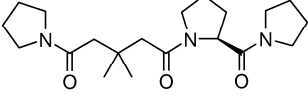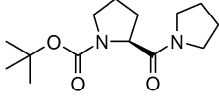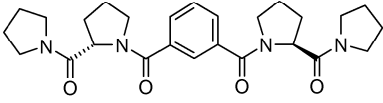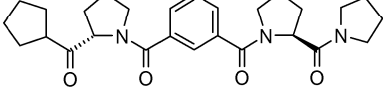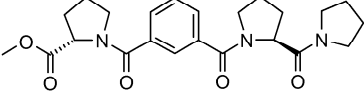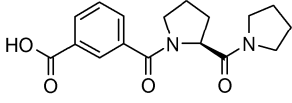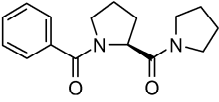
This result showed that the L-prolyl-pyrrolidine part of the compound in the series of compounds **9** had the same structure-activity relationships as earlier described *N*-acyl-L-prolyl-pyrrolidine type of POP inhibitors. However, the P3 site and beyond in compounds of series **9** was very unusual as compared to earlier described POP inhibitors.

The compounds were developed further as described in Chapters 5 and 6 resulting in three very potent compounds **12**, **15** and **16** (Table 2). Reducing the size of these molecules from the opposite side to the P1 site clearly showed that all parts of the molecules are essential for the high inhibitory activity (Table 2). As compared to the smaller molecules the differences are not very large, but they are consistently in favour of compounds **12**, **15** and **16**. Although compound **14** has an oxygen atom in the chain, it serves as a good comparison to compound **12**. As compared to SUAM-1221 **2**

compounds **12**, **15** and **16** are less potent by factors of 6, 13 and 7, respectively, against pig brain POP. However, the difference between compound **15** and SUAM-1221 **2** against rat brain POP was smaller.

Table 2. A comparison of the inhibitory activity of dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amide type of POP inhibitors and smaller fragments of the structures.

Compound	Structure	IC_{50} [nM] pig	IC_{50} [nM] rat
2		2	3
12		13	
13		23	
14		29	45
15		26	9
16		14	
17		56	20
18		54	
19		66	

When the P1 pyrrolidinyl group was replaced by 2(*S*)-formylpyrrolidinyl, 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups the potency of these compounds reached the same range as for the typical substrate-like compounds **1** (Table 1). Compounds **20-22** had IC$_{50}$ values in the range 0.3-1.5 nM against pig brain POP.
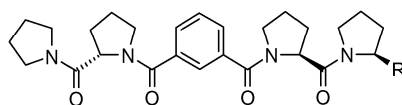
20a: CHO
20b: CN
20c: COCH$_2$OH
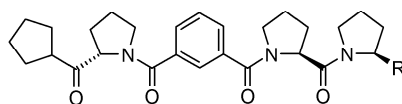
21a: CN
21b: COCH$_2$OH
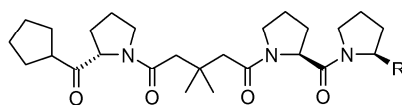
22a: CN
22b: COCH$_2$OH

Compound **6**, presented in Chapter 6, was a combination of the two new types of POP inhibitors presented in Chapters 4 and 5. Although this compound had only a moderate IC$_{50}$ value of 78 nM against pig brain POP, it is clearly the most atypical POP inhibitor presented in this thesis.
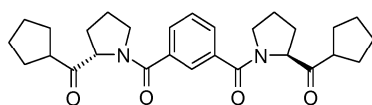
6

1.4. Increasing the rigidity of the inhibitor structure by modifying the P2 site

The central position of the P2 site in substrate-like POP inhibitors makes the modification of this site important. An important general objective in drug design is to make more rigid analogues of active compounds. The rigid analogues have a limited conformational freedom, which gives valuable information of the binding site. Potent rigid analogues are also likely to be more selective towards the target. The rigidity can be introduced by linking two parts of the molecule together, by introducing bulky substituents to the structure or by using more rigid building blocks.

In Chapter 8 a 5-alkyl substituent was introduced to the L-proline moiety at the P2 site of a POP inhibitor with an *N*-acyl-L-prolyl-pyrrolidine structure. As described in Chapter 1, several L-proline analogues have been used successfully as replacements of the L-prolyl group at the P2 site of POP inhibitors. Nevertheless, a 5-alkyl-L-proline moiety with a bulky alkyl group, which limits the conformational freedom of the inhibitor structure, had not been studied.

In the series of compounds based on Boc-L-prolyl-pyrrolidine **14**, with an IC_{50} value of 29 nM against pig brain POP, the 5(*R*)-*tert*-butyl derivative **23a** and the 5(*S*)-*tert*-butyl derivative **23b** had IC_{50} values of 2.2 and 9.2 nM, respectively, against pig brain POP. The 5(*R*)-*tert*-butyl substituent increased the potency strongly in this series of compounds. In the series of compounds based on a SUAM-1221 **2**, with an IC_{50} value of 2.2 nM against pig brain POP, the 5(*R*)-*tert*-butyl derivative **24a**, 5(*R*)-methyl derivative **24b** and the 5(*S*)-methyl derivative **24c** had IC_{50} values in the range 0.7-1.4 nM against pig brain POP. In this series of compounds the 5-substituent did not have a significant effect on the inhibitory activity. The most likely explanation for this result is that the conformation of a bulky Boc group is strongly affected by the bulky 5-alkyl substituent, whereas the conformation of a flexible 4-phenylbutanoyl is not. The potency of the compounds could be increased with a 2(*S*)-(hydroxyacetyl)pyrrolidinyl group at the P1 site. The resulting compound **25** had an IC_{50} value of 0.26 nM against pig brain POP.

14: R = H
23a: R = 5(*R*)-*t*-Bu
23b: R = 5(*S*)-*t*-Bu

2: R = H
24a: R = 5(*R*)-*t*-Bu
24b: R = 5(*R*)-Me
24c: R = 5(*S*)-Me

25

1.5. Physico-chemical properties of the new compounds

Different physico-chemical properties, such as the pK_a value, log P (or log D) value, the molecular weight and water solubility, are important parameters that can be used to make a first prediction of the pharmacokinetic properties of compounds. The targeted POP is in the brain, which adds the additional requirement to the compounds that they have to penetrate the blood-brain barrier.

In the present study only one compound had a pK_a value in the range 0-14, which meant that the pK_a value was not a relevant parameter in this study. The optimal log P value for a compound that is targeted to the brain is between 2 and 3. However, the log P values for two well known centrally active POP inhibitors SUAM-1221 **2** and JTP-4819 **1e** are 1.8 and 0.2, respectively, which may indicate that the lower limit of 2 is not very strict. Regarding the molecular weight, 500 was considered an upper limit for a compound that has to penetrate the blood brain barrier. No quantitative limits were set for the water solubility.

The most potent compounds in Chapter 4, compounds **5a** and **5b**, were very promising *in vitro*, but they were expected to be extremely lipophilic due to their hydrocarbon rich structure. Indeed, they were not water soluble and there was even a difficulty in dissolving them in methanol. Therefore, the development of these compounds was not continued further and their log P values were not determined.

5a: R = cyclopentyl
5b: R = Ph

One of the most potent compounds in Chapter 5, compound **15**, had a log P value of -0.2 (Table 3). Therefore, further development of these type of compounds in Chapter 6 was mainly focused on increasing the log P value. The log P value was increased by replacing the nitrogen atoms of the pyrrolidinyl end groups with carbon atoms, resulting in compounds **16** and **6**.

Table 3. Log P values of selected compounds presented in Chapters 4 and 5.

Compound	Structure	log P
15		-0.2
16		1.1
6		2.7
21a		0.8
21b		0.2
22b		0.7

The log P value for the more potent series of compounds with 2(*S*)-cyanopyrrolidinyl and 2(*S*)-(hydroxyacetyl)pyrrolidinyl groups at the P1 site were also studied. As compared to compound **16**, compounds **21a** and **21b** showed that the 2(*S*)-cyano group did not reduce the log P value much, whereas the 2(*S*)-hydroxyacetyl group did. Replacing the central isophthalic acid residue by a 3,3-dimethylglutaric acid residue improved the log P also for the 2(*S*)-hydroxyacetyl substituted compound, as indicated by compound **22b**.

In Chapter 8 different 5-alkyl substituents were added to the L-prolyl group at the P2 site of *N*-acyl-L-prolyl-pyrrolidine type of POP inhibitors. Including a 5-*tert*-butyl substituent to the L-prolyl group at the P2 site increased the log P by as much as 1.5 log units. SUAM-1221 **2** and compound **24a** had log P values of 1.8 and 3.3, respectively. A combination of the 5-*tert*-butyl substituent at the L-prolyl group at the P2 site and a 2(*S*)-(hydroxyacetyl)pyrrolidinyl group at the P1 site resulted in compound **25** with an

excellent log P value of 2.3. As compared to JTP-4819 **1e** compound **25** is equipotent, but its log P value is 2.1 log units higher. However, JTP-4819 **1e** has been reported to be centrally active and whether or not the increased log P value for compound **25** is relevant requires further pharmacological studies.

2:   R = H
24a: R = 5(*R*)-*t*-Bu

25
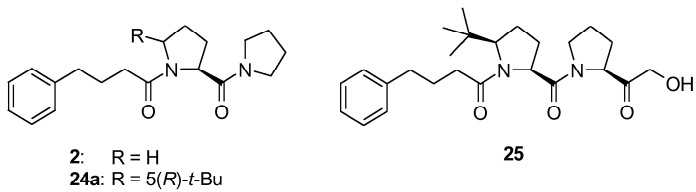
2. SUMMARY OF THE SYNTHESIS AND CHARACTERIZATION OF THE NEW COMPOUNDS

In medicinal chemistry the main synthetic objective is to obtain certain compounds and new synthetic procedures are only developed when needed. The purity of the compounds is essential for obtaining reliable biological data. This includes also the stereochemical purity, since biological systems are primarily asymmetric. Therefore, the applied synthetic procedures were selected among reactions where the configuration of new stereocentres could be controlled and the racemization of existing stereocentres could be avoided.

The most important contributions to synthetic chemistry in the thesis are the diastereoselective synthetic procedures of various 5-alkyl-L-prolines, described in Chapters 7 and 8. The 5-alkyl-L-prolines are important L-proline analogues, which may also have a wide range of potential applications in peptidomimetics in general. Both the (2S,5R)- and the (2S,5S)-diastereomers were synthesized by diastereoselective synthetic procedures, where the diastereoselectivity was induced by the existing stereocentre at the 2-position of the starting material. The procedures yielded (2S,5R)-N-Boc-5-methyl-L-proline methyl ester, (2S,5R)-N-Boc-5-tert-butyl-L-proline methyl ester, (2S,5S)-N-Boc-5-methyl-L-proline ethyl ester and (2S,5S)-N-Boc-5-tert-butyl-L-proline ethyl ester as products. After flash chromatography the (2S,5R)-diastereomers were obtained pure, whereas the (2S,5S)-diastereomers contained a small amount of the (2S,5R)-diastereomer as an impurity. However, the (2S,5S)-diastereomers were used in the synthesis for the end products without further purification. Before reaching the end product several flash chromatography purifications were made, and the impurity of the (2S,5R)-diastereomer was removed during these purifications. The diastereomeric purity was verified by NMR.

Amide bonds cannot rotate freely due to their partial double bond character. In general, the *trans* isomer of an N-amide bond of an natural amino acid is energetically favoured. However, the *cis* and *trans* isomers of an N-amide bond of L-proline are energetically comparable. The main consequence of this phenomenon is that many compounds containing an N-acyl-L-prolyl group are mixtures of the *cis* and *trans* isomers at rt (Figure 1).
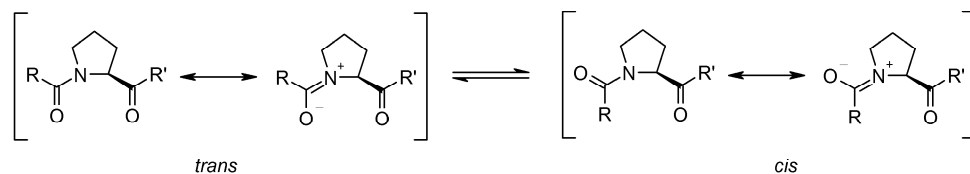
Figure 1. The *cis* and *trans* isomers of an *N*-acyl-L-prolyl group.

Consequently, the NMR spectra of these compounds show separate shifts for the *cis* and *trans* isomers. It is impossible (or at least very difficult) to deduce from one single NMR experiment if the double shifts come from the *cis* and *trans* isomers of an *N*-acyl-L-prolyl group or from a diastereomeric impurity. The best method to exclude the possibility of a diastereomeric impurity is to measure the NMR spectrum in two different solvents. A change in the ratio between the two isomers indicates that they are not diastereomers, but *cis* and *trans* isomers of an *N*-acyl-L-prolyl group. However, compounds with several *N*-acyl-L-prolyl groups give often very complicated spectra. In Chapters 5 and 6 the compounds could have up to three *N*-acyl-L-prolyl groups (or other 2(*S*)-substituted *N*-acyl-pyrrolidine moieties), and for some of these compounds their NMR spectra could not be assigned. Especially the broad band decoupled ^{13}C NMR spectrum becomes difficult to assign if there is more than one major *cis-trans* isomer.

This ESI ionization technique in mass spectrometry gives usually only the $(M+H)^+$ peak in the mass spectrum. Many compounds also had a smaller additional peaks corresponding to $(M+Na)^+$, $(2M+H)^+$ or another adduct of the compound. However, only the $(M+H)^+$ peak was reported for such compounds.

Most of the compounds containing a L-proline moiety were amorphous and hygroscopic compounds. Many compounds contained a little water after purification and evaporation overnight *in vacuo* at 0.1 mbar. Therefore, it was common that 0.1-1.0 water molecules per product molecule had to be added in the calculations of the elemental analysis in order to obtain a calculated value within 0.4% of the found value for C, H and N.

3. CONCLUSIONS

This thesis describes the design, synthesis, characterization and *in vitro* evaluation of new POP inhibitors. For some compounds also the log P values were determined in order to make a first prediction of the blood-brain barrier penetrability. The starting point for this study was the substrate-like POP inhibitors with an *N*-acyl-L-prolyl-pyrrolidine structure. The design of the new POP inhibitors was mainly focused on increasing the structural diversity of POP inhibitors.

The following conclusions can be made:

1. The 2(*S*)-substituent of the pyrrolidinyl group at the P1 site cannot be replaced by a simple 2(*S*)-acyl group. Without an activating group the keto function will decrease the *in vitro* inhibitory activity as compared to the unsubstituted pyrrolidinyl group at the P1 site.

2. The pyrrolidinyl group at the P1 site could be replaced by a cyclopentyl or a phenyl group, resulting only in a small decrease of the inhibitory activity. These compounds are a new type of POP inhibitors which lack one of the most typical structural features of POP inhibitors, the pyrrolidinyl group at the P1 site.

3. Introducing a 5-alkyl substituent to the L-prolyl group at the P2 site, introduces a steric hindrance to the *N*-acyl group at the P3 site. This conformational rigidity increased the inhibitory activity for some combinations of 5-alkyl substituents and *N*-acyl groups. The 5(*R*)-*tert*-butyl substituent in combination with an *N*-Boc group resulted in a 13 fold increase of the inhibitory activity.

4. A new type of POP inhibitors based on a dicarboxylic acid *bis*(L-prolyl-pyrrolidine) amide structure was introduced. These compounds show that the lipophilic P3 site can be replaced by a relatively polar group without a large effect on the inhibitory activity.

5. Because the compounds are targeted to the brain, the log P value was used as a first prediction of the blood-brain barrier penetrability of a compound. Although the log P values for all compounds were not determined, there are clearly potent compounds that either fulfilled the criteria of a log P value between 2 and 3 or came very close the lower limit of 2.

6. New synthetic procedures were developed for various 5-alkyl-substituted prolines. These are useful proline analogues which can be used widely in peptidomimetics.

4. FUTURE PERSPECTIVES

The results from the first clinical studies of POP inhibitors are still awaited. It is too early to draw any conclusions of the clinical studies based on the few articles that have been published so far.

One major drawback might be that the currently tested compounds inhibit more effectively peripheral POP than brain POP. Therefore, new POP inhibitors may still have to be developed in order to increase the brain POP inhibition as compared to the peripheral POP inhibition.

POP is widely distributed and many of its substrates are biologically active. On the basis of these two arguments alone the future research on POP and its inhibition is very important. The emergence of new implications, such as the inhibition of a parasitic POP as a target in the treatment of Chagas disease, will raise new challenges for the development of POP inhibitors. In the case of inhibiting the parasitic POP there is a demand for new more selective POP inhibitors to be developed.

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