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# Prolyl endopeptidase inhibitors: a new class of memory enhancing drugs

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## Introduction

The hope of maintaining an intact capacity for memorization in the aging population is one of the most outstanding challenges for modern neurosciences. Although considerable progress has been made in the past few years, especially in the knowledge of central processes of memory, there is still a great need for new memory-improving treatments. Mnemocognitive disorders associated with normal or pathological cerebral aging are being detected much more frequently. Increasing life expectancy and occurrence of neurodegenerative pathologies (Alzheimer's, Parkinson's and Huntington's diseases) justify increased efforts in the search for new biological targets implicated in neurodegenerative processes and memory loss. One of these targets is the enzyme prolyl endopeptidase (PEP: EC 3.4.21.26), first isolated in the human uterus and later purified from lamb kidney. It was subsequently named post-proline cleaving enzyme (PPCE) because of its specificity for cleaving a peptide bond at the C-terminal side of a proline residue (1). More recently, PEP was denominated POP for prolyl-oligopeptidase (2). This enzyme is widely distributed in various mammalian tissues such as the brain, liver and kidney (3).

In the central nervous system, PEP degrades proline-containing neuropeptides such as vasopressin, substance P (SP) and thyrotropin releasing hormone (TRH) (4), involved in the processes of learning and memory. Moreover, cognitive deficits in Alzheimer's patients were reported to show improvement with TRH, and one can postulate that PEP inhibitors could prevent memory loss and increase attention span in patients suffering from senile dementia. More recently, PEP-like immunoreactivity was detected and associated with amyloid β-peptide-like immunoreactivity, suggesting that PEP could be implicated in amyloidogenesis (5). Although the activity of PEP is very low as the "cleaving enzyme" of amyloid precursor protein, this finding has attracted great interest in the plausible inhibitory activity of PEP inhibitors in brain amyloidosis. Recently, protective activity of SP against the neurodegenerative effect of β-amyloid was demonstrated in the adult rat (6). Since SP is known to be a substrate for PEP, it is expected that an inhibitor of this enzyme might possess neuroprotective activity by inhibiting SP degradation, thus elevating its cerebral content.

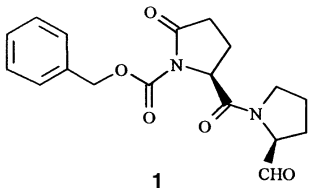
In 1987, Yoshimoto *et al*. reported that an *in vitr o*PEP inhibitor had *in viv o*antiamnesic properties in the passive avoidance learning test using scopolamine-induced amnesia in the rat (7). Likewise, novel molecules likely to interact with this enzyme are intensively awaited.

The purpose of this paper is to review the synthetic low-molecular weight PEP inhibitors.

## Prolyl endopeptidase inhibitors

Over the last two decades, the potential clinical utility of PEP inhibitors has generated substantial research efforts by numerous pharmaceutical companies and in many academic groups, especially in Japan. Peptidic derivatives as well as naturally occurring inhibitors have been reviewed by Higuchi (8) and will not be discussed herein.

It is known that PEP cleaves its natural substrates at the C-terminal side of a proline residue (1, 9). This property has been extensively exploited in the design of substrate analog type inhibitors. Since the first report in 1977 by Yoshimoto at Nagasaki University, numerous low-molecular weight inhibitors of PEP have been described which contain a proline or a proline analog residue. Z-Gly-Pro-$CH_2Cl$ was reported as an irreversible inhibitor which alkylated the active site of the enzyme. In general, irreversible inhibitors contain either a chloroacetyl or a diazoacetyl moiety, which reacts covalently with the enzyme (9). Reversible inhibitors have also been extensively studied, and in the majority of them, the chemical functionality used to react with serine-554 in the active site of the enzyme is a formyl group (10).



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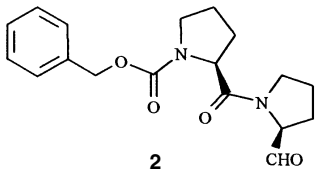


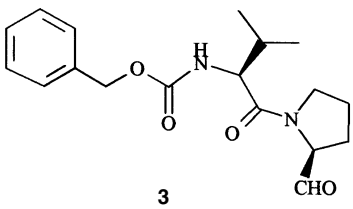
2

Z-Pyroglutamyl-prolinal **1** and Z-Pro-prolinal **2** (11) were first described by the Nagasaki group as potent PEP inhibitors with subnanomolar $K_i$s on a bovine brain as well as on a *Flavobacterium* enzymatic preparation. Compound **2** was also found to be a potent inhibitor on a rabbit brain PEP preparation with a $K_i$ of 14 nM (12).

Another series of prolinal-containing derivatives was prepared by Nishikata (13), and the most potent inhibitor on a *F. meningosepticum* preparation was found to be Z-Val-prolinal **3**. Conversion of the aldehyde function into a primary alcohol or a carboxylic acid moiety was extremely deleterious for inhibitor potency.

Following these reports, the antiamnesic effect of the prolinal family of compounds was reported in the passive avoidance model in the rat, where inhibitors prevented the induction of amnesia by scopolamine at an i.p. dose of 1 µM (14). It was also shown that the aldehyde functionality often favored oily-like unstable derivatives; hence, the aldehyde function was suppressed, and the resulting compounds were also found to be potent PEP inhibitors. The enzyme is most probably interacting with the amide group, the nitrogen atom being part of a 5-membered ring, often a pyrrolidine or a thiazolidine system. Derivatives from this second family were found to be potent inhibitors of bovine brain PEP; for example, Z-thio-Pro-thiazolidine **4** showed a $K_i$ value of 0.36 nM (14, 15).

Interaction of compound **4** with the active site of the enzyme was studied, and it was postulated that the carbonyl group between P1 (thiazolidine) and P2 (thioproline) subsites was essential for interacting with the enzyme through hydrogen bonding, thus participating strongly in inhibitor-enzyme fixation. Furthermore, it was confirmed that the presence of a bulky lipophilic group in P3 (benzyloxycarbonyl) favored the enzyme-inhibitor interaction (15).



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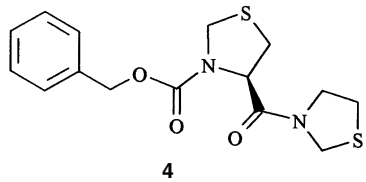

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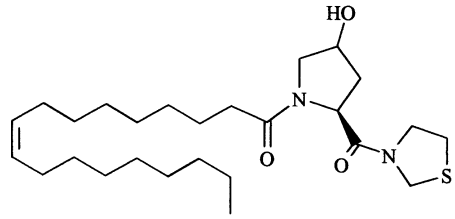

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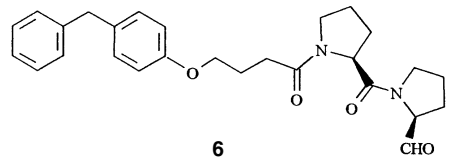
Suntory was one of the first pharmaceutical companies to be involved in the discovery of a PEP inhibitor. Several patents were filed, among them one describing fatty acyl proline derivatives exemplified herein by SUAM-14748 **5** (16). In 1990, Saito from the Suntory Research Center in Osaka reported the synthesis and PEP inhibitory activity of a series of acyl-peptidyl-prolinal derivatives as well as their nootropic properties (17). These compounds were found to inhibit PEP (from bacterial or bovine brain source) in the nM range, with the prolyl-prolinal derivatives being the most potent, among them 4-(4-benzylphenoxy)butyryl-Pro-Pro-H **6** ($IC_{50}$ *Flavobacterium* = 0.2 nM) and 4-(4-benzylphenoxy)butyryl-Val-Pro-H **7** ($IC_{50}$ bovine brain = 2 nM). In the scopolamine-induced passive avoidance response test, the retention time was increased after treatment with the prolinal derivatives (i.p. doses of 0.01-1 mg/kg). It is noteworthy that the best compounds **8** and **9** presented a dose-dependent, bell-shaped curve.



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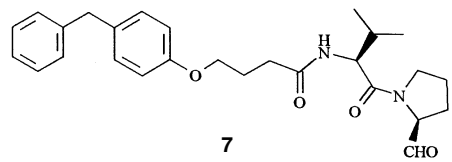

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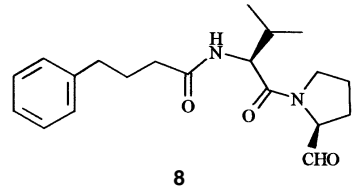

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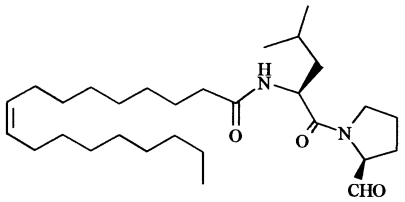
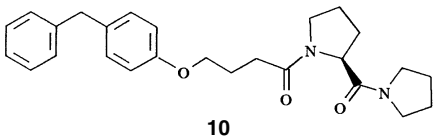
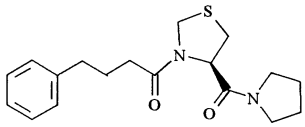


**9**

One year later, the same group reported the preparation and activity of several acyl-peptidyl-pyrrolidine derivatives (18). Again, the most effective inhibitors had a central proline residue, from which was appended an arylalkyl or aryloxyalkyl side chain interacting with the P3 subsite. **10** and **11** were the most potent inhibitors on *Flavobacterium* ($IC_{50}$ = 1.4 nM) and bovine brain ($IC_{50}$ = 67 nM), respectively.

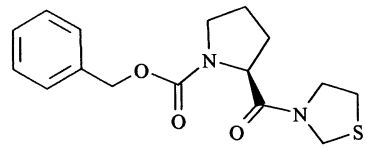


**10**



**11**

The large number of compounds tested allowed the same authors (17, 18) to propose a comparison of the active sites of both the *Flavobacterium* and the bovine brain enzymes. They postulated that the main difference between the two sites was located in the S3 region, the bovine enzyme requiring specifically a bulky *ortho*-substituted aryloxyalkyl moiety while the microorganism enzyme favored a long hydrophobic fatty acid side chain. Again, two inhibitors were evaluated in the passive avoidance test: compounds **12** (SUAM-1221) and **13** prevented the induction of amnesia at i.p. doses of 5 and 1 mg/kg, respectively. The authors concluded that PEP may play a role in the regulation of learning and memory consolidation in the brain, and as such, inhibitors of this enzyme might be possible candidates for nootropic agents.

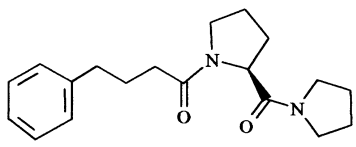


**14**

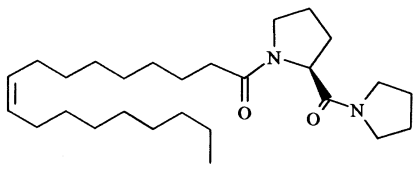
In 1984, another Japanese pharmaceutical company, Yakult Honsha, patented the antiamnesic activity of two irreversible inhibitors, Z-Gly-Pro-$CH_2Cl$ and Z-Gly-Pro-$CH_2N_2$, on passive avoidance learning (19). They also published a new use for Z-Pro-prolinal, initially described by Yoshimoto (11), which produced substantial inhibition of syncytia formation at 300 µM in HIV-infected cells (20). In 1987, the same company filed a patent claiming derivatives of **14**, which are very similar to those mentioned above, exemplified by compound **4** (21).

In order to improve the originality of their molecules, researchers at Yakult Honsha developed a series of PEP inhibitors where the aldehyde function interacting with the enzyme was replaced by a dialkyl acetal moiety, very probably acting as a prodrug. This new class of compounds is exemplified herein by the diethylacetal derivative **15** (22, 23). Compound **15** inhibited bovine brain PEP with a $K_i$ of 2.2 µM. Interestingly, the corresponding dimethylacetal was two orders of magnitude less potent, with a $K_i$ of 4300 µM. Antiamnesic activity of both compounds was evaluated *in vivo* in the step-down passive avoidance test in the rat, where **15** showed a significant activity after oral dosing of 1-100 mg/kg.

Since 1986, Ono Pharmaceuticals has filed several patents (24-28). Their lead compound is the prolinal derivative ONO-1603 **16**, which has been described as acting in part by central vasopressin modulation. In rats, the compound improved impairment of acquisition and retention of scopolamine-, ischemia- and electroconvulsive shock-induced passive avoidance response (29).



**12**



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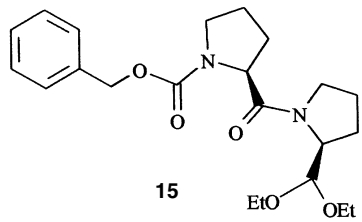
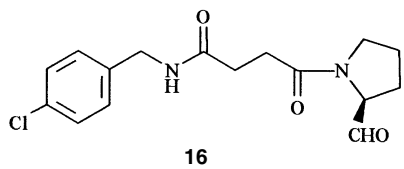
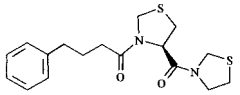
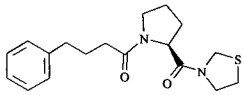
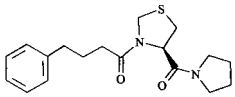


**15**



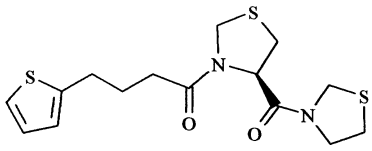
**16**

*Table I: PEP inhibition with Zeria's dipeptidyl inhibitors.*

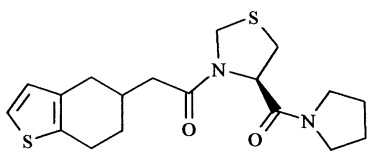
| Compound | Structure | Inhibition potency (canine brain PEP) $IC_{50}$ (nM) |
|---|---|---|
| **17** |  | 170 |
| **18** |  | 410 |
| **19** |  | 310 |

According to the Prous Science Trilogy database, this compound is in phase II clinical trials as a memory enhancer for the treatment of senile dementia.

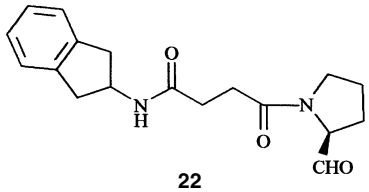
One of the most prolific companies in terms of patent filing in the field of PEP inhibition has been Zeria Pharmaceuticals. Their first-generation inhibitors exemplified by **17** were close analogs of SUAM-1221 **12** (30). Modifications of the two 5-membered rings led to the discovery of the potent inhibitors **18** and **19**, shown in Table I. Compound **17** was studied in the mouse on the prevention of inhibition of long-term memory fixation by cycloheximide, where amnesia reversal was evaluated and found to be 74.8% at the i.p. dose of 3 mg/kg. Improvement in the *in vitro* inhibitory potency was achieved when the phenyl ring of the side chain was replaced by a thiophene ring, *e.g.*, compound **20** gave an $IC_{50}$ of 17 nM (31). Several other patents describe compounds exemplified herein by **21-23**, in which the side chain is part of a bicyclic ring system (32-37). Nitrile derivative **23** gave an $IC_{50}$ of 0.55 nM on a canine brain PEP preparation (38).



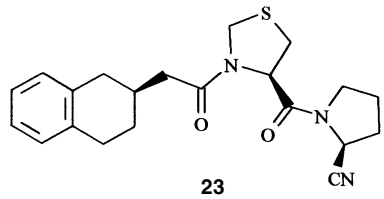
**20**



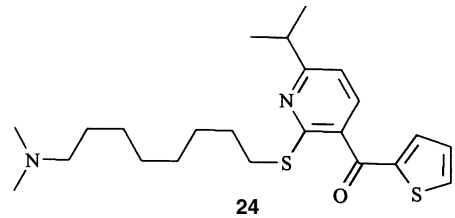
**21**



**22**



**23**



**24**

In 1993, Yoshitomi Seiyaku Pharmaceuticals disclosed the structure of diarylketones as PEP inhibitors (39, 40). The lead compound, Y-29794 **24** was described as "a nonpeptide prolyl endopeptidase inhibitor that can penetrate into the brain" (41). This compound was shown to be a competitive, selective and reversible PEP inhibitor with a $K_i$ of 0.95 nM. *Ex vivo* dose-dependent and long-lasting PEP inhibition was observed after oral administration to rats (Fig. 1). Moreover, **24** at the oral dose of 3 mg/kg was found to increase the TRH-induced acetylcholine release in rat hippocampus, suggesting that the action of TRH was potentiated due to PEP inhibition by Y-29794. In order to study the biodistribution of Y-29794, the synthesis of $^{14}C$-labeled **24** was performed and reported (42). Four other patents were filed in collaboration with Japan Tobacco and will be reviewed below with publications from the latter company.

Biseibutsu Kagaku emerged in the field of PEP inhibition in 1988 when they patented actinonine derivatives such as **25** (43). Soon afterwards, researchers of this company disclosed α-ketoamide structures such as **26**,

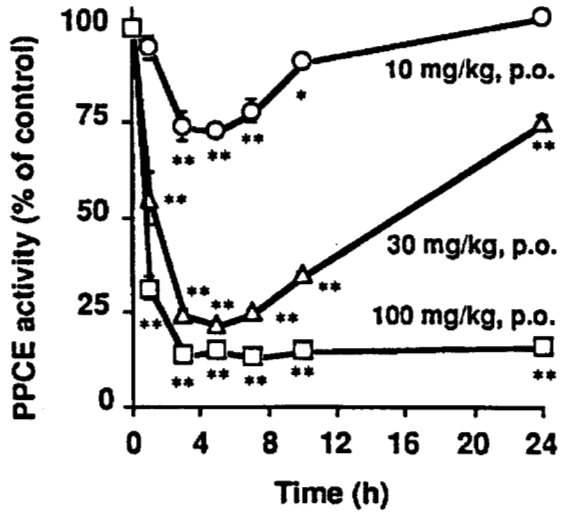
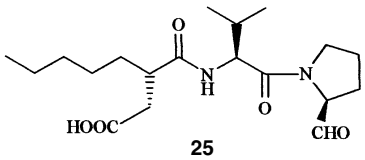
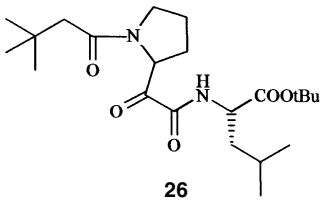


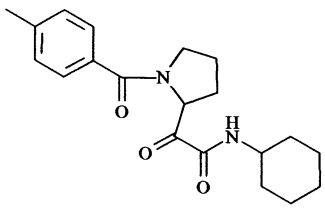

Fig. 1. *Ex vivo* hippocampal PEP inhibition after oral administration of Y-29794 to rats.



**25**



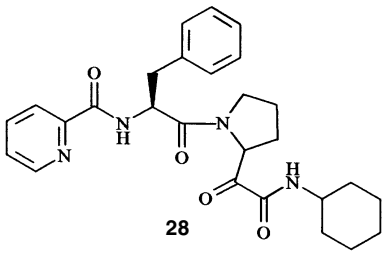
**26**



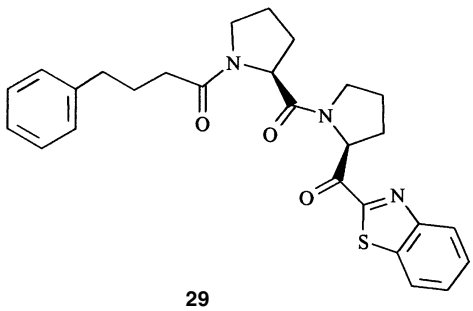
**27**

able to inhibit the enzyme with an $IC_{50}$ of <0.01 µg/ml (44). In the early 1990s, in collaboration with the Microbial Chemical Research Foundation in Tokyo, two new series of α-ketoamide PEP inhibitors were claimed, exemplified herein by **27** ($IC_{50}$ = 0.022 µg/ml against pig kidney enzyme) and **28**. Compound **28** was found to decrease the enzymatic activity by 94.3% in a mouse brain preparation (45, 46).

The first patent by Meiji Seika in the area of PEP inhibition was filed in collaboration with Biseibutsu Kagaku and described compounds exemplified by **25** (43). More recently, a collaborative effort between researchers in the United States and Japan has led to new PEP inhibitors in which the carbonyl function interacting with the enzyme



**28**



**29**

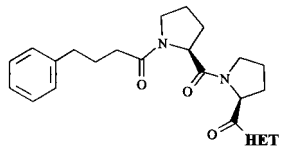
was activated by an heteroaromatic ring system (47). Compound **29** was specifically claimed as a potent PEP inhibitor showing an $IC_{50}$ of 1.02 ng/ml compared to 14.5 ng/ml for SUAM-1221 **12**, both on porcine kidney enzyme. A summary of this work was published in two papers (48, 49), where the chemistry employed for the preparation of this class of inhibitors was described in detail and pharmacological results were illustrated by *in vitro* enzymatic inhibition data.

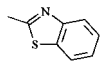
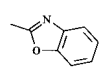
α-Ketobenzoxazoles containing pseudopeptides have recently been shown to act as site-directed elastase inhibitors (50). PEP, like elastase, is a serine protease, and the authors postulated that the heterocyclic ring would stabilize the enzyme-inhibitor complex through formation of a hydrogen bond with an histidyl residue. Hydrophobicity obtained with this α-ketoheterocycle was also required in order to penetrate the central nervous system. An overview of the inhibitory activity of this class of compounds is given in Table II.

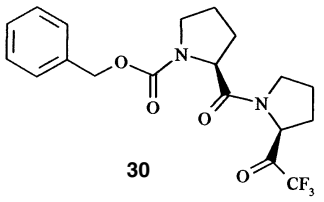
Interestingly, PEP inhibitory potency is very sensitive to the position of the two heteroatoms in the 5-membered ring. If both heteroatoms are actually required for activity (thiophene and pyrrole are inactive), aromaticity appears to be of less importance, since the thiazoline-containing inhibitor is still quite potent. Finally, introduction of the fused phenyl ring and likewise the nature of the bivalent heteroatom have virtually no effect on *in vitro* potency. $IC_{50}$ of the prolinal analog is 8.7 nM.

In three of the four patents filed by Hoechst in 1987, the carbonyl group interacting with the enzyme is activated by a trifluoro or a difluoromethyl group giving rise to inhibitors **30-32** (51-53). The fourth patent describes compound **33**, a vinyl analog of known structure (54). No biological results were given in these publications.

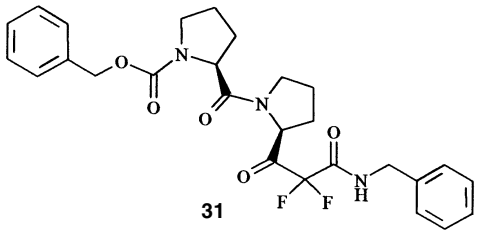
*Table II: PEP inhibition values for Meiji's α-ketoheterocycle inhibitors.*



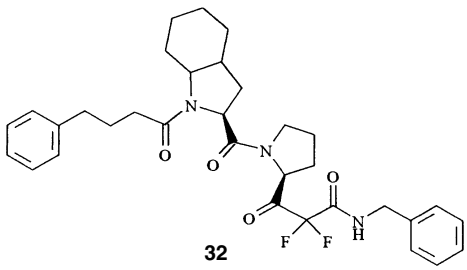
| HET | $IC_{50}$ (nM) | HET | $IC_{50}$ (nM) |
|---|---|---|---|
|  | 5.0 |  | 1260 |
|  | 1090 |  | 21300 |
|  | 6.2 |  | 3.8 |
|  | 4.0 |  | 5.6 |



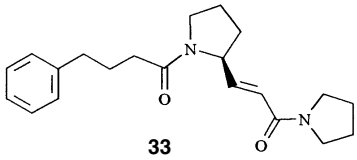
**30**



**31**



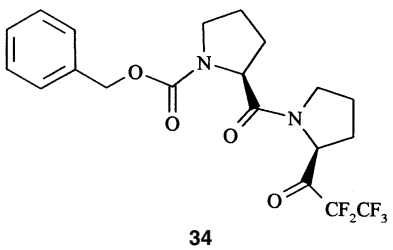
**32**



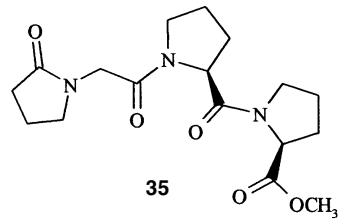
**33**

Merrell Dow also patented inhibitors which contain polyfluoroalkyl activated ketones. They focused their work on pentafluoroethyl and heptafluoropropyl ketones appended on the classical diproline backbone. Typically, compound **34** inhibited purified enzyme isolated from bovine brain with an $IC_{50}$ of 1 nM. No results were provided for *in vivo* evaluation of the compounds. Furthermore, these inhibitors are reported to be preferred for non-oral administration, which could be a severe limitation with respect to the long-term administration expected in chronic degenerative diseases (55).

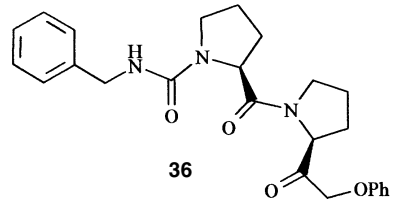
The first patent filed by Japan Tobacco described compounds exemplified by **35** (56). Four subsequent patents were filed in collaboration with Yoshitomi Pharmaceuticals and disclosed a series of PEP inhibitors,



**34**



**35**



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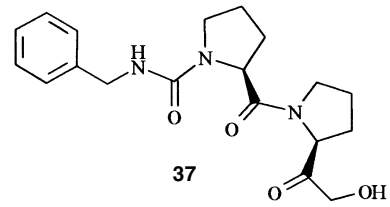
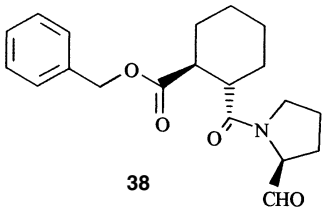


**37**

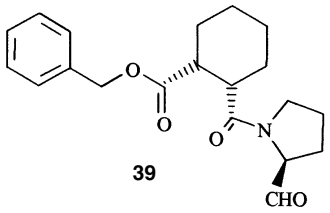
the leader of which was JTP-3399 **36** (57-60). The active metabolite of this compound was found to be JTP-4819 **37**, which was intensively studied and is currently in phase II clinical studies.

Recently, the preclinical pharmacology of JTP-4819 was extensively reviewed (61). Briefly, JTP-4819 inhibited PEP in a concentration-dependent manner in the cytosolic fraction of rat brain with an $IC_{50}$ value of 0.83 nM. *Ex vivo* experiments in the rat showed that orally administered JTP-4819 (3 mg/kg) significantly reduced PEP activity in several brain regions after 1 and 3 hours. JTP-4819 inhibited the degradation of proline-containing neuropeptides (SP, AVP, TRH) secondary to PEP inhibition (62). Furthermore, neuropeptide levels were increased in the cerebral cortex and hippocampus of aged rats after oral treatment by JTP-4819 (63-65). Interestingly, the JTP product inhibited degradation by PEP of a mimic of the amyloid β-peptide (Aβ), Suc-Ile-Ala-MCA, with an $IC_{50}$ of 0.32 nM. This result suggests that one of the enzymes generating Aβ from its precursor, the β-amyloid precursor protein, could be PEP, and that the formation of Aβ could be regulated by a specific PEP inhibitor.

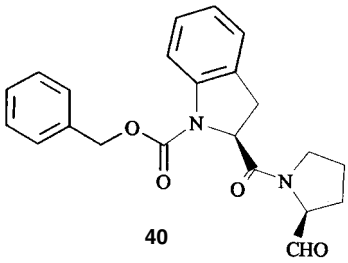
*In vivo*, JTP-4819 improved performance in several memory and learning-related tests, including Morris water maze in aged rats, nucleus basalis lesioned rats, MCA occlusion in rats (66), passive avoidance task in scopolamine-treated rats (62), *etc.* Furthermore, JTP-4819 has been shown to reverse central cholinergic dysfunction. At daily oral doses of 1 and 3 mg/kg, it reversed the age-related increase of choline acetyltransferase activity in the cerebral cortex and the decrease of [$^3$H]-choline uptake in the hippocampus (67). The authors suggested



**38**



**39**



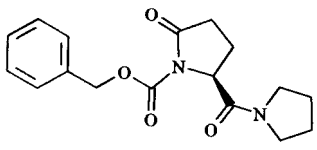
**40**

that this compound could be of potential interest in the treatment of Alzheimer's disease (68).

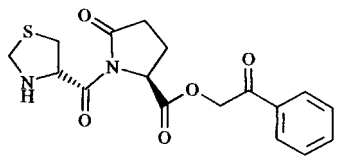
In the early 1990s, Pfizer filed a patent disclosing the structures of aromatic pyrrolidine and thiazolidine amides (69). This work is exemplified here by the three compounds **38-40**, which proved to be potent PEP inhibitors, with $K_i$s of 3, 3 and 2.4 nM, respectively (70, 71).

In 1989, Kissei filed four patents in the field of PEP inhibitors, one of which describes the amide analog **41** of pyroglutamic acid **1** (72). The other three patents describe *inverso* compounds, where the P'1 heterocycle is a 5-substituted thiazolidine as in compounds **42** and **43** (73, 74), or a 2-substituted furan as in compound **44** (75).

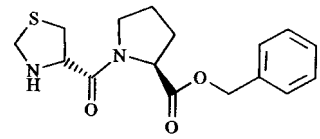
When we embarked on the project of PEP inhibition here at the Servier Research Institute, we envisioned that it would be possible to replace the central proline residue



**41**



**42**



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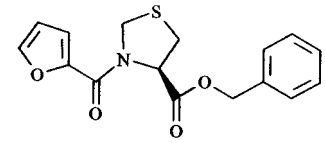
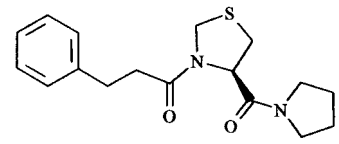
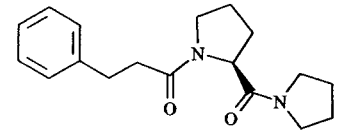


**44**



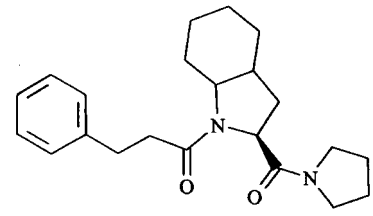
**45**
$IC_{50}$ = 67 nM



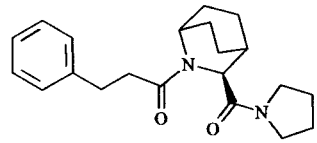
**12** (SUAM 1121)
bovine brain PEP: $IC_{50}$ = 190 nM

by some nonnatural analogs and thus obtain potent PEP inhibitors. Saito had already shown that replacement of the central proline by a thioproline could be important in gaining additional inhibitory potency (compare **45** to SUAM-1221 **12**) (18).

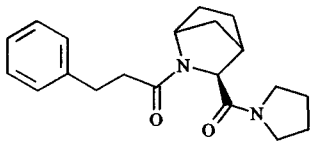
Previously, we had shown that it was possible to retain inhibitory potency when a proline moiety was replaced by the nonnatural analogs PHI ((2*S*,3a*S*,7a*S*)-perhydroindole 2-carboxylic acid), ABO ((3*S*)-2-azabicyclo[2.2.2]octane-3-carboxylic acid) or ABH ((3*S*)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid) in this case in the structures of serine or metalloproteinase inhibitors (76). In a recent paper reporting our efforts in the discovery of new PEP inhibitors (77), we have shown that in several different chemical series, the use of these amino acids was beneficial to improving the *in vitro* PEP inhibitory activity. Examples are compounds **46-48**, which gave $IC_{50}$



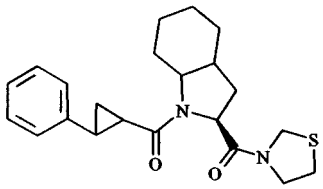
**46**



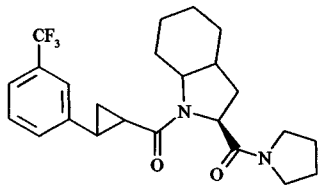
47



48



49




50

values on a *Flavobacterium* preparation of 34, 50 and 27 nM, respectively, when compared to the thioproline derivative **45** ($IC_{50}$ = 540 nM).

A large number of variations have also been effected to replace the pyrrolidine ring and to modulate the butyroyl side chain. For example, introduction of a cyclopropyl ring within this chain gave derivatives **49** (internal company code for this compound is S 17092-1) and **50**, which proved to be exceptionally potent inhibitors when tested on a rat cortex preparation ($IC_{50}$s = 1.3 and 0.9 nM, respectively).

In order to obtain strong PEP inhibition *in vitro* as well as *ex vivo*, stereochemistry of the cyclopropyl stereogenic centers has to be (*R,R*). The preparation of the substituted aryl cyclopropyl carboxylic acids was performed using Oppolzer's chemistry as shown in Scheme 1 (78).

These carboxylic acids were easily coupled with the central amino acid, already bearing the 5-membered heterocycle at the C-terminal position, to give the desired inhibitors. As illustrated in Figure 2, *ex vivo* studies showed that PEP inhibition was much more longer-lasting with compounds exemplified here by **51** when compared to reference compound **45**. Higher inhibitory potency was obtained mainly with the PHI-containing derivatives, culminating with an $ID_{50}$ of 1 mg/kg after oral administration for compound **52**.

*In vivo*, S 17092-1 **49** was found to inhibit scopolamine-induced amnesia in the passive avoidance test in the rat with an i.p. and p.o. $ID_{50}$ of 0.3 and 1 mg/kg,

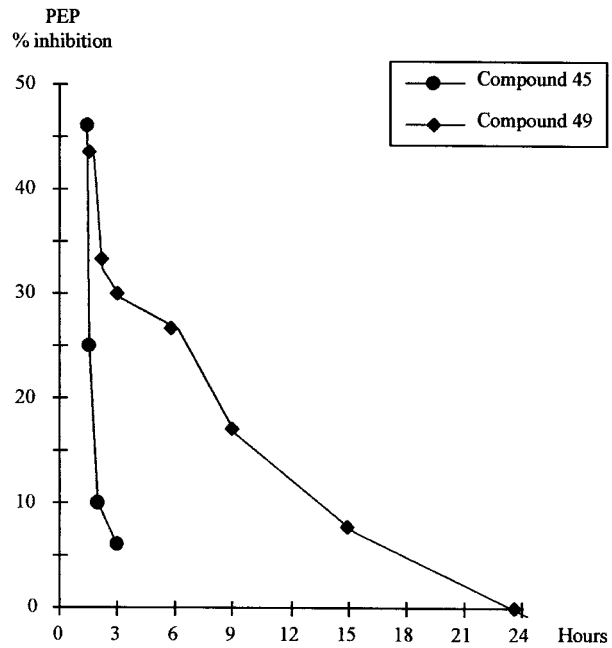
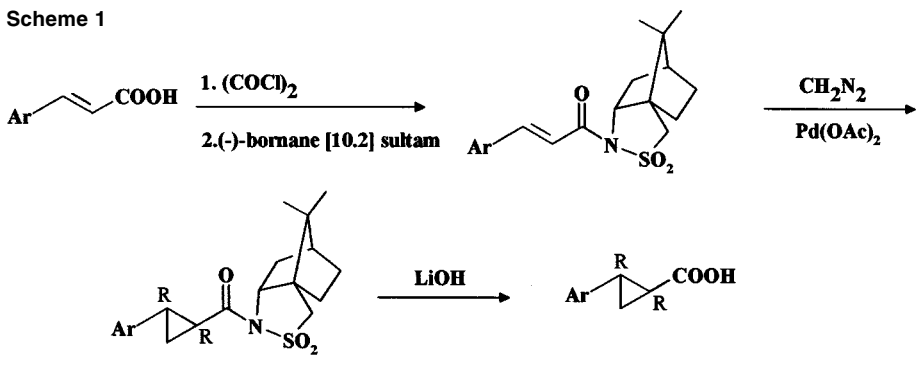
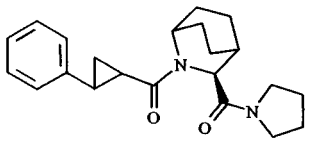


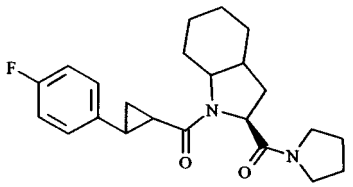

Fig. 2. *Ex vivo* evaluation of PEP inhibitory potency of compound **45** (30 mg/kg i.p.) and compound **49** (5 mg/kg i.p.).

**Scheme 1**





**51**

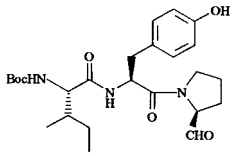
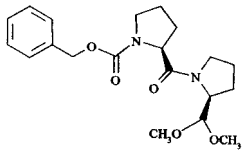
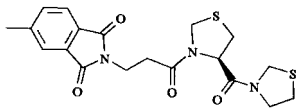
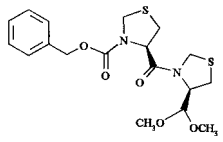
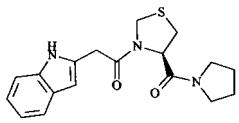
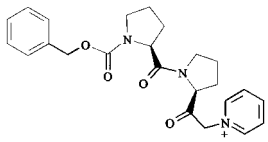
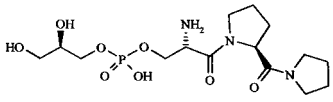


**52**

respectively. In the test of social memory in the rat, compound **50** at the oral dose of 10 mg/kg prevented time-dependent spontaneous forgetting with an effect similar to that of vasopressin. Finally, chronic treatment with **49** (10 mg/kg/day for 2 weeks) antagonized age-associated memory deficits in the test of spontaneous alternation in the mouse (79). Moreover, potentiation of SP-induced grooming behavior by compound **49** suggested that these memory-enhancing effects could be due in part to an increase of SP cognitive activity through modulation of neuropeptide catabolism (80).

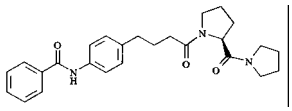
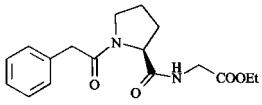
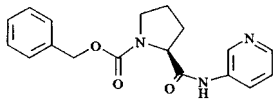
Other pharmaceutical companies and academic institutions have also been involved in the search for PEP inhibitors but to a lesser extent than those cited above.

*Table III: PEP inhibitors from other pharmaceutical institutions.*

| Company | Compound | Activity | Ref. |
|---|---|---|---|
| Ajinomoto |  | $IC_{50}$ = 100 nM | 85 |
| Antwerpen University |  | $IC_{50}$ = 120 nM human monocytes | 86, 87 |
| Chinoin |  | $IC_{50}$ = 0.36 nM rat brain | 88, 89 |
| Kyushu University, Fukuoka |  ZTAA-1 | Improves the impairment of performance in passive avoidance task in basal forebrain-lesioned rats | 90, 91 |
| |  Z.321 | Augments the potentiation of synaptic transmission in rat hippocampus | 92 |
| Hans Knoell Institute and Karolinska Institute |  | $K_i$ = 1.8 nM *Flavobacterium meningosepticum* | 93-97 |
| Magis Farm. |  | Scopolamine-induced amnesia in the passive avoidance test in rats | 98 |

(Continued)

T*able III: Continued.*

| Company | Compound | Activity | Ref. |
|---|---|---|---|
| Merck |  | $IC_{50}$ = 4.4 nM rat brain | 99 |
| Russian American Institute |  | Scopolamine-induced amnesia in the passive avoidance test in rats | 100 |
| Sigma-Tau |  | Electroconvulsive shock-induced amnesia in the passive avoidance test in mice | 101 |

These studies are presented in Table III. All inhibitors are structurally related to prolinal or pyrrolidine-proline derivatives. A recent report also mentioned that PEP inhibitory activity had been discovered in sake, the traditional Japanese rice-derived alcoholic beverage (81).

Several theoretical studies on PEP inhibition have also been performed. A conformational analysis study led to the proposition of a 3-dimensional QSAR model, the validity of which was evaluated by analyzing inhibitory activity of reference as well as new PEP inhibitors (82, 83). The interaction of Z-Pro-prolinal **2** was recently investigated using NMR studies. The authors confirmed that this transition-state analog forms a tetrahedral intermediate with catalytic serine, rather than with a reactive cysteine (84).

## Conclusions

We have reviewed the synthetic low-molecular weight inhibitors of prolyl endopeptidase, a serine protease implicated in the catabolism of proamnesic neuropeptides. In most cases, the compounds are proline-derived with a lipophilic arylalkyl side chain on the N-terminal side and a 5-membered saturated heterocycle on the C-terminal side of the amino acid. *In vitro*, all compounds have been shown to be potent inhibitors of PEP within the nanomolar range. *In vivo*, they have been found to reverse memory and learning impairment after oral administration in several animal models. Thus, one may hypothesize that PEP inhibitors could well be of therapeutic value for the improvement of general cognitive behavior in the elderly. To date, only 3 compounds have entered clinical studies, *i.e.*, ONO-1603, JTP-4819 and S 17092-1, and results are eagerly awaited.

## Acknowledgements

The authors are indebted to Drs. Pierre Lestage and Cécile Lebrun from the Cerebral Pathology Division for conducting the biochemical and pharmacological studies. The participation of Dr. Georges Rémond, Mr. Alain Benoist, Dr. Yolande Hervé and Mrs. Daniele Pommier in the medicinal chemistry program is greatly appreciated. Finally, we thank Dr. Angela Morris for critical reading of the manuscript.

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