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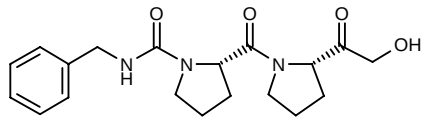
CCC: 0377-8282/98

JTP-4819

Cognition Enhancer
Prolyl Endopeptidase Inhibitor

N-Benzyl-2(*S*)-[2(*S*)-(2-hydroxyacetyl)pyrrolidin-1-ylcarbonyl]pyrrolidine-1-carboxamide

N-Benzylcarbamoyl-L-prolyl-L-prolyl-methanol



$C_{19}H_{25}N_3O_4$ Mol wt: 359.42

CAS: 162203-65-8

EN: 211400

Synthesis

JTP-4819 can be obtained by two related ways:

1) The reaction of *tert*-butoxycarbonyl-L-prolinal (I) with trimethylsulfonium iodide (II) and potassium *tert*-butoxide gives the epoxide (III), which is treated with benzyl alcohol and NaH to perform epoxide ring opening and simultaneous cyclization to yield the oxazolidinone (IV). The hydrolysis of (IV) with KOH affords compound (V), which is condensed with *N*-(benzylaminocarbonyl)-L-proline (VI) (prepared *in situ* with benzyl isocyanate (VII) and L-proline (VIII) in the presence of triethylamine) by means of diphenyl phosphoryl azide (DPPA) giving the dipeptide analog (IX). The oxidation of (IX) with P_2O_5 in DMSO yields the benzylated JTP-4819 product (X), which is finally deprotected by hydrogenolysis with H_2 over Pd/C (1). Scheme 1.

2) The reaction of *tert*-butoxycarbonyl-L-proline (XI) with diazomethane and triethylamine in ether gives the diazoketone (XII), which by treatment with acetic acid at 100 °C yields the acetoxyketone (XIII). The deprotection of (XIII) with trifluoroacetic acid affords the unprotected α-hydroxyketone (XIV), which is condensed with *N*-(benzylaminocarbonyl)-L-proline (VI) by means of 1-hydroxybenzotriazole (HOBT) giving the acetylated dipeptide analog (XV). Finally, this compound is deacetylated with K_2CO_3 in methanol/water (2). Scheme 2.

Description

Colorless needles, m.p. 139 °C, $[\alpha]_D$ –125.6° (c 1.01, MeOH)(1).

Introduction

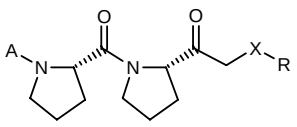
Several enzyme targets have been proposed for the design of new cognition-enhancing drugs. One of these is the serine protease enzyme prolyl endopeptidase (PEP, [EC 3.4.21.26]), which is widely distributed in various mammalian tissues such as brain, liver and kidney (3). In the central nervous system, prolyl endopeptidase degrades proline-containing neuropeptides such as vasopressin, substance P and thyrotropin-releasing hormone, which are involved in the processes of learning and memory (4). Thus, several companies have shown interest in the design of prolyl endopeptidase inhibitors. A review on prolyl endopeptidase inhibitors has been recently published in this journal (5).

In order to obtain new prolyl endopeptidase inhibitors with enhanced activity, scientists at Japan Tobacco attempted to make a hydrogen bonding interaction between an inhibitor and the hydrogen on the imidazole ring of the histidine residue. Thus, compounds were designed of general structure [I] having a heteroatom (X) for the hydrogen bonding at the β-position of ketone which reacts with the serine residue (6). From these studies, JTP-4819 was selected for further evaluation.

Pharmacological Actions

JTP-4819 inhibited prolyl endopeptidase with high specificity and in a concentration-dependent manner in the cytosolic fraction of rat brain (IC_{50} = 0.83 nM). The title compound also strongly inhibited enzyme activity (IC_{50} = 0.65 nM) after incubation with rat plasma, indicating stability in plasma (7). The preclinical pharmacology of JTP-4819 has been extensively described (1, 7-15).

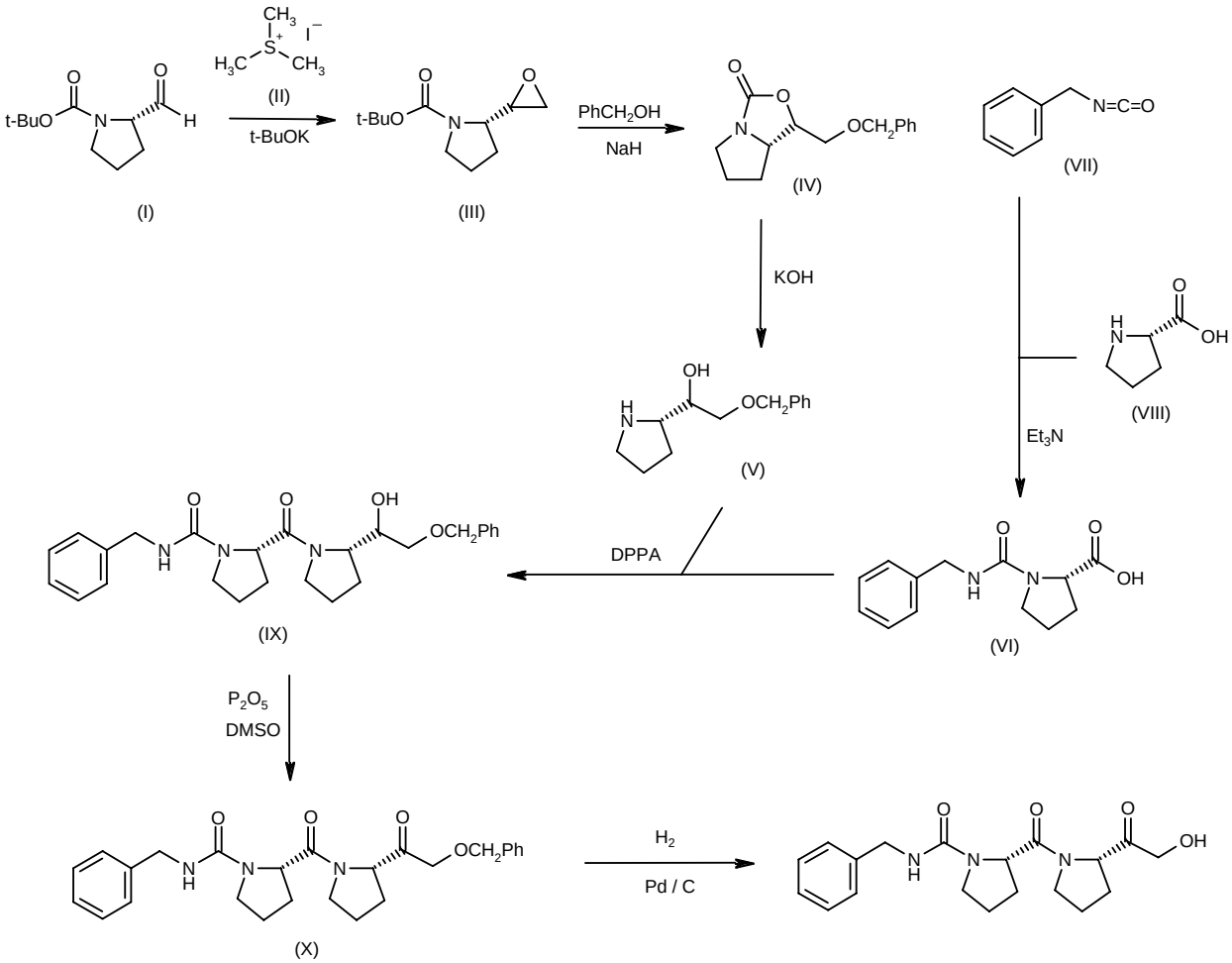
Table I summarizes the prolyl endopeptidase-inhibitory activity of JTP-4819 and other selected compounds from the Prous Science MFLine database.



[I]

T. Wroblewski, J. Silvestre, J. Castañer. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of JTP-4819

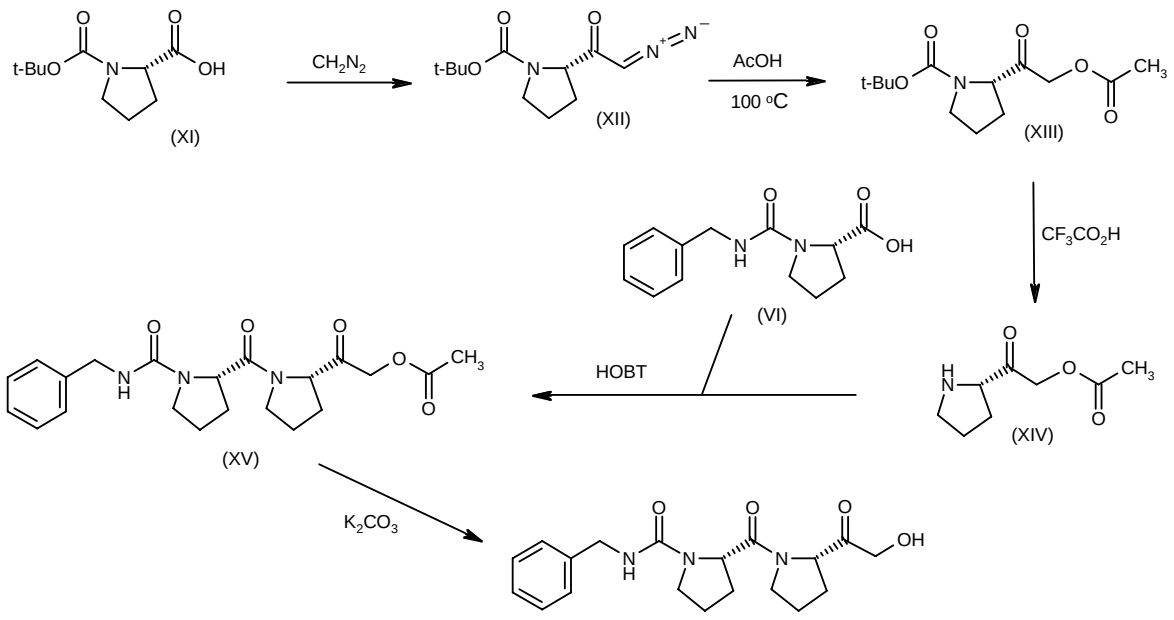


Degradation of substance P, arginine vasopressin and thyrotropin-releasing hormone was inhibited by JTP-4819 with IC_{50} values of 3.4, 2.1 and 1.4 nM, respectively, when evaluated in supernatants from cerebral cortex, while IC_{50} values determined in assays with hippocampal homogenate supernatants were 3.3, 2.8 and 1.9 nM, respectively. When administered orally to rats, 1 and 3 mg/kg of JTP-4819 increased substance P- (SP-LI) and arginine vasopressin-like immunoreactivity (AVP-LI) in the cerebral cortex and hippocampus, and increased thyrotropin-releasing hormone-like immunoreactivity (TRH-LI) in the hippocampus was observed after the 3 mg/kg dose. IC_{50} values from these experiments imply that increased cortical and hippocampal SP-LI and AVP-LI, as well as hippocampal TRH-LI, are mediated by JTP-4819 primarily by inhibiting PEP, and secondarily by blocking the degradation of SP, AVP and TRH (8).

A single oral dose of JTP-4819 (3 mg/kg) restored levels of TRH-LI in the cerebral cortex of aged rats in a dose-dependent manner, while doses of 0.3-3 mg/kg had a similar effect in the hippocampus. Repeated oral doses of 1 mg/kg administered during 21 days significantly increased TRH-LI in the cerebral cortex, while doses of 0.3 and 1 mg/kg produced a similar effect in the hippocampus. These results suggest that memory deficits in aged rats may be due to decreased levels of neuropeptides such as TRH and substance P, and that JTP-4819 may improve the functioning of TRH-responsive neurons by inhibiting prolyl endopeptidase (10).

JTP-4819 inhibited prolyl endopeptidase activity in supernatants of brain homogenates from young and aged rats with IC_{50} values of 0.7 and 0.8 nM, respectively. Enzyme immunoassays showed that SP-LI in the cerebral cortex of aged rats increased after a single oral dose of JTP-4819 (3 mg/kg), while SP-LI in the hippocampus

Scheme 2: Synthesis of JTP-4819



was not affected. Repeated oral dosing of JTP-4819 (1 mg/kg during 21 days) increased SP-LI in the cerebral cortex and restored it in the hippocampus of aged rats. AVP-LI in the hippocampus and cerebral cortex of aged rats showed a tendency to increase after single (1 mg/kg) and repeated (1 mg/kg for 21 days) oral doses of JTP-4819. These results also support the hypothesis that JTP-4819 improves SP-LI in the cerebral cortex and hippocampus of aged rats by inhibiting prolyl endopeptidase (11).

JTP-4819 inhibited the C-terminal but not the N-terminal cleavage by NG108-15 cell lysate (a neuroblastoma-glioma hybrid cell line) of two synthetic substrates corresponding to the C- and N-termini of amyloid-β protein, with an IC_{50} value of 0.6 nM. Western blot analysis showed that the drug also inhibited serum deprivation-induced increase in amyloid-β protein concentrations in the culture medium of NG108-15 cells in a concentration- and time-dependent manner. This inhibition had no effect on concentrations of amyloid precursor protein in the culture medium. Using immunoblotting with specific antisera, the observed increase in amyloid-β protein was shown to correspond to portion 1-40 of amyloid-β protein, with a concomitant decrease in portion 1-42 of the peptide, suggesting that the increase may be due to the conversion of amyloid-β protein 1-42 to 1-40. Taken together, these observations implicate prolyl endopeptidase as an important factor in the production of amyloid-β protein, and suggest that inhibitors of this enzyme, such as JTP-4819, can modulate amyloid-β protein secretion (12).

Evaluated in an eight-arm radial maze, JTP-4819 administration during 34-41 days (3.0 mg/kg p.o.) significantly improved learning impairment in rats with dorsal hippocampal lesions and subjected to 18 acquisition trials. In a second experiment, dorsal hippocampal lesions were produced in rats following achievement of learning, resulting in a marked decrease in performance. While performance recovered with the number of trials, administration of JTP-4819 (3.0 mg/kg p.o.) reduced the number of trials necessary to recover learning criterion. Choline acetyltransferase (ChAT) activity and [^{3}H]-pirenzepine binding analyzed in the residual hippocampus and cerebral cortex following the behavioral experiments were not affected by drug treatment. On the basis of these results and previous findings, it is suggested that JTP-4819 may improve learning and relearning impairment in this model by enhancing neuropeptide function via inhibition of prolyl endopeptidase, although enhancement of central cholinergic function cannot be completely dismissed as a mechanism of action (13).

In rats with learning impairment induced by middle cerebral artery occlusion, JTP-4819 (0.1 and 1 mg/kg p.o.) significantly prolonged step-through latency for the passive avoidance response. In rats receiving the vehicle, this parameter was notably shorter as compared to sham-operated rats. The drug significantly reduced prolonged escape latency in rats with middle cerebral artery

Table I: IC_{50} values of prolyl endopeptidase inhibitors shown to be active in animal models.

Compound	IC_{50} (nM)	Enzyme Source	Ref.
4'-Amino-SUAM 1221	3.1	Mouse brain	18
	6.2	Whole brain	18
	5.0	Mouse kidney	18
Eurystatin A	8.8	*Flavobacterium meningosepticum*	19
	187.2	Rabbit brain	19
Eurystatin B	4.3	*Flavobacterium meningosepticum*	19
	66.2	Rabbit brain	19
JTP-4819*	0.70	Whole rat brain	11
	0.80	Whole rat brain	11
	0.83	Whole rat brain	7
	0.58	Rat cerebral cortex	8
	0.61	Rat hippocampus	8
	4.50	*Flavobacterium meningosepticum*	20
	5.43	*Flavobacterium meningosepticum*	7
Lipohexin	25000	*Flavobacterium meningosepticum*	21
Ono-1603	50.0	Porcine kidney	22
Poststatin	54.9	Porcine kidney	23
S-17092-1*	1.3	Rat cerebral cortex	24
SUAM-1221	4.7	Mouse brain	18
	7.2	Whole rat brain	18
	8.0	Mouse kidney	18
	30.0	Whole rat brain	25
	49.0	Canine brain	26
	94.0	*Flavobacterium meningosepticum*	27
	190.0	Bovine brain	27
Y-29794	3.0	Whole rat brain	28
	3500	Canine brain	26
Z-Pro-prolinal	0.74	Bovine brain	29
	0.66	Bovine brain	30
	1.10	Canine brain	26
ZTTA-1	0.12	Whole rat brain	31
Z-Val-prolinal	2.5	Bovine brain	30
	11.9	*Flavobacterium meningosepticum*	19
	30.0	*Flavobacterium meningosepticum*	32
	47.9	Rabbit brain	19

*Under clinical development. Source: Prous Science MFLine database.

occlusion when evaluated in the Morris water maze task. Ischemic infarct and atrophy size were not affected by repeated drug treatment, suggesting that the observed effects of JTP-4819 on the passive avoidance response and Morris water maze may be due to improvements in memory and learning. In addition, JTP-4819 treatment (0.3-3 mg/kg p.o.) for 15 days restored reduced levels of TRH-LI, but had no effect on cortical and hippocampal SP-LI or AVP-LI. This observation indicates that JTP-4819 improves memory in rats with middle cerebral artery occlusion mainly by restoring and enhancing cortical thyrotropin-releasing hormone, followed by strong and specific prolyl endopeptidase inhibition (14).

Aged rats with impaired spatial memory improved their memory deficits after treatment with JTP-4819 (1 mg/kg p.o.) for 14 days, as indicated by decreased escape latency and path length in the Morris water maze task. Administration of the drug (1 and 3 mg/kg p.o.) for 21 days also normalized age-related alterations of ChAT activity in the cerebral cortex and [^{3}H]-choline uptake in the hippocampus in aged rats, while single doses had no effect on these parameters. These results again suggest that JTP-4819 improves age-related deficits in spatial memory, and that it partly recovers central cholinergic dysfunction by a mechanism involving prolyl endopeptidase-mediated degradation of substance P, arginine vasopressin and thyrotropin-releasing hormone (15).

Clinical Studies

To determine its safety profile and evaluate its pharmacokinetics, JTP-4819 was given to 28 healthy male

volunteers at single doses of 30, 60 and 120 mg, and at multiple doses of 60 mg 3 times daily, and was compared to placebo. In addition, a crossover study was carried out with a single 60-mg dose to determine the effect of food on the bioavailability of the drug. A gradual increase in plasma cholinesterase activity was observed in subjects given the multiple dose, exceeding normal levels on days 4-8 and normalizing following completion of the study. This effect was assumed to be treatment-related, but should be evaluated further. No abnormalities in objective symptoms or laboratory findings were observed. C_{max} values for the 30-, 60- and 120-mg doses were determined to be 474, 887 and 1649 ng/ml, respectively, with a half-life of approximately 2 h. AUC increased in a dose-related manner, while cumulative urinary recovery was estimated to be 66%. None of these parameters was affected by food intake. Significantly increased plasma substance P concentrations were observed in subjects given 30- and 120-mg doses; however, the increase was small and not dose-related. Changes in plasma arginine vasopressin, thyrotropin-releasing hormone and somatostatin were not detected, suggesting that these compounds may be metabolized by prolyl endopeptide isozymes present at sites other than the central nervous system and are not inhibited by JTP-4819. Drug accumulation in plasma following multiple dosing was not observed. The results indicate that JTP-4819 is safe, does not produce adverse effects and demonstrates acceptable pharmacodynamics and pharmacokinetics in this group of subjects (16).

JTP-4819 is undergoing phase II clinical trials in Japan for the treatment of Alzheimer's disease (17).

Manufacturers

Japan Tobacco, Inc. (JP); Yoshitomi Pharmaceutical Industries, Ltd. (JP).

References

1. Toide, K., Shinoda, M., Iwamoto, Y., Fujiwara, T., Abe, H., Uchida, I. *A novel prolyl endopeptidase inhibitor, JTP-4819, for the treatment of Alzheimer's disease: Review of preclinical pharmacology.* CNS Drug Rev 1996, 2: 343-62.

2. Kobayashi, K., Akamatsu, M., Yata, S., Abe, H., Toide, K., Kogayu, M., Uchida, I. (Japan Tobacco, Inc.; Yoshitomi Pharm. Ind., Ltd.). *Cpd. with prolyl endopeptidase inhibitor activity and pharmaceutical use thereof.* EP 670309, JP 95512967, US 5536737, WO 9412474.

3. Yoshimoto, T., Ogita, K., Walter, R., Koida, M., Tsuru, D. *Post proline cleaving enzyme.* Biochem Biophys Acta 1979, 569: 184-92.

4. Kovacs, G.L., Bohus, B., Versteeg, D.H.G., De Kloet, R., De Wied, D. *Effect of oxytocin and vasopressin on memory consolidation: Sites of action and catecholaminergic limbic-midbrain structures.* Brain Res 1975, 175: 303-14.

5. De Nanteuil, G., Portevin, B., Lepagnol, J. *Prolyl endopeptidase inhibitors: A new class of memory enhancing drugs.* Drugs Fut 1998, 23: 167-79.

6. Kobayashi, K., Abe, H., Toide, K., Uchida, I. *Novel prolyl endopeptidase inhibitors: JTP-3399 and JTP-4819. Design, synthesis and pharmacological activity on central nervous system.* 13th Int Symp Med Chem (Sept 19-23, Paris) 1994, Abst O6.

7. Toide, K., Iwamoto, Y., Fujiwara, T., Abe, H. *JTP-4819: A novel prolyl endopeptidase inhibitor with potential as a cognitive enhancer.* J Pharmacol Exp Ther 1995, 274: 1370-8.

8. Toide, K., Fujiwara, T., Iwamoto, Y., Shinoda, M., Okamiya, K., Kato, T. *Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on neuropeptide metabolism in the rat brain.* Naunyn-Schmied Arch Pharmacol 1996, 353: 355-62.

9. Toide, K., Shinoda, M., Iwamoto, Y., Fujiwara, T., Okamiya, K., Uemura, A. *A novel prolyl endopeptidase inhibitor, JTP-4819, with potential for treating Alzheimer's disease.* Behav Brain Res 1997, 83: 147-51.

10. Shinoda, M., Okamiya, K., Toide, K. *Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on thyrotropin-releasing hormone-like immunoreactivity in the cerebral cortex and hippocampus of aged rats.* Jpn J Pharmacol 1995, 69: 273-6.

11. Toide, K., Okamiya, K., Iwamoto, Y., Kato, T. *Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on prolyl endopeptidase activity and substance P- and arginine-vasopressin-like immunoreactivity in the brains of aged rats.* J Neurochem 1995, 65: 234-40.

12. Shinoda, M., Toide, K., Ohsawa, I., Kohsaka, S. *Specific inhibitor for prolyl endopeptidase suppresses the generation of amyloid β protein in NG108-15 cells.* Biochem Biophys Res Commun 1997, 25: 641-5.

13. Miyazaki, A., Toide, K., Sasaki, Y., Ichitani, Y., Iwasaki, T. *Effect of a prolyl endopeptidase inhibitor, JTP-4819, on radial maze performance in hippocampal-lesioned rats*. Pharmacol Biochem Behav 1998, 59: 361-8.

14. Shinoda, M., Matsuo, A., Toide, K. *Pharmacological studies of a novel prolyl endopeptidase inhibitor, JTP-4819, in rats with middle cerebral artery occlusion*. Eur J Pharmacol 1996, 305: 31-8.

15. Toide, K., Shinoda, M., Fujiwara, T., Iwamoto, Y. *Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on spatial memory and central cholinergic neurons in aged rats.* Pharmacol Biochem Behav 1997, 56: 427-34.

16. Umemura, K., Kondo, K., Ikeda, Y., Kobayashi, T., Urata, Y., Nakashima, M. *Pharmacokinetics and safety of JTP-4819, a novel specific orally active prolyl endopeptidase inhibitor, in healthy male volunteers.* Brit J Clin Pharmacol 1997, 43: 613-8.

17. Japan Tobacco, Inc. Company Communication March 16, 1998.

18. Atack, J.R., Suman-Chauhan, N., Dawson, G., Kulagowski, J.J. *In vitro and in vivo inhibition of prolyl endopeptidase.* Eur J Pharmacol 1991, 205: 157-63.

19. Kamei, H., Ueki, T., Obi, Y., Fukagawa, Y., Oki, T. *Protective effects of eurystatins A and B, new prolyl endopeptidase inhibitors, on scopolamine-induce amnesia in rats.* Jpn J Pharmacol 1992, 60: 377-80.

20. Toide, K., Iwamoto, Y., Fujiwara, T., Okamiya, K. *A novel PEP inhibitor, JTP-4819, activates peptidergic-cholinergic neurotransmissions as a cognitive enhancer: Implications and mechanism of action*. In: Proceedings of the 6th International

Conference in Neuroscience, A. Louilot, T. Durkin, U. Spampinato and M. Cadet (Eds.), Universite de Bordeaux I, Talence, 1994, 273-4.

21. Christner, C., Zerlin, M., Gräfe, U., Stephan, H., Küllertz, G., Fischer, G. *Lipohexin, a new inhibitor of prolyl endopeptidase from Moeszia lindteri (HKI-0054) and Paecilomyces sp. (HKI-0055; HKI-0096): II. Inhibitory activities and specificity.* J Antibiot 1997, 50: 384-9.

22. Tsuda, M., Muraoka, Y., Nagai, M., Aoyagi, T., Takeuchi, T. *Poststatin, a new inhibitor of prolyl endopeptidase. VII. N-Cycloalkylamide analogues.* J Antibiot 1996, 49: 909-20.

23. Aoyagi, T., Nagai, M., Ogawa, K., Kojima, F., Okada, M., Ikeda, T., Hamada, M., Takeuchi, T. *Poststatin, a new inhibitor of prolyl endopeptidase, produced by Streptomyces viridochromogenes MH534-30F3.* J Antibiot 1991, 44: 949-55.

24. Portevin, B., Benoist, A., Rémond, G., Hervé, Y., Vincent, M., Lepagnol, J., De Nanteuil, G. *New prolyl endopeptidase inhibitors: In vitro and in vivo activities of azabicyclo-[2.2.2]octane, azabicyclo[2.2.1]heptane, and perhydroindole derivatives.* J Med Chem 1996, 39: 2379-91.

25. Kánai, K., Erdö, S., Susán, E., Fehér, M., Sipos, J., Podányi, B., Szappanos, A., Hermecz, I. *Prolyl endopeptidase inhibitors: N-Acyl derivatives of L-thioproline-pyrrolidine*. Bioorg Med Chem Lett 1997, 7: 1701-4.

26. Tanaka, Y., Niwa, S., Nishioka, H., Yamanaka, T., Torizuka, M., Yoshinaga, K., Kobayashi, N., Ikeda, Y., Arai, H. *New potent prolyl endopeptidase inhibitors: Synthesis and structure-activity relationships of indan and tetralin derivatives and their analogues*. J Med Chem 1994, 37: 2071-8.

27. Saito, M., Hashimoto, M., Kawaguchi, N., Shibata, H., Fukami, H., Tanaka, T., Higuchi, N. *Synthesis and inhibitory activity of acyl-peptidyl-pyrrolidine derivatives toward post-proline cleaving enzyme: A study of subsite specificity.* J Enzym Inhib 1991, 5: 51-75.

28. Nakajima, T., Ono, Y., Kato, A., Maeda, J.-I., Ohe, T. *Y-297994 - A non-peptide prolyl endopeptidase inhibitor that can penetrate into the brain*. Neurosci Lett 1992, 141: 156-60.

29. Tsuru, D., Yoshimoto, T., Koriyama, N., Furukawa, S. *Thiazoline derivatives as potent inhibitors specific for prolyl endopeptidase.* J Biochem 1988, 104: 580-6.

30. Katsube, N., Tateishi, N., Ohuchida, S. *Prolyl oligopeptidase inhibitor: ONO-1603.* 2nd Symp Med Chem Approach Alzheimer's Dis (Aug 28-30, Strasbourg) 1995.

31. Shishido, Y., Furushiro, M., Tanabe, S., Nishiyama, S., Hashimoto, S., Ohno, M., Yamamoto, T., Watanabe, S. *ZTTA, a postproline cleaving enzyme inhibitor, improves cerebral ischemia-induced deficits in a three-panel runway task in rats.* Pharmacol Biochem Behav 1996, 55: 333-8.

32. Nishikata, M., Yokosawa, H., Ishii, S.-I. *Synthesis and structure of prolinal-containing peptides, and their use as specific inhibitors of prolyl endopeptidases.* Chem Pharm Bull 1986, 34: 2931-6.