Synthesis of Prolyl Endopeptidase Inhibitors and Evaluation of Their Structure–Activity Relationships: *In Vitro* Inhibition of Prolyl Endopeptidase from Canine Brain

Heihachiro ARAI,* Hiroyasu NISHIOKA, Seiichi NIWA, Takeshi YAMANAKA, Yoshiaki TANAKA, Koji YOSHINAGA, Naomi KOBAYASHI, Naoyoshi MIURA, and Yugo IKEDA

Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512–1 Oshikiri, Kohnan-machi, Ohsato-gun, Saitama 360–01, Japan. Received December 21, 1992

By chemical modification of a known prolyl endopeptidase (PEP) inhibitor (*N*-[*N*-(4-phenylbutanoyl)-L-prolyl]pyrrolidine; SUAM-1221), several arylalkanoyl derivatives (V-1—27) were synthesized and tested for *in vitro* inhibitory activity towards PEP from canine brain. Among them, 4-(2-thienyl)butanoyl derivatives (V-24—27) showed more potent PEP-inhibitory activity than SUAM-1221. The structure–activity relationships of these compounds are discussed.

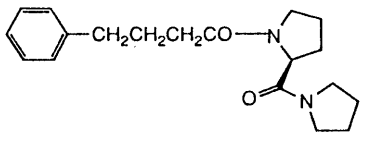
Keywords prolyl endopeptidase; inhibitor; structure–activity relationship; 4-phenylbutanoyl derivative; 4-(2-thienyl)butanoyl derivative

Prolyl endopeptidase (PEP) [EC 3.4.21.26][1] is highly active in the brain and readily degrades proline-containing oligopeptides such as thyrotropin releasing hormone,[2] luteinizing hormone releasing hormone,[3] angiotensin II,[4] bradykinin,[5] substance P,[6] and neurotensin.[7] The enzyme also degrades vasopressin, a cyclic nonapeptide hormone, which controls blood pressure and acts as an antidiuretic hormone. The hormone and its metabolites have been reported to play an important role in learning and memory processes.[8-10]

Recently, many potent PEP inhibitors such as *Z*-Gly–Pro–CH_2Cl,[11] *Z*-Pro–prolinal,[12] phenylbutanoyl-Pro–prolinal,[13] and related compounds[13-17] including a non-peptide PEP inhibitor[18] have been reported.

Yoshimoto *et al.*[14] first reported that compounds capable of inhibiting PEP were effective for preventing experimental amnesia induced by scopolamine in rats and inferred that PEP inhibitors have functions in the fixation of memory. They also found that the anti-amnesic effects of such compounds were approximately parallel to the inhibitory activities towards PEP *in vitro*. It has been reported that aniracetam, which is being developed as a nootropic drug, showed inhibitory activity against PEP.[19] Furthermore, Saito *et al.*[13] reported that PEP plays an important role in the regulation of learning and memory consolidation in brain and inhibitors of the enzyme were suggested to be possible candidates for anti-amnesic drugs. These lines of evidence have prompted us to search for novel PEP inhibitors.

N-[*N*-(4-Phenylbutanoyl)-L-prolyl]pyrrolidine (SUAM-1221, A), in Chart 1, is a potent PEP inhibor.[20] Therefore, we started chemical modification of the compound (A) in an attempt to find more potent analogues. In this paper, we describe the syntheses of the analogues of A and their structure–activity relationships.



SUAM-1221 (A)

Chart 1

Synthesis

The compounds (V-1—27) were prepared as outlined in Chart 2 and their biological activities are listed in Table I.

Compounds (II) were prepared from the corresponding *N*-BOC-prolines (Ia) or *N*-BOC-thioprolines (Ib) by treatment with pivaloyl chloride in the presence of triethylamine followed by condensation with pyrrolidine or thiazolidine. Hydrolysis of the *N*-BOC compounds (II) with hydrochloric acid gave III. Arylalkanoic acids (IV) were condensed with these compounds (III) in the presence of pivaloyl chloride and triethylamine to give the desired compounds (V-1—6, 11—13, 16 and 18—27).

Other compounds (V-7—10, 14, 15 and 17) were synthesized by a different route (method B). 4-Phenylbutanoyl chloride (VI) was treated with the proline derivatives (VII e—g) in the presence of sodium carbonate to afford the *N*-butanoylprolines (VIII e—g), which were condensed with the cyclic amines (IX h—l) by a similar method to that used for the preparation of II from I, giving rise to V-7—10, V-14, V-15 and V-17. The yields and physical and analytical data of V are collected in Table I.

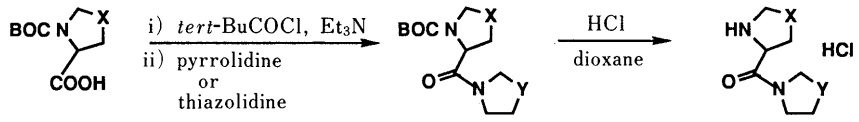
Discussion

It is well known that PEP inhibitors having a formyl,[12,13,16,17,21] chloroacetyl,[11] or diazoacetyl[22] group exert very potent PEP inhibition. Since they are chemically reactive, they inhibit PEP in an irreversible manner and may be unstable.

We tried to synthesize less chemically reactive and more stable compounds since we hoped to find a PEP inhibitor suitable for clinical use. Consequently, we selected SUAM-1221 (A), which is a potent reversible PEP inhibitor, as our lead compound. By chemical modification of the compound, various new compounds (V) were synthesized. The PEP-inhibitory activity of these compounds was assayed using PEP purified from canine brain, and the results are summarized in Table I.

To estimate the effect of the number of methylene groups in the phenylalkanoyl series on the inhibitory activity, we examined five compounds (V-1, 2, 3,[20b] 4,[20] 5) having

method A

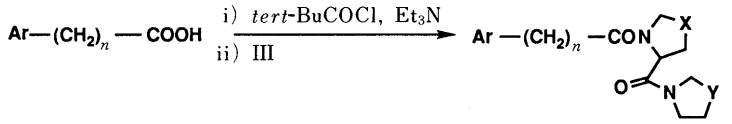


I (L-a, b; D-a, b)
a : X=CH_2
b : X=S

II (L-a–d, D-a–d)

III (L-a–d, D-a–d)

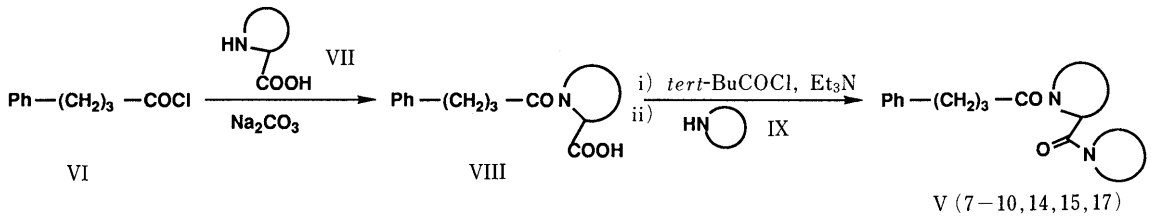
a : X=Y=CH_2 b : X=CH_2, Y=S c : X=S, Y=CH_2
d : X=Y=S



IV

V (1–6, 11–13, 16, 18–27)

method B



VI

VIII

V (7–10, 14, 15, 17)

VIIe : L-proline
f : L-thioproline
g : L-pipecolinic acid

IXh : oxazolidine
i : 3-pyrroline
j : 1,2,3,6-tetrahydropyridine
k : 2-pyrrolidinone
l : thiazolidine

Chart 2

different numbers of methylene groups. Among them, V-4(A) having three methylene groups ($n=3$) showed the strongest inhibitory activity.

On the basis of this result, the 4-phenylbutanoyl group was fixed, and L- or D-proline, L- or D-thioproline, or L-pipecoline was introduced as the amino acid moiety, and pyrrolidine, thiazolidine, oxazolidine, pyrroline, piperidine, 1,2,3,6-tetrahydropyridine, or pyrrolidinone as the amine moiety, ring (B), of the prolyl amide of V, respectively.

The activities of the compounds (V-6 and 8) possessing a sulfur atom and a double bond in the pyrrolidine ring were slightly increased as compared with that of V-4. However, compound V-7 having an oxygen atom and V-9 and V-10 having a 1,2,3,6-tetrahydropyridine or 2-pyrrolidinone moiety in ring B, showed decreased inhibitory activities. In contrast, V-12[20c] and V-13[20b] having a sulfur atom in ring A of V-4 and V-6, showed increased activity compared with V-4 and V-6, respectively. The inhibitory activity of V-14 having an oxygen atom in ring B was decreased, like that of V-7. On the other hand, the inhibitory activities of V-11 and V-16 having D-proline and D-thioproline as the amino acid moiety were drastically diminished, as in the case of *Z*-D-Pro-pyrrolidine or -thiazolidine reported by Yoshimoto *et al.*[20b]

We also examined the effect of a substituent at the *para*-position on the phenyl ring in V-12. The inhibitory activities of the substituted compounds (V-18, V-19 and V-20) were at almost the same level of 10^{-8} M. However, the activities of V-21, V-22 and V-23 were less by one order of magnitude. On the other hand, the inhibitory activities of V-24—27, in which the phenyl groups of V-4, V-6, V-12 and V-13 were replaced by 2-thienyl groups, remained in the 10^{-8} M range, and were superior to that of V-4, the lead compound (A).

In the case of the series of 4-phenylbutanoyl and 4-(2-thienyl)butanoyl derivatives, introduction of a sulfur atom in ring A and/or B resulted in inceased inhibitory activity. These results are in agreement with those of Yoshimoto *et al.*[20b]

In the present study, we have demonstrated that several compounds (V-6, 8 and 17—20) showed inhibitory activity nearly equal to that of compound A, and V-24—27 had even more potent inhibitory activities. We are investigating these compounds to assess their usefulness as nootropics.

Experimental

Melting points were determined on a Yamato melting point apparatus or a Büchi 535 digital melting point apparatus and are uncorrected. IR spectra were measured with a JASCO A102 or a Shimadzu DR-8000 spectrophotometer. ^{1}H-NMR spectra were taken on a JEOL JNM RMX60 or a GSX270 spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured with a JEOL JMS DX300 or a DX302 instrument. Optical rotations were recorded with a JASCO DIP370 digital polarimeter in methanol.

TABLE I. Structures, Physical Properties, and PEP-Inhibitory Activities of the Synthesized Compounds (V)
Ar–$(CH_2)_n$–CO–(A)–CO–(B)

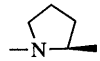
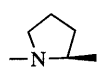
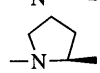
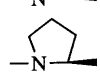
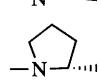
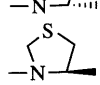
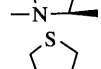
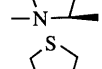
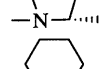
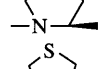
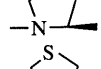
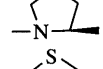
Compd. No.	Ar	n	A	B	Yield (%)	mp (°C) (Recryst. sol.)	$[\alpha]_D$ (in MeOH)	Formula	Analysis Calcd (Found) C	H	N	PEP inhibition IC_{50} (μM)
1	Ph	0			70	Oil	−62.6 (c=0.99)	$C_{16}H_{20}N_2O_2$	272.1524 (272.1517)			1.84
2	Ph	1			68	Oil	−57.1 (c=1.01)	$C_{17}H_{22}N_2O_2$	286.1681 (286.1672)			0.71
3	Ph	2			74	Oil	−53.9 (c=1.00)	$C_{18}H_{24}N_2O_2$	300.1838 (300.1819)			0.53
4	Ph	3			73	Oil	−30.0 (c=1.10)	$C_{19}H_{26}N_2O_2$	314.2020 (314.2007)			0.076
5	Ph	4			62	Oil	−33.0 (c=1.00)	$C_{20}H_{28}N_2O_2$	328.2151 (328.2161)			0.25
6	Ph	3			45	53—55 (IPE–n-hexane)	−23.0 (c=1.00)	$C_{18}H_{24}N_2O_2S$	65.03 (65.24	7.28 7.33	8.43 8.42)	0.041
7	Ph	3			23	Oil	−19.8 (c=1.02)	$C_{18}H_{24}N_2O_3$	316.1777 (316.1769)			0.21
8	Ph	3			56	Oil	−25.0 (c=1.16)	$C_{19}H_{24}N_2O_2$	312.1837 (312.1849)			0.057
9	Ph	3			65	Oil	−44.2 (c=1.13)	$C_{20}H_{26}N_2O_2$	326.1989 (326.1994)			3.36
10	Ph	3			28	80—81 (IPE–n-hexane)	−45.2 (c=1.01)	$C_{19}H_{24}N_2O_3$	69.49 (69.58	7.37 7.50	8.53 8.41)	57.1
11	Ph	3			40	Oil	24.5 (c=1.24)	$C_{18}H_{24}N_2O_2S$	332.1558 (332.1566)			21.5
12	Ph	3			48	100—102 (AcOEt–IPE)	−104.4 (c=1.01)	$C_{18}H_{24}N_2O_2S$	65.03 (64.79	7.28 7.44	8.43 8.35)	0.031
13	Ph	3			46	80—82 (AcOEt–IPE)	−93.0 (c=1.00)	$C_{17}H_{22}N_2O_2S_2$	58.25 (58.48	6.33 6.42	7.99 8.07)	0.021
14	Ph	3			20	71—72.5 (IPE–n-hexane)	−83.0 (c=1.01)	$C_{17}H_{22}N_2O_3S$	61.05 (61.04	6.63 6.65	8.38 8.68)	0.13
15	Ph	3			48	Oil	−99.3 (c=1.01)	$C_{19}H_{26}N_2O_2S$	346.1714 (346.1707)			6.89
16	Ph	3			50	80—81 (AcOEt–IPE)	94.0 (c=1.00)	$C_{17}H_{22}N_2O_2S_2$	58.25 (58.55	6.33 6.14	7.99 8.15)	102.0
17	Ph	3			60	Oil	−16.5 (c=1.06)	$C_{20}H_{28}N_2O_2$	328.2151 (328.2148)			0.061
18	4-Cl–Ph	3			53	107—108 (AcOEt–IPE)	−91.2 (c=1.01)	$C_{18}H_{23}ClN_2O_2S$	58.92 (59.07	6.32 5.93	7.64 7.52)	0.063
19	4-OH–Ph	3			5	109—111 (EtOH–AcOEt)	−97.3 (c=1.00)	$C_{18}H_{24}N_2O_3S$	62.04 (62.22	6.94 6.97	8.04 8.14)	0.063
20	4-Me–Ph	3			73	75—76 (IPE)	−103.0 (c=1.05)	$C_{19}H_{26}N_2O_2S$	65.86 (65.91	7.56 7.68	8.09 7.91)	0.047
21	4-OMe–Ph	3			52	73.5—74.5 (AcOEt–IPE)	−95.7 (c=1.02)	$C_{19}H_{26}N_2O_3S$	62.96 (62.99	7.23 7.12	7.73 7.68)	0.115
22	4-NO_2–Ph	3			58	93.5—94.5 (AcOEt–IPE)	−85.9 (c=1.00)	$C_{18}H_{23}N_3O_4S$	57.28 (57.29	6.14 6.18	11.13 11.10)	0.152
23	4-NH_2–Ph	3			27	183—184 (EtOH–ether)	−85.6 (c=1.01)	$C_{18}H_{25}ClN_3O_2S$	56.31 (56.20	6.83 7.01	10.95 10.72)	0.743
24		3			73	Oil	−32.1 (c=1.01)	$C_{17}H_{24}N_2O_2S$	320.1558 (320.1566)			0.034
25		3			73	Oil	−19.5 (c=1.04)	$C_{16}H_{22}N_2O_2S_2$	338.1123 (338.1122)			0.027

TABLE I. (continued)

Compd. No.	Ar	n	A	B	Yield (%)	mp (°C) (Recryst. sol.)	$[\alpha]_D$ (in MeOH)	Formula	Analysis Calcd (Found) C	H	N	PEP inhibition IC_{50} (μM)
26		3			96	Oil	−99.7 (c=1.00)	$C_{16}H_{22}N_2O_2S_2$	338.1123 (338.1123)			0.029
27		3			73	Oil	−86.4 (c=1.02)	$C_{15}H_{20}N_2O_2S_3$	356.0687 (356.0678)			0.017

Synthesis of II. General Procedure A solution of pivaloyl chloride (20 mmol) in chloroform (10 ml) was added dropwise to a stirred solution of I (20 mmol) and triethylamine (22 mmol) in chloroform (40 ml) over a 15—20 min period at 0—5 °C. The reaction mixture was stirred for an additional 1 h. A solution of pyrrolidine (20 mmol) and triethylamine (22 mmol) in chloroform (20 ml) was then added. Stirring was continued for 3 h at room temperature and then the mixture was successively washed with water, 30% citric acid, saturated aqueous $NaHCO_3$, and water. The chloroform layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The resulting solid residue was recrystallized from an appropriate solvent.

II-L-a: Yield, 88%. mp 84—85 °C (from isopropyl ether (IPE)–AcOEt). $[\alpha]_D^{23}$ −36.4° (c=1.00). IR (KBr): 1700 (C=O), 1649 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.40 (4.5H, s), 1.46 (4.5H, s), 1.77—2.20 (8H, m), 3.35—3.77 (6H, m), 4.35 (0.5H, dd, J=5, 8 Hz), 4.88 (0.5 H, dd, J=3, 7 Hz). *Anal.* Calcd for $C_{14}H_{24}N_2O_3$: C, 62.66; H, 9.01; N, 10.44. Found: C, 62.76; H, 9.28; N, 10.59.

II-L-b: Yield, 81.2%. mp 71—72.5 °C (from IPE). $[\alpha]_D^{23}$ −26.6° (c=1.00). IR (KBr): 1700 (C=O), 1642 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.41 (4.5H, s), 1.46 (4.5H, s), 1.80—2.00 (2H, m), 2.02—2.25 (2H, m), 2.94—3.04 (1H, m), 3.04—3.15 (1H, m), 3.36—3.58 (3H, m), 3.83—3.94 (1H, m), 4.35—4.80 (3H, m). *Anal.* Calcd for $C_{13}H_{22}N_2O_3S$: C, 54.52; H, 7.74; N, 9.78. Found: C, 54.32; H, 7.96; N, 9.78.

II-L-c: Yield, 85.7%. mp 107—108 °C (from IPE–*n*-hexane). $[\alpha]_D^{24}$ −146.2° (c=1.00). IR (KBr): 1700 (C=O), 1645 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.84—2.02 (4H, m), 3.14 (1H, dd, J=6, 11 Hz), 3.29 (1H, dd, J=6, 11 Hz), 3.38—3.80 (4H, m), 4.52 (1H, d, J=9 Hz), 4.60—5.00 (2H, m). *Anal.* Calcd for $C_{13}H_{22}N_2O_3S$: C, 54.52; H, 7.74; N, 9.78. Found: C, 54.64; H, 7.88; N, 9.51.

II-L-d: Yield, 74%. mp 108—110 °C (from AcOEt–IPE). $[\alpha]_D^{23}$ −124.1° (c=1.00). IR(KBr): 1700 (C=O), 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.40 (9H, s), 2.95—3.36 (4H, m), 3.65—4.18 (2H, m), 4.45—5.10 (5H, m). *Anal.* Calcd for $C_{12}H_{20}N_2O_3S_2$: C, 47.35; H, 6.62; N, 9.20. Found: C, 47.48; H, 6.87; N, 9.35.

II-D-a: Yield, 73.2%. mp 88—90 °C (from IPE–*n*-hexane). $[\alpha]_D^{23}$ +37.1° (c=1.00). IR (KBr): 1697 (C=O), 1647 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.40 (4.5H, s), 1.45 (4.5H, s), 1.76—2.23 (8H, m), 3.35—3.78 (6H, m), 4.33—4.37 (0.5H, m), 4.95—4.51 (0.5H, m). *Anal.* Calcd for $C_{14}H_{24}N_2O_3$: C, 62.66; H, 9.01; N, 10.44. Found: C, 62.38; H, 8.82; N, 10.57.

II-D-b: Yield, 79.2%. mp 69—69.5 °C (from IPE–*n*-hexane). $[\alpha]_D^{22}$ +29.7° (c=1.01). IR (KBr): 1693 (C=O), 1647 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.41, 1.46 (total 9H, each s), 1.45—2.00 (2H, br), 2.00—2.25 (2H, m), 2.95—3.16 (2H, m), 3.35—3.73 and 4.01—4.11 (total 3H, each m), 3.80—3.90 (1H, m), 4.32—4.78 (3H, m). *Anal.* Calcd for $C_{13}H_{22}N_2O_3S$: C, 54.52; H, 7.74; N, 9.79. Found: C, 54.81; H, 7.90; N, 9.89.

II-D-c: Yield, 87%. mp 107—109°C (from IPE). $[\alpha]_D^{23}$ +143.3° (c= 1.00). IR (KBr): 1700 (C=O), 1648 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.45 (9H, m), 1.80—2.05 (4H, m), 3.11—3.17 (1H, m), 3.26—3.33 (1H, m), 3.38—3.80 (4H, m), 4.52 (1H, d, J=9 Hz), 4.60 (2H, m). *Anal.* Calcd for $C_{13}H_{22}N_2O_3S$: C, 54.52; H, 7.74; N, 9.78. Found: C, 54.28; H, 7.90; N, 9.59.

II-D-d: Yield, 70.5%. mp 109—111 °C (from AcOEt–IPE). $[\alpha]_D^{22}$ +123.0° (c=1.00). IR (KBr): 1695 (C=O), 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.46 (9H, m), 2.95—3.20 (3H, m), 3.31 (1H, dd, J=7, 11 Hz), 3.65—4.15 (2H, m), 4.43—5.05 (5H, br m). *Anal.* Calcd for $C_{12}H_{20}N_2O_3S_2$: C, 47.34; H, 6.62; N, 9.20. Found: C, 47.54; H, 6.51; N, 8.99.

Synthesis of III. General Procedure A compound II (5 g) was dissolved in a mixture of 4 N HCl–dioxane (30 ml). The solution was stirred for 2 h at 60°C and then evaporated to dryness *in vacuo*. The resulting residue was recrystallized from an appropriate solvent to give III, except for III-L-b (see below).

III-L-a: Yield, 85.7%. mp 85—88 °C (from $CHCl_3$–IPE). $[\alpha]_D^{23}$ −74.1° (c=1.00). IR (KBr): 1646 (C=O) cm^{-1}. *Anal.* Calcd for $C_9H_{17}ClN_2O$: C, 52.80; H, 8.37; N, 13.69. Found: C, 52.91; H, 8.12; N, 13.69.

III-L-b: The residue was dissolved in 10% Na_2CO_3 and extracted with chloroform. The extract was dried with $MgSO_4$ and evaporated *in vacuo*. The resulting residue was crystallized by addition of a solution of oxalic acid in ethanol. The precipitated solid was collected and recrystallized from ethanol. Yield, 65%. mp 141—143 °C. $[\alpha]_D^{23}$ −85.6° (c=1.00). IR (KBr): 1648 (C=O), 1720 (C=O) cm^{-1}. *Anal.* Calcd for $C_{10}H_{16}N_2O_5$: C, 43.47; H, 5.84; N, 10.14. Found: C, 43.55; H, 5.79; N, 10.04.

III-L-c: Yield, 97%. mp 170—172 °C (from EtOH–Et_2O). $[\alpha]_D^{24}$ −166.0° (c=1.00). IR (KBr): 1650 (C=O) cm^{-1}. *Anal.* Calcd for $C_8H_{15}ClN_2OS$: C, 43.14; H, 6.79; N, 12.58. Found: C, 43.41; H, 6.98; N, 12.58.

III-L-d: Yield, 93%. mp 194 °C (dec.) (from MeOH–IPE). $[\alpha]_D^{23}$ −166.5° (c=1.00). IR (KBr): 1650 (C=O) cm^{-1}. *Anal.* Calcd for $C_7H_{13}ClN_2OS_2$: C, 34.92; H, 5.44; N, 11.64. Found: C, 35.04; H, 5.20; N, 11.66.

III-D-a: Yield, 92%. mp 88—90 °C (from $CHCl_3$–IPE). $[\alpha]_D^{23}$ +73° (c=1.00). IR (KBr): 1645 (C=O) cm^{-1}. *Anal.* Calcd for $C_9H_{17}ClN_2O$: C, 52.80; H, 8.37; N, 13.69. Found: C, 52.60; H, 8.05; N, 13.97.

III-D-b: yield, 80%. mp 59—62 °C (from $CHCl_3$–ether). $[\alpha]_D^{23}$ +69.9° (c=1.12). IR (KBr): 1648 (C=O) cm^{-1}. *Anal.* Calcd for $C_8H_{15}ClN_2OS$: C, 46.47; H, 7.31; N, 13.55. Found: C, 46.25; H, 7.09; N, 13.78.

III-D-c: Yield, 82%. mp 168—170 °C (from EtOH–Et_2O). $[\alpha]_D^{24}$ +161.5° (c=1.01). IR (KBr): 1648 (C=O) cm^{-1}. *Anal.* Calcd for $C_8H_{15}ClN_2OS$: C, 43.14; H, 6.79; N, 12.58. Found: C, 43.48; H, 6.51; N, 12.58.

III-D-d: yield, 82% mp 180.5—182 °C (dec.) (from methanol). $[\alpha]_D^{23}$ +165° (c=1.06). IR (KBr): 1651 (C=O) cm^{-1}. *Anal.* Calcd for $C_7H_{13}ClN_2OS_2$: C, 34.92; H, 5.44; N, 11.64. Found: C, 34.77; H, 5.40; N, 11.49.

Synthesis of V (1—6, 11—13, 16, 18—27). General Procedure (Method A) Pivaloyl chloride (20 mmol) was added dropwise to a stirred solution of an arylalkanoic acid (IV) (20 mmol) and thietylamine (22 mmol) in chloroform (40 ml) at 0—5 °C. Stirring was continued for 1 h, then a suspension of III (20 mmol) and triethylamine (22 mmol) in chloroform (30 ml) was added to the solution. The reaction mixture was stirred for 3 h at room temperature and successively washed with 10% HCl, water, saturated aqueous $NaHCO_3$, and brine. The chloroform layer was drid over $MgSO_4$ and evaporated *in vacuo*. The resulting residue was chromatographed over silica gel using $CHCl_3$–MeOH (20 : 1) as an eluent to give V. Physical and analytical data of V are listed in Table I.

V-1: IR (KBr): 1628 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.50—2.60 (8H, m), 3.24—4.02 (6H, m), 4.28 (0.2H, dd, J=4, 8 Hz), 4.85 (0.8H, dd, J=6, 8 Hz), 7.35—7.44 (3H, m), 7.57—7.61 (2H, m).

V-2: IR (neat): 1638 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.78—2.34 (8H, m), 3.28—3.87 (6H, m), 3.70 (2H, s), 4.37 (0.3H, dd, J=3, 8 Hz), 4.67 (0.7H, dd, J=4, 8 Hz).

V-3: IR (neat): 1638 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.78—2.39 (8H, m), 2.52—2.73 (2H, m), 2.94—3.00 (2H, m), 3.30—3.50 (3H, m), 3.55—3.70 (2H, m), 3.82—3.90 (1H, m), 4.22—4.26 (0.1H, m), 4.65 (0.9H, dd, J=4, 7 Hz), 7.16—7.32 (5H, m).

V-5: IR (neat): 1644 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.63—1.72 (4H, m), 1.74—2.15 (8H, m), 2.20—2.41 (2H, m), 2.60—2.65 (2H, m), 2.37 (1H, dt, J=7, 12 Hz), 3.14 (1H, dt, J=7, 10 Hz), 3.47 (1H, dt, J=7, 10 Hz), 3.57 (1H, dt, J=7, 12 Hz). 3.68 (1H. ddd, J=5, 8, 10 Hz), 3.83 (1H, dt, J=7, 12 Hz), 4.63 (0.9H, dd, J=4, 8 Hz), 4.42 (0.1H, dd, J=4, 8 Hz). 7.10—7.16 (5H, m).

V-6: IR (neat): 1635 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.44—2.40 (8H, m), 2.65 (2H, t, J=6 Hz), 2.85—4.83 (7H, m), 4.53 (2H, m), 7.16 (5H, br s).

V-11: IR (KBr): 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.53—2.40 (8H, m), 2.66 (2H, t, J=6 Hz), 2.86—4.83 (7H, m), 4.56 (2H, br s), 7.16 (5H,

br s).

V-12: IR (KBr): 1635 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.53—2.37 (6H, m), 2.23 (2H, t, J=6 Hz), 2.67 (2H, t, J=6 Hz), 3.01—3.98 (6H, m), 4.55 (2H, br s), 5.02 (1H, t, J=7 Hz), 7.14 (5H, br s).

V-13: IR (KBr): 1650 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.64—2.60 (4H, m), 2.64 (2H, t, J=6 Hz), 2.79—4.26 (6H, m), 4.52 (4H, br s), 5.03 (1H, t, J=7 Hz), 7.15 (5H, br s).

V-16: IR (KBr): 1650 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 2.00 (2H, quint, J=7 Hz), 2.37 (2H, t, J=7 Hz), 3.68 (2H, t, J=7 Hz), 2.98—3.32 (4H, m), 3.66—3.95 (1H, m), 4.15—4.25 (1H, m), 4.47—4.65 (4H, m), 5.05—5.13 (1H, m), 7.16—7.31 (4H, m).

V-18: IR (KBr): 1641 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.83—2.10 (6H, m), 2.37 (2H, t, J=7 Hz), 2.64 (2H, t, J=7 Hz), 3.15 (1H, dd, J=7, 11 Hz), 3.28 (1H, dd, J=7, 11 Hz), 3.14 (1H, dt, J=7, 12 Hz), 3.43 (1H, dt, J=7, 10 Hz), 3.56 (1H, dt, J=7, 12 Hz), 3.84 (1H, dt, J=7, 10 Hz), 4.57 (1H, d, J=9 Hz), 4.65 (1H, d, J=9 Hz), 5.05 (1H, t, J=7 Hz), 7.13 (2H, dt, J=2, 8 Hz), 7.25 (2H, dt, J=2, 8 Hz).

V-19: IR (KBr): 1638 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.80—2.10 (6H, m), 2.34 (2H, t, J=7 Hz), 2.48—2.59 (2H, m), 3.15 (1H, dd, J=7, 12 Hz), 3.28 (1H, dd, J=7, 12 Hz), 3.39—3.48 (2H, m), 3.54—3.63 (1H, m), 3.83—3.91 (1H, m), 4.56 (1H, d, J=9 Hz), 4.66 (1H, d, J=9 Hz), 5.01 (1H, d, J=9 Hz), 5.10 (1H, t, J=9 Hz), 6.72 (2H, d, J=8 Hz), 6.80 (1H, br), 6.96 (2H, d, J=8 Hz).

V-20: IR (KBr): 1649 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.80—2.07 (6H, m), 2.31 (3H, s), 2.36 (2H, t, J=7 Hz), 2.63 (2H, t, J=7 Hz), 3.16 (2H, dd, J=7, 11 Hz), 3.27 (2H, dd, J=7, 11 Hz), 3.36 (2H, m), 3.52—3.61 (1H, m), 3.81—3.90 (1H, m), 4.53 (1H, d, J=8 Hz), 4.64 (1H, d, J=8 Hz), 5.06 (1H, t, J=7 Hz), 7.08 (4H, s).

V-21: IR (KBr): 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.83—2.04 (6H, m), 2.35 (2H, t, J=7 Hz), 2.61 (2H, t, J=7 Hz), 3.14 (1H, dd, J=7, 11 Hz), 3.27 (1H, dd, J=7, 11 Hz), 3.36—3.46 (2H, m), 3.51 (1H, m), 3.78—3.89 (1H, m), 3.78 (3H, s), 4.59 (2H, dd, J=9, 22 Hz), 5.05 (1H, t, J=7 Hz), 6.82 (2H, d, J=9 Hz), 7.10 (2H, d, J=9 Hz).

V-22: IR (KBr): 1650 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.82—2.11 (6H, m), 2.42 (2H, t, J=7 Hz), 2.79 (2H, t, J=7 Hz), 3.11—3.62 (5H, m), 3.79—3.88 (1H, m), 4.63 (2H, dd, J=9, 20 Hz), 5.05 (1H, t, J=7 Hz), 7.37 (2H, d, J=9 Hz), 8.14 (2H, d, J=9 Hz).

V-23 (as the HCl Salt): IR (KBr): 1640 (C=O), 1620 (C=O) cm^{-1}. NMR (DMSO-d_6) δ: 1.74—1.93 (6H, m), 2.31—2.64 (4H, m), 2.93—3.62 (6H, m), 4.31—4.97 (3H, m), 7.24—7.31 (4H, m), 10.2—10.6 (3H, br).

V-24: IR (neat): 1643 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.80—2.13 (9H, m), 2.15—2.45 (3H, m), 2.90 (2H, t, J=7 Hz), 3.28—3.48 (3H, m), 3.52—3.68 (2H, m), 3.79—3.87 (1H, m), 4.64 (1H, dd, J=4, 8 Hz), 6.81 (1H, dd, J=1, 3 Hz), 6.90 (1H, dd, J=3, 5 Hz), 7.10 (1H, dd, J=1, 5 Hz).

V-25: IR (neat): 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.49—2.58 (8H, m), 2.73—3.28 (2H, m), 2.92 (2H, t, J=7 Hz), 3.36—4.04 (4H, m), 4.42—4.91 (1H, m), 4.60 (2H, s), 6.78—7.24 (3H, m).

V-26: IR (neat): 1649 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.83—2.09 (6H, m), 2.42 (2H, t, J=7 Hz), 2.90 (2H, t, J=7 Hz), 3.14 (1H, dd, J=6, 11 Hz), 3.36—3.46 (2H, m), 3.57 (1H, dt, J=7, 12 Hz), 3.85 (1H, dt, J=6, 10 Hz), 4.58 (1H, d, J=9 Hz), 4.65 (1H, d, J=9 Hz), 5.05 (1H, t, J=7 Hz), 6.81 (1H, d, J=3 Hz), 6.91 (1H, dd, J=3, 5 Hz), 7.11 (1H, d, J=5 Hz).

V-27: IR (neat): 1647 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 2.04 (2H, dt, J=6, 6 Hz), 2.42 (2H, t, J=6 Hz), 2.90 (2H, t, J=6 Hz), 2.95—3.32 (4H, m), 3.65—4.00, 4.15—4.24 (total 2H, each m), 4.50—4.68 (4H, m), 4.85—4.90, 5.14—5.30 (total 1H, each m), 6.81 (1H, dd, J=1, 3 Hz), 6.91 (1H, dd, J=3, 5 Hz), 7.12 (1H, dd, J=1, 5 Hz).

Synthesis of VIII e—g. General Procedure (Method B) γ-Phenylbutanoyl chloride (20 mmol) was added dropwise to a stirred solution of VII (20 mmol) in 10% Na_2CO_3 (40 ml) under ice cooling. Strirring was continued for 1 h, then the mixture was acidified with 10% HCl. The resulting precipitate was collected and recrystallized from AcOEt–IPE.

VIII e: Yield, 77%. mp 100.5—101 °C. $[\alpha]_D^{24}$ −88.4° (c=1.01). IR (KBr): 1730, 1605 cm^{-1}. NMR ($CDCl_3$) δ: 1.92—2.36 (8H, m), 2.68 (2H, t, J=7 Hz), 3.31—3.63 (2H, m), 4.53—4.56 (1H, m), 7.15—7.30 (5H, m), 9.40—9.70 (1H, br). *Anal.* Calcd for $C_{15}H_{19}NO_3$: C, 60.19; H, 6.13; N, 5.01. Found: C, 60.33; H, 5.97; N, 5.16.

VIII f: Yield, 73%. mp 85—85.5 °C. $[\alpha]_D^{23}$ −55.7° (c=1.00). IR (KBr): 1730, 1605 cm^{-1}. NMR ($CDCl_3$) δ: 1.95—2.06 (2H, m), 2.25—2.40 (2H, m), 2.60—2.70 (2H, m), 3.19—3.37 (2H, m), 4.41—4.78 (2H, m), 4.66 (0.5H, dd, J=4, 7 Hz), 5.08 (0.5H, dd, J=4, 7 Hz), 7.16—7.30 (5H, m), 9.41 (1H, s). *Anal.* Calcd for $C_{14}H_{17}NO_3S$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.02; H, 7.25; N, 5.56.

VIII g: Yield, 76%. mp 131—133 °C. $[\alpha]_D^{23}$ −44.5° (c=1.02). NMR ($CDCl_3$) δ: 1.33—1.50 (2H, m), 1.53—1.80 (3H, m), 1.90—2.06 (2H, m), 2.29 (1H, br d, J=12 Hz), 2.39 (2H, t, J=7.5 Hz), 2.67 (2H, t, J=7, 5 Hz), 3.23—3.26 (1H, m), 3.65 (1H, br d, J=12 Hz), 5.40 (1H, d, J=5 Hz), 7.14—7.32 (5H, m), 9.59 (1H, br). *Anal.* Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.07. Found: C, 69.54; H, 7.41; N, 4.87.

Synthesis of V (7—10, 14, 15, 17). Geneal Procedure Pivaloyl chloride (20 mmol) was added dropwise to a stirred solution of VIII (20 mmol) and triethylamine (22 mmol) in chloroform (40 ml) over a 10 min period under ice-cooling. Stirring was continued for 1 h, then a solution of IX (20 mmol) and triethylamine (20 mmol) in chloroform (10 ml) was added dropwise. The mixture was stirred for 3 h at room temperature and successively washed with 10% HCl, H_2O, saturated aqueous Na_2CO_3, and brine. The $CHCl_3$ layer was dried and evaporated *in vacuo*. The residue was chromatographed over silica gel using $CHCl_3$–MeOH (20 : 1) as the eluent to give V. The physical data of V are listed in Table I.

V-7: IR (KBr): 1645 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.94—2.04 (2H, m), 2.36 (2H, t, J=7 Hz), 2.67 (2H, t, J=7 Hz), 3.15—3.31 (2H, m), 3.48—3.56 and 3.68—3.72 (total 2H, each m), 3.99—4.17 (2H, m), 4.50—5.27 (5H, m), 7.15—7.30 (5H, m).

V-8: IR (neat): 1620 (C=O) cm^{-1}. IR ($CDCl_3$) δ: 1.70—2.50 (8H, m), 2.50—2.80 (2H, m), 3.33—3.76 (2H, m), 4.05—4.40 (3H, m), 4.55—4.75 (2H, m), 5.70—5.95 (2H, m), 7.10—7.40 (5H, m).

V-9: IR (neat): 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.43—2.80 (10H, m), 3.03—4.10 (8H, m), 4.50—4.93 (1H, m), 5.30—5.93 (2H, m), 7.00 (5H, s).

V-10: IR (KBr): 1735 (C=O), 1690 (C=O), 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.57—2.87 (14H, m), 3.28–3.99 (4H, m), 5.28—5.62 (1H, m), 7.14 (5H, m).

V-14: IR (neat): 1640 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.89—2.40 (8H, m), 2.64 (2H, t, J=7 Hz), 3.38—3.75 (4H, m), 3.99—4.16 (2H, m), 4.27—4.31, 4.53—4.57 (total 1H, each m), 4.92—5.01, 5.28—5.29 (total 2H, each m), 7.14—7.29 (5H, m).

V-15: IR (neat): 1645 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.42—1.81 (6H, m), 2.00 (2H, quint, J=7 Hz), 2.39 (2H, t, J=7 Hz), 2.68 (2H, t, J=7 Hz), 3.06 (1H, dd, J=5, 11 Hz), 3.27 (1H, dd, J=8, 11 Hz), 3.42—3.66 (4H, m) 4.58 (1H, d, J=19 Hz), 4.62 (1H, d, J=19 Hz), 5.39 (1H, dd, J=5, 8 Hz), 7.14—7.32 (5H, m).

V-17: IR (neat): 1630 (C=O) cm^{-1}. NMR ($CDCl_3$) δ: 1.59—2.02 (12H, m), 2.35 (2H, t, J=7 Hz), 2.66 (2H, t, J=7 Hz), 3.29—3.74 (6H, m), 5.30—5.31 (1H, m), 7.14—7.29 (5H, m).

***In Vitro* Experiments. 1) Enzyme** PEP was purified from canine brains by the method of Tanaka *et al.*[23] The purified enzyme was homogeneous as judged by polyacrylamide gel electrophoresis in the presence or absence of sodium dodecyl sulfate. The enzyme activity was measured using *Z*–Gly–Pro–*p*-nitroanilide (*Z*–Gly–Pro–*p*NA) as a substrate according to the method of Yoshimoto *et al.*[24] One unit of enzyme activity was defined as the amount of the enzyme which released 1 μmol of *p*-nitroaniline per min at 37°C. The specific activity of the enzyme was over 62 units/mg protein.

2) Measurement of PEP-Inhibitory Potency A test compound was dissolved in dimethylsulfoxide (DMSO) at various concentrations. The solution (10 μl) was incubated for 10 min at 37 °C with purified PEP (2.7 3.6×10^{-3} units) in 990 μl of 0.1 M Tris–HCl buffer (pH 7.6) containing 1 mM EDTA, 1 mM 2-mercaptoethanol and 0.05 mg/ml gelatin. To the mixture of the compound and the enzyme, 0.1 ml of 2.5 mM *Z*–Gly–Pro–*p*NA in 40% dioxane was added and the mixture was incubated for 10 min at 37 °C. The enzyme reaction was stopped by adding 0.1 ml of 5% Triton X-100 in 50% acetic acid and then the absorbance at 410 nm was measured. The potency of inhibitory activity was represented by the IC_{50} value, which was defined as the concentration of the test compound that resulted in 50% inhibition of the enzyme with respect to the DMSO control.

Acknowledgements We are grateful to Dr. T. Iwakuma, General Manager of the Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., for his encouragement and helpful suggestions.

References

1) R. Walter, H. Shlank, J. D. Glass, I. L. Schwartz, T. D. Kerenyi, *Science*, **173**, 827 (1971).
2) P. C. Orlowski, E. Wilk, S. Pearce, S. Wilk, *J. Neurochem.*, **33**, 461 (1979).
3) H. Knisatschek, K. Bauer, *Biol. Chem.*, **254**, 10936 (1979).
4) L. B. Hersh, J. F. Mckelvy, *Brain Res.*, **168**, 553 (1979).
5) J. H. Rupnow, W. L. Taylor, J. E. Dixon, *Biochemistry*, **18**, 1206 (1979).
6) W. L. Taylor, J. E. Dixon, *Biochem. Biophys. Res. Commun.*, **94**, 9

(1980).
7) M. S. Kreiclor, A. Winokur, N. R. Krieger, *Neuropeptide*, **1**, 455 (1981).
8) D. de Wied, B. Bohus, *Nature* (London), **22**, 1484 (1966).
9) S. S. Wang, *Biochem. Biophys. Res. Commun.*, **48**, 1511 (1971).
10) G. L. Kovacs, B. Bohus, D. H. G. Versteeg, E. R. De Kloet, D. De Wied, *Brain Res.*, **175**, 303 (1979).
11) T. Yoshimoto, R. C. Orlowski, R. Walter, *Biochemistry*, **16**, 2942 (1977).
12) *a*) S. Wilk, M. Orlowski, *J. Neurochem.*, **41**, 69 (1983); *b*) T. C. Friedman, M. Orlowski, S. Wilk, *ibid.*, **42**, 237 (1984).
13) M. Saito, M. Hashimoto, N. Kawaguchi, N. Fukami, T. Tanaka, N. Higuchi, *J. Enzyme Inhibition*, **3**, 163 (1990).
14) T. Yoshimoto, K. Kado, F. Matsubara, N. Koriyama, H. Kaneto, D. Tsuru, *J. Pharmacobio-Dyn.*, **10**, 730 (1987).
15) H. Yokosawa, M. Nishikata, S. Wilk, *J. Biochem.*, **95**, 1819 (1984).
16) M. Nishikata, H. Yokosawa, S. Ishii, *Chem. Pharm. Bull.*, **34**, 2931 (1986).
17) T. Yoshimoto, K. Kawahara, F. Matsubara, K. Kado, D. Tsuru, *J. Biochem.*, **98**, 975 (1985).
18) T. Nakajima, Y. Ono, A. Kato, J. Maeda, T. Ohe, *Neuroscience Lett.*, **141**, 156 (1992).
19) *a*) A. Kubota, T. Nakajima, T. Okada, T. Hayashi, K. Nakamura, *Jpn. J. Pharmacol.*, **33**, Suppl. 67p (1983); *b*) T. Nakajima, S. Kubota, K. Nakamura, *Nippon Yakurigaku Zasshi*, **82**, 154p (1983).
20) *a*) N. Higuchi, M. Saito, M. Hashimoto, H. Hukami, T. Tanaka, Japan. Patent 201877 (1987) [*Chem. Abstr.*, **108**, 75852*m* (1988)]; *b*) T. Yoshimoto, D. Tsuru, N. Yamamoto, R. Ikesawa, S. Furukawa, *Agric. Biol. Chem.*, **55**, 37 (1991); *c*) M. Saito, M. Hashimoto, N. Kawaguchi, H. Shibata, H. Fukami, T. Tanaka, N. Higuchi, *J. Enzyme Inhibition*, **5**, 51 (1991).
21) D. Tsuru, T. Yoshimoto, N. Koriyama, S. Furukawa, *J. Biochem.*, **104**, 580 (1988).
22) H. Knisatschek, K. Bauer, *Biochem. Biophys. Res. Commun.*, **134**, 888 (1986).
23) Y. Tanaka, N. Kobayashi, K. Yoshinaga, N. Miura, Y. Ikeda, *Clin. Neuropharmacol.*, **15**, Suppl. 1, Pt. B, 290p (1992).
24) T. Yoshimoto, M. Fischl, R. C. Orlowski, R. Walter, *J. Biol. Chem.*, **253**, 3708 (1978).