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

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By chemical modification of a known prolyl endopeptidase (PEP) inhibitor (N-[N-(4-phenylbutanoyl)-1-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] is highly active in the brain and readily degrades proline-containing oligopeptides such as thyrotropin releasing hormone, luteinizing hormone releasing hormone, angiotensin II, bradykinin, substance P, and neurotensin. 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.

Recently, many potent PEP inhibitors such as Z-Gly-Pro-CH₂Cl, Z-Pro—prolinol, phenylbutanoyl-Pro—prolinol, and related compounds including a non-peptide PEP inhibitor have been reported.

Yoshimoto et al. 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. Furthermore, Saito et al. 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)-1-prolyl]pyrrolidine (SUAM-1221, A), in Chart 1, is a potent PEP inhibitor. 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.

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-butanolprolines (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 formal, chloroacetyl, or diazoacetyl 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 phenylalkanooyl series on the inhibitory activity, we examined five compounds (V-1, 2, 3, 4, 5) having

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Chart 1
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 \(L\)-piperidine was introduced as the amino acid moiety, and pyrrolidine, thiazolidine, oxazolidine, pyrroline, pyrrolidine, 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 and V-13 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 \(Z\)-Pro-pyrrolidinone or \(-\)thiazolidine reported by Yoshimoto et al.208)

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 increased inhibitory activity. These results are in agreement with those of Yoshimoto et al.208)

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.
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<th>Compd. No.</th>
<th>Ar</th>
<th>n</th>
<th>A</th>
<th>B</th>
<th>Yield (%)</th>
<th>mp (°C) (Reccryst. sol.)</th>
<th>$[\alpha]_D$ (in MeOH)</th>
<th>Formula</th>
<th>Analysis Calcd (Found)</th>
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<td>N</td>
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<td>N</td>
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<td>N</td>
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<td>C_{18}H_{32}N_{2}O_{4}S_{2}</td>
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<td>N</td>
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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 pyridinium (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 NaHCO₃ and water. The chloroform layer was dried over anhydrous MgSO₄ and evaporated in vacuo. The resulting solid resin was recrystallized from an appropriate solvent.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Ar</th>
<th>n</th>
<th>A</th>
<th>B</th>
<th>Yield (%)</th>
<th>mp (°C) (Recryst. sol.)</th>
<th>[α]₀</th>
<th>(MeOH)</th>
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<td>3</td>
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<td>H₂C₆H₄N₂O₄S₂</td>
<td>C₂₆H₂₂N₂O₄S₂</td>
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Table I. (continued)
Synthesis of V—10, 14, 15, 17. General Procedure: Pivaloyl chloride (20 mmol) was added dropwise to a stirred solution of VII (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$_2$O, saturated aqueous Na$_2$CO$_3$, and brine. The CHCl$_3$ layer was dried and evaporated in vacuo. The residue was chromatographed over silica gel using CHCl$_3$-MeOH (2:1) as the eluent to give V. The physical data of V are listed in Table 1.

V: IR (neat): 1620 (C=O) cm$^{-1}$. IR (CDCl$_3$) $\delta$: 1.70—2.50 (8H, m), 2.50—2.80 (20H, 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 (3H, 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).

VIII: IR (neat): 1620 (C=O) cm$^{-1}$. IR (CDCl$_3$) $\delta$: 1.70—2.50 (8H, m), 2.50—2.80 (20H, 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 (3H, 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).

In Vivo 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-nitroaniline (6 x Z-Gly-Pro-pNA) as 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 x 10$^{-5}$ 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–pNA substrate was added. 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.

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References: