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Prolyl endopeptidase inhibitors

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Abstract

Selective prolyl endopeptidase inhibitors were elaborated by modification of the structure of SUAM-1221, by using a CoMFA study and protein crystallography. The most active representatives of ω-(*N*-hetaryl)alkanoylprolylpyrrolidines, containing 2- or 3-methylene chain links have high activity (IC_{50} $10^{-9} \sim 10^{-11}$) and exhibit significant in vivo activities. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Prolyl endopeptidase inhibitors; ω-(*N*-Hetaryl)alkanoylprolylpyrrolidines; Synthesis; CoMFA; Protein crystallography

1. Introduction

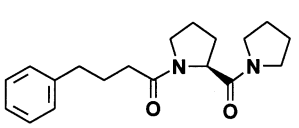
The prolyl endopeptidase (PEP, EC 3.4.21.26) is a member of a recently described serine oligopeptidase family. PEP, also known as prolyl oligopeptidase, first was described in 1971. Since that time it has been proved that this enzyme is widely distributed in different organs (including the brain) of mammals, and it is found both in cytosolic and membrane-bound forms. There are several lines of evidence suggesting that PEP plays a pivotal role in the breakdown of proline-containing neuropeptides due to the specificity for cleaving at the carboxyl side of the proline residue. Among them thyreotrop releasing hormone (TRH), neurotensine, Substance P and vasopressin are related to learning and memory functions.

2. Results and discussion

Based on the above observations, a novel potential mechanism arose for preventing and/or treating amnesia by disclosing the peptidergic neuronal imbalance, and potent and selective prolyl endopeptidase inhibitors are expected to have therapeutic value for treating progressive memory deficits and cognitive dysfunction related to aging and neurodegenerative diseases of the central nervous system.

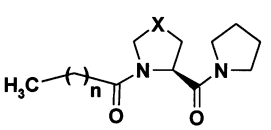
We initiated a project to find potent prolyl endopeptidase inhibitors with good pharmacokinetic properties. As a lead compound SUAM-1221 (**1**) was selected [1], and the replacement of the phenyl group was carried out with different *N*-alkanoyl moieties to increase the hydrophobic interactions with the enzyme. A series of *N*-acyl derivatives of prolylpyrrolidine (**2**) and thioprolylpyrrolidine (**3**) were prepared conventionally and evaluated in vitro against rat brain PEP.

A link between the activity and length of the straight alkyl chain has been established. The most active derivatives contain six methylene groups, and they have similar activities than SUAM-1221, indicating that there is an optimal lipophilicity of the side chain.



(**1**) IC_{50} = 40 nM

SUAM-1221



(**2**) X=CH_2; n=6; IC_{50} = 48 nM

(**3**) X= S ; n=6; IC_{50} = 11 nM

In the next step, a substituted amino group, mainly as a part of heterocyclic ring system, was introduced into the ω-position of the alkyl chain, resulting in derivatives with similar or higher activities. By the application of either synthetic route 1 or a synthetic route 2 (Scheme 1) a series of ω-(*N*-heterocyclic)-alkanoyl-prolylpyrrolidines (**4**) were prepared.

All synthesized molecules were tested against rat brain homogenate PEP enzyme. The enzyme reactions

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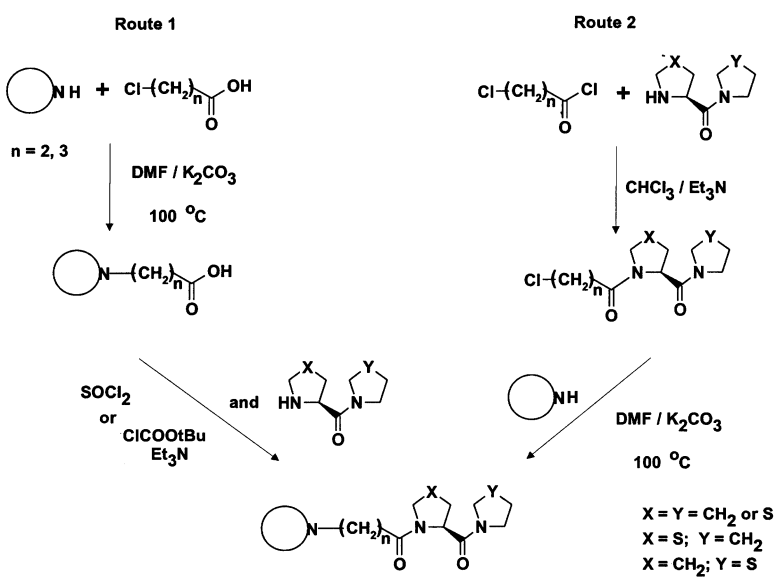
were performed at room temperature for 15 min in the presence of *Z*-glycyl-prolyl-7-amino-4-methylcoumarine as a highly specific substrate of PEP, and the formation of 7-amino-4-methylcoumarine was detected spectrofluorometrically at excitations of 370 and 440 nm emission wavelengths. The inhibitory activity was first tested at 10^{-7} mol/l inhibitor concentration and then the IC_{50} values were determined for compounds that exhibited more than 50% inhibition. Besides the in vitro activity determination, absorption properties in the CaCO-2 model and metabolic stability of the compounds were determined using appropriate in vitro methods.

In all active compounds the proline group was considered in its natural (*S*) configuration except derivatives containing an *R*-prolyl moiety that were totally inactive. Further structural changes at the proline–pyrrolidine moiety, e.g. the replacement of one of the pyrrolidine methylene groups with a sulfur atom, a sulfoxide or sulfone group enhanced the inhibitory activity and modified the pharmacokinetic properties. Depending upon the *N*-heterocyclic group the optimal chain length contains two or three methylene units.

A 3D-QSAR technique, the comparative molecular field analysis (CoMFA), may serve as a useful tool to gain insight into the mechanism of inhibitory action and to predict the inhibitory activity of compounds. Therefore, the CoMFA model may also allow the design and synthesis of more efficient and selective inhibitor molecules. Within the selected series, CoMFA studies were carried out to characterize the sensitive parts of the heterocycle for side-chain optimization. A set of 44 previously synthesized and tested prolyl endopeptidase inhibitors were investigated using CoMFA. CoMFA models were developed for two conceivable alignments of the molecules: one based on a template structure in its global conformational energy minimum (bent form), and the other based on a template structure in a similar conformation to related molecules from the co-crystallized enzyme-inhibitor complex (linear form). Good CoMFA models were obtained for both alignments with q^2 values of 0.709 for the bent form and 0.652 for the linear form. The CoMFA models were validated by dividing the set of molecules into a training set and a validation set: the r^2 value between the measured and predicted inhibitory activities of the molecules in the validation set was 0.773 for the bent form, and 0.645 for the linear form. The derived CoMFA models reveal several interaction sites between the inhibitor molecules and the PEP enzyme, but do not differentiate between the bent and linear forms.

The fine tuning of the pharmacophore moiety (e.g. the prolylpyrrolidine part of the molecules) was achieved by substitution of the 4-methylene group of proline and 3-methylene group of pyrrolidine moieties with either a sulfur atom or its oxygenated derivatives such as sulfoxides and sulfones. Furthermore, a new isostere, a 4,4′-difluoroproline, in combination with *N*-phthalimido-β-alanine derivatives contribute towards identifying orally active PEP inhibitors. Parallel to this work, the three-dimensional structure of prolyl endopeptidase isolated from pig brain was determined by protein crystallography [2] and further data for the structural optimization were gained from three-dimensional structures of the co-crystals of different inhibitors and prolyl endopeptidase.

As a result of this research the activity of SUAM-1221 was increased by three orders of magnitude. The



Scheme 1.

ex vivo and in vivo activities of the most active inhibitors were demonstrated on different animal models. Further profiling is needed to indicate whether these compounds can be useful in clinical investigation.

References

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