Aldehydic peptides inhibiting renin

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Aldehydic peptides in which the C terminal residue is leucinal or phenylalaninal were synthesized. These compounds exhibited potent inhibition of renin activity and appeared to be precursors of transition state analogues for renin-catalysed amide bond hydrolysis.

Renin *Peptidyl aldehyde* *Enzyme inhibitor*

1. INTRODUCTION

Pepstatin A, isovaleryl-L-valyl-L-valyl-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, was isolated in [1]. It strongly inhibits several carboxyl proteases including pepsin and renin [2–6]. Many studies have been performed to explain its inhibitory potency towards carboxyl proteases [7–10] and it has been proposed that the statin [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid] residue of pepstatin is an analogue of the transition state for the enzyme reaction [7,11–13]. Authors in [10] have studied a keto analogue of statin. This peculiar inhibitor acts as an analogue of the substrate, undergoing nucleophilic addition from either water or hydroxylic ligands from the enzyme to give a tetrahedral intermediate, directly observed by ^{13}C NMR, mimicking the aspartyl proteases' transition state.

To test very short inhibitors of renin, we decided to synthesize aldehydic peptides. Indeed, the structure of these products represents an ultimate shorting of the substrate, exhibiting an enhanced electrophilicity whereas the corresponding aldehyde hydrates resemble the shortest transition state analogue as shown in scheme 1.

Our preceding studies [14] having shown that the presence of an aromatic residue in subsite S^3 was mandatory for a good recognition by renin, whereas the presence of a histidine in subsite S^2 was not so critical, we decided to test a series of tripeptide aldehydes of the type P-A-Y-Xh where P is a Boc or Z protecting group, A is an aromatic residue, Xh is either leucinal or phenylalaninal and Y is a variable residue.

Many *N*-peptidylamino aldehydes are potential inhibitors towards some classes of proteolytic enzymes [15–22]. Authors in [21] have synthesized new renin inhibitors by replacing the carboxyl group of the Leu^{10} residue at the C-terminus of angiotensin I with an aldehyde group. Z-leucinal semicarbazone (Z-Leu-Sem) was prepared from Z-Leu-OEt by reduction with diisobutylaluminium hydride, followed immediately by treatment with semicarbazide. The removal of the semicarbazide protecting group was performed by treatment with formalin–hydrogen chloride. The IC_{50} for hog kidney renin was found to be in the range of 100 μM indicating a poor affinity of these inhibitors towards enzyme.

This method of preparation of aldehydic peptides is thus far the only one known; it was not safe for the conservation of chemical and optical integrity.

2. METHODS

Recently we published a new method for the easy and racemization-proof preparation of *N*-acylamino aldehydes [23]. Notably, this method

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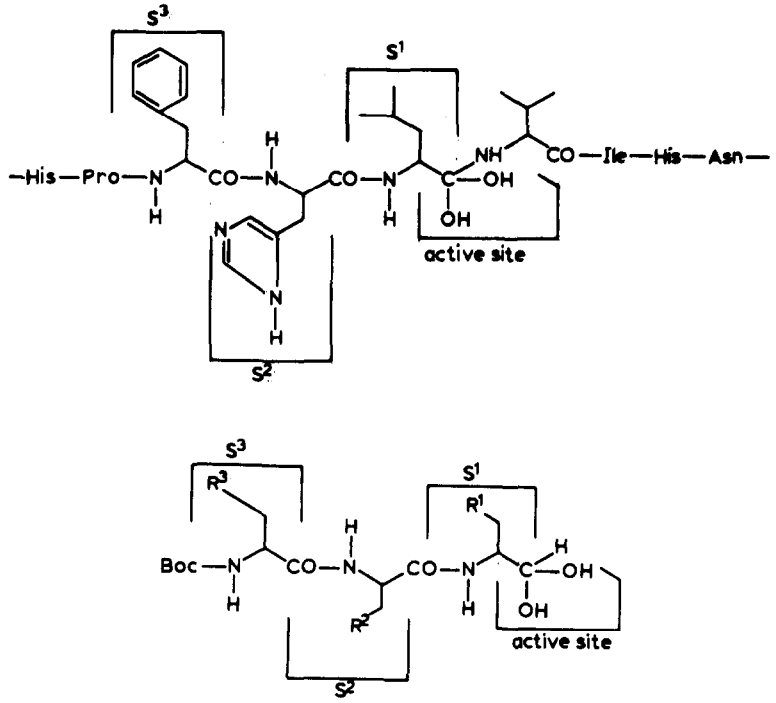
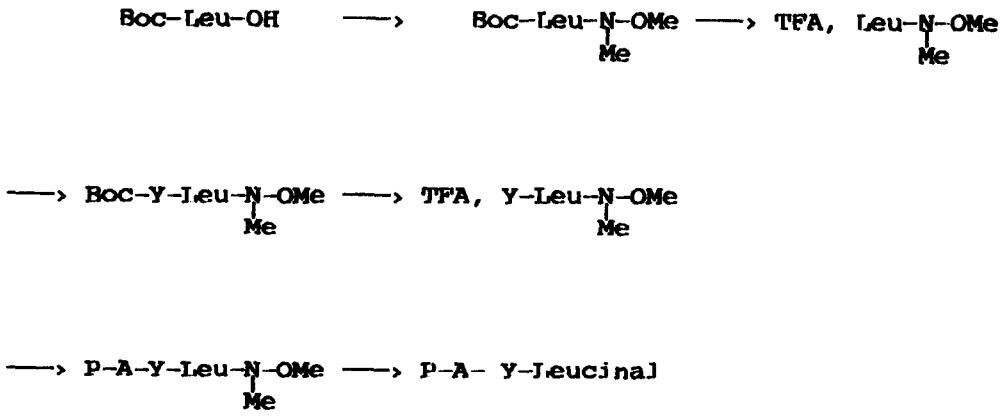


Scheme 1. (Bottom) Tripeptide aldehyde hydrates as transition state analogues. (Top) The transition state and the main recognition subsites.

does not require any purification of the crude product which can be obtained free from reduced alcohol contamination. We have found that the same method can be used to prepare peptides containing the aldehydic function at the C-terminus. The preparation of these peptides is described in [24]; the synthetic pathway is summarised in scheme 2.

The enantiomeric purity of the aldehyde residue was established by the presence of a single aldehydic proton as observed by ^{1}H NMR at 360 MHz.

All the products can be produced rapidly in high yield and chemical purity. The limitation of the method lies in the failure of the attempted preparation of histidine, containing products which were



Scheme 2.

degraded under strongly reducing conditions.

Inhibition of human plasma renin activity was measured as in [25] under both acidic and physiological conditions. Porcine pepsin inhibition was measured according to known methods [2].

3. RESULTS AND DISCUSSION

The measurements for 12 tripeptide aldehydes and one tetrapeptide are reported in table 1. They can be compared to the results for some single acylamino aldehydes, and other tripeptides bearing other C-terminal functional groups.

Every tripeptide aldehyde with an aromatic residue in position S^3 behaves as an inhibitor as shown by items 7, 9–17 and 19. The aldehydic function is essential, as the corresponding alcohol 3, acid 4, hydrazide 5 or methyl ketone 6 are completely devoid of inhibitory activity.

The inactivity of Boc-alaninal 1 and Boc-phenylalaninal 2 demonstrates that the inhibition is not the result of a non-specific reaction with protein amino or hydroxyl groups. The inactivity of Boc-Val-Val-leucinal demonstrates that the inhibitor must recognise the aromatic subsite S^3.

Though the inhibitory activity is much lower than the activities of recently described statin containing peptides or substrate analogues [14,26,27], some of these peptide aldehydes, 7, 9, 11, 14, 16 and 19 have an equivalent or better activity at physiological pH than pepstatin which was the reference compound to this time. In contrast to the behaviour of pepstatin and renin inhibitors homologous to pepstatin, the inhibition decreased only a few percent from the optimal pH of the enzyme to physiological pH.

The best tripeptidic inhibitors were compared to pepstatin. So we could see that at physiological pH, the aldehydic tripeptide 11 presented an activity towards renin 6.5-times greater than pepstatin. On the other hand, all these products were more specific, being much less inhibitory towards pepsin than was pepstatin.

Clearly, some of these aldehydic peptides are good renin inhibitors: they are the shortest and the simplest yet known.

Table 1

Item no.	Inhibitors (X)	$-\log IC_{50}$ renin		$IC_{50}(X)/IC_{50}(P)$		$-\log IC_{50}$ pepsin
		pH 6	pH 7.4	pH 6	pH 7.4	pH 2
	Pepstatin (P)	5.88	4.88			7.82
1	Boc-alaninal	<4	<4			4.40
2	Boc-phenylalaninal	<4	<4			4.70
3	Boc-Phe-Phe-leucinol	<4	<4			<4
4	Boc-Phe-Phe-Leu	<4	<4			<4
5	Boc-Phe-Phe-Leu-$NHNH_2$	<4	<4			<4
6	Boc-Phe-Phe-Leu-Me	<4	<4			<4
7	Boc-Phe-Phe-leucinal	5.79	4.96	0.81	1.2	<4
8	Boc-Val-Val-leucinal	<4	<4			4.85
9	Z-Phe-Val-leucinal	5.40	5.26	0.33	2.4	5.18
10	Z-Phe-Phe-leucinal	4.92	<4			<4
11	Z-Trp-Val-leucinal	5.77	5.70	0.77	6.5	5.52
12	Z-Tyr-Val-leucinal	4.70	<4			5.15
13	Z-Phe-Pro-leucinal	<4.70	<4			<4
14	Boc-Phe-Leu-leucinal	5.96	5.38	1.2	3.2	<4
15	Z-Phe-Ile-leucinal	5.10	4.82	0.17	0.87	5.24
16	Boc-Trp-Val-leucinal	5.45	5.27	0.37	2.5	<4
17	Boc-Phe-Ala-leucinal	5.06	4.88	0.15	1.0	<4
18	Boc-Pro-Phe-Phe-leucinal	4.85	4.72		0.68	<4
19	Boc-Phe-Leu-phenylalaninal	5.43	5.68	0.35	6.3	<4

The relatively high inhibition constants presented by these products in spite of the presence of only very few residues for subsite recognition (3 residues only, instead of 5–8 in the inhibitors recently described), can be explained if these inhibitors act as transition state analogues. Indeed the aldehydic function is susceptible to rapid transformation to the corresponding hydrate, either during the plasmatic transport, or in the active site itself. Scheme 1 shows clearly the structural homology between the tripeptide aldehyde hydrate and the tetrahedral intermediate near the transition state.

In conclusion we believe that this family of inhibitors is the basis of a very practical tool for exploring modifications at the subsites S^1, S^2 and S^3 of renin, needing only rapid synthesis of short tripeptide aldehydes.

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