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New fluoroketones as human renin inhibitors

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Renin inhibition has been evaluated for a new class of fluorinated ketones, true analogues of peptides that have been retroinverted at the C-terminal position. The readily formed hydrate of the ketone is proposed to mimic the tetrahedral intermediate that occurs during the enzyme-catalyzed hydrolysis of amide linkage. From this series of compounds it appears that the number of reverted amide bonds is crucial in terms of activity. Furthermore, a shortening of the C-terminal part of our peptide analogues and the replacement of the leucine residue in P_1 by a cyclohexylalanine leads to the tripeptide analogue **12** a potent renin inhibitor ($IC_{50} = 3.5 \times 10^{-9}$ M).

Enzyme inhibitor; Renin; Fluoroketone; Retroamide bond

1. INTRODUCTION

The renin-angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte homeostasis [1,2]. Renin (EC 3.4.99.19), an aspartyl protease, is the enzyme responsible for cleaving a decapeptide fragment from the N-terminal portion of angiotensinogen. This highly specific and rate limiting step initiates the renin-angiotensin converting enzyme cascade.

The search for an orally active inhibitor of human renin for the treatment of hypertension has intensified greatly over the past few years [3].

It has been known for some time that much of the catalytic specificity and efficiency of aspartate proteases derives from binding interactions that occur throughout the extended active site [4]. As a consequence, the minimum synthetic substrate known for renin is an octapeptide [5], and renin inhibitors have tended to be rather large. This seemingly necessary size and the peptide character of renin inhibitors, led to compounds that had serious problems of metabolism, oral bioavailability and duration of action [5]. Thus, the challenge in this field is to minimize those features. Steady progress toward this goal has been made over the past three years.

In the present work we wish to report a new series of small peptide analogues, whose original structure is the result of the combination of fluoroketone and retroamide type bonds [6]. The potency of fluoroketone derivatives as inhibitors of hydrolytic enzymes has been well documented and illustrated [7]. In the case of zinc metallo- and aspartyl proteases, they are assumed to be transition state analogues due to their high propensity to act in their carbonyl hydrated form. As a result, several renin inhibitors bearing difluorostatone or perfluoroketone have been reported recently [8,9]. The potent inhibitory action of the latter peptides prompted us to synthesize new extended binding type fluoroketones [10,11]. These inhibitors incorporate a dipeptide surrogate of leucine-glycine or cyclohexylalanine-glycine.

Our approach lies in the design of fluorinated peptide analogues that have been retroinverted at the C-terminus. These compounds are chemically stable [12]. Moreover, enhanced resistance to biodegradation processes might be an additional benefit of this type of structural modification. The inhibitory potency of these compounds against human plasma renin is presented here.

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2. MATERIALS AND METHODS

2.1. *Renin assay*

A human plasma pool containing 10 mM EDTA stored at 0°C and rich in renin, was incubated for 90 min at 37°C, pH 6.0, in the presence of 1.5 mM PMSF (phenyl methyl sulfonyl fluoride) and increasing concentrations of inhibitors. The inhibitors were added to the assay medium from a stock solution in Me_2SO. The presence of 1% Me_2SO in the final incubation mixture had no significant effect on the renin activity.

The radioimmunoassay for angiotensin I was carried out with a commercially available kit (clinical assays: SB REN2, available from CEA).

Plasma renin activity values for assays containing inhibitor were compared to control assays to estimate the percent of inhibition. The inhibition results were expressed as IC_{50} values (concentration of test product causing 50% inhibition of plasma renin activity).

2.2. *Chemical synthesis*

The compounds described in this study were synthesized by the following methods: Cbz-L-α-aminoaldehyde was condensed with ethyl bromodifluoroacetate in the presence of activated zinc. The resulting α,α-difluoro-β-hydroxyester was converted in two steps to the corresponding β,β-difluoro-γ-hydroxyamine by the procedure in [12]. The 1,4-diamine was isolated in its diprotected form. The N-Cbz-N'-Boc-1,4-diamine was deprotected sequentially and coupled to carboxylic acids (on the carboxyl terminus) and N-protected dipeptides (on the amino terminus) [12] to yield the precursor difluoroalcohol. Oxidation of the difluoroalcohols to the final difluoroketones was performed using pyridinium dichromate [12].

Complete details of the chemical synthesis will be reported elsewhere.

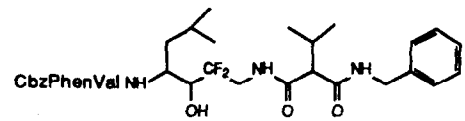
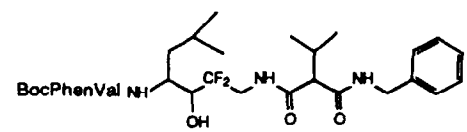
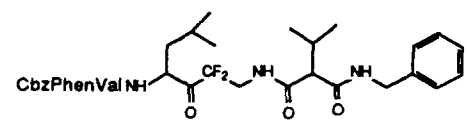
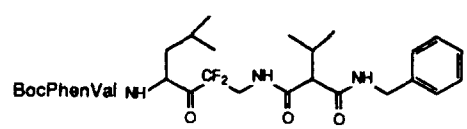
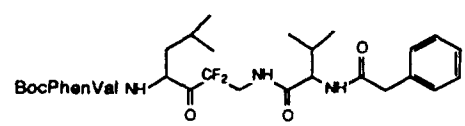
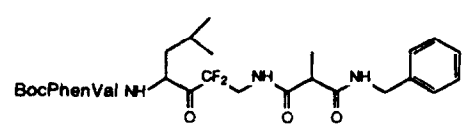
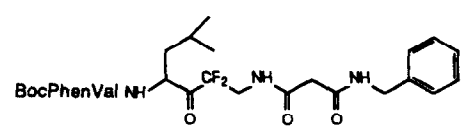
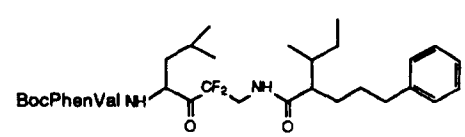
3. RESULTS AND DISCUSSION

The peptides analogues that were prepared in this study along with the corresponding IC_{50} values against human plasma renin are shown in tables 1 and 2. The choice of the general amino acid sequence of the reported peptide analogues was dictated by both the enzyme specificity and inhibitor synthesis requirements. Regarding the latter consideration, a norvaline residue was incorporated in place of the normally occurring P_2 histidine, since it is known that this replacement should not affect the binding [13].

General comments on this type of peptide are 2-fold. First, as observed previously [7–11] for other types of fluorinated renin inhibitors, ketones **3** and **4** are more potent than their corresponding alcohols (**1** and **2**, respectively). Secondly, as anticipated [13,14], the nature of the amino terminal substituent plays a role in the affinity, the Boc derivative **4** being 6 times more potent than the Cbz

Table 1

Inhibition of human plasma renin

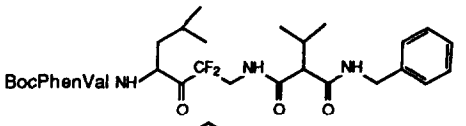
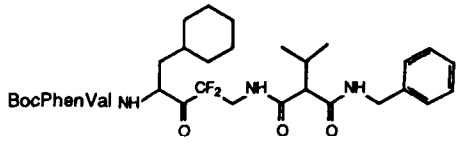
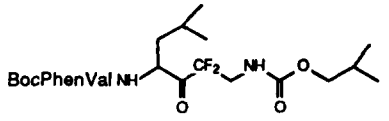
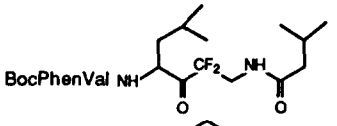
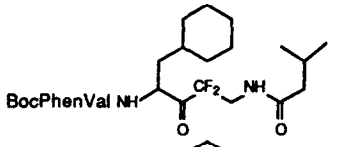
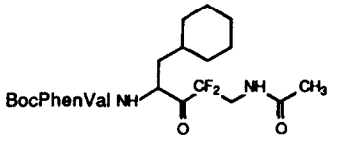
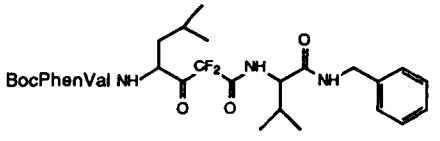
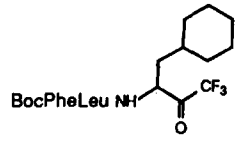
	Human angiotensinogen	Phe P_3	His P_2	Leu P_1	Val P'_1	Ile P'_2	His P'_3	IC_{50} (nM)
1								80000
2								75000
3								10000
4								1500
5								>50000
6								1400
7								400
8								1450

3. Inhibitor **4** was our lead compound for the structural modifications which follow.

Introducing additional retroamide bonds [15] between the P' residues of the peptides led to the important observation that the total number of such modifications seems to be important in terms of activity. In this respect, fluoroketone **5**, which bears two retroamide peptide bonds, is much less

Table 2

Inhibition of human plasma renin

	P_3 P_2 P_1 P'_1 P'_2 P'_3	IC_{50} (nM)
4		1500
9		25
10		250
11		40
12		3.5
13		30
14		30
15		250*

* See [17]

active than fluoroketone **4** bearing only one retroamide linkage.

Interestingly, compound **8** which is lacking one peptide bond at the carboxy terminus, is equipotent to **4** suggesting that this particular amide is not implicated in any hydrogen bonding.

Changes in the size of the side chain of the P'2 residue induces less dramatic changes in the affinity. The replacement of the isopropyl side chain of P'2 in inhibitor **4** by a methyl (**6**) or a hydrogen (**7**) had a very small effect on IC_{50} values with compound **7** being only three times more potent than **6** or **4**. This might suggest a very slight steric effect of P'2 in **4** or **6**.

The structure activity relationship at the P1 site was also investigated. In agreement with a previously reported result [16], an enormous improvement in affinity was observed by substituting the isobutyl side chain in P1 of **4** by a cyclohexyl methylene moiety. Compound **9** is 60 times more effective than **4**. We can assume that this particular side chain fits the S1 subsite of the active site in an optimal fashion. This result is impressive when compared to the disappointing observation of Thaisrivong et al. [8] for difluorostatone containing inhibitors, in which such a modification had no effect. Based on these two contradictory results, we can anticipate that the two families of fluorinated ketones bind differently to renin. Although the concept of difluorostatone has generated some of the most potent inhibitors of human renin (compare **4** to **14**), the interesting effect of the incorporation of the cyclohexyl methyl side chain in the P1 position of our retroinverted fluoroketone leads to an inhibitor equipotent to the difluorostatone containing peptide (compare **9** to **14**).

An additional aim of our work was to further define the importance of the carboxy terminal part of this type of molecule in terms of activity. The most interesting feature in this series of peptide analogues was that a shortening of the carboxy terminus as in ketone **10** or **11** yielded inhibitors 6 and 40 times more active than **4**, respectively. Therefore, we investigated some further modifications on this part of our inhibitors. It is clear that the isovaleryl substituent at the carboxy terminus of **11** and **12**, when compared to an isobutyloxycarbonyl moiety in **10** or an acetyl group in **13**, provides the best fit with the enzyme. This is logical if we consider the isovaleryl side chain as a good mimic of the P'2 isoleucine residue of human angiotensinogen. Moreover this would suggest that this side chain binds tightly to the S'2 subsite of human renin.

Finally, the combination of the shortening of the carboxy terminus and the replacement of the P_1

leucine of **4** by a cyclohexylalanine residue produced the tripeptide analogue **12**, which inhibits renin at nanomolar concentrations.

At this stage of our study it is of interest to compare the difluoroketone containing peptide **12** with the recently published tripeptide trifluoromethyl ketone **15** [17]. These two derivatives are based on a quite similar peptide sequence, allowing a direct comparison. The additional interactions provided by the retroamide and the isovaleryl side chain make **12** about 70 times more active than **15**. This result reinforces the concept of extended-binding type inhibitors for renin inhibition.

In conclusion, the small size of our peptide analogues and the presence of a retroamide bond that should enhance both the chemical and the proteolytic stabilities provide a new possibility for the development of orally active renin inhibitors. Our progress in this respect, as well as in vivo studies, will be the subject of future reports.

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