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# Highly potent and specific inhibitors of human renin

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We have designed and synthesized a series of small peptides containing a perfluoroalkyl ketone group at the C-terminal position of the angiotensin I sequence as inhibitors of human renin. From this series of compounds, **8** and **10** showed strong inhibition of human renin ($IC_{50} = 3 \times 10^{-9}$, $7 \times 10^{-9}$ M, respectively). Compound **10** did not inhibit pepsin and cathepsin D at $10^{-4}$ M. Comparison of the $IC_{50}$ of compound **8** and compound **11** ($8.7 \times 10^{-7}$ M) demonstrated the marked effect of the perfluoropropyl group on the potency of inhibition on renin, presumably due to the strong electron-withdrawing effect causing the ketone in **8** to exist predominantly as the hydrate – thus mimicking the tetrahedral transition state during hydrolysis of the scissile $Leu^{10}$–$Val^{11}$ amide bond.

Enzyme inhibitor; Renin; Perfluoroalkyl ketone; Specificity

## 1. INTRODUCTION

Renin is the enzyme which cleaves a decapeptide fragment from the N-terminal portion of angiotensinogen, thereby initiating the renin–angiotensinogen-converting enzyme cascade. The important role played by the renin-angiotensin system (RAS) [1] in the etiology of some forms of cardiovascular disease has recently been demonstrated by the successful development of ACE inhibitors as clinically useful agents for the treatment of hypertension and congestive heart failure [2].

Intense research efforts to block the RAS at the first catalytic step have resulted in a continued search to discover an orally active renin inhibitor [3]. During the past several years a large number of structurally diverse peptide analogs have been described as renin inhibitors. Kokubu et al. [4] reported that small peptidic aldehydes at the C-terminal position of angiotensin I are potent inhibitors of human renin, while Szelke et al. were the first to describe renin inhibitors containing a reduced amide [5] and a hydroxyethylene isostere [6] at the scissile $Leu^{10}$–$Val^{11}$ amide bond. Renin inhibitors incorporating the amino acid statine [7] and more recently difluorostatine and difluorostatone [8] have also been described. The high level of interest in preparing fluoro ketone analogs as inhibitors of proteolytic enzymes [8–10] prompted us to report our work in this area. Here, we describe a series of small peptides containing a perfluoroalkyl ketone moiety incorporated at the C-terminal position of angiotensin I. The inhibitor potency of these compounds against human renin and their specificity against other aspartic proteinases are also presented.

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## 2. MATERIALS AND METHODS

### 2.1. *Renin assay*

Purified human renal renin [11] was assayed utilizing pure human angiotensinogen [12] at pH 6.0 in maleate buffer. Test compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO and 0.5% in BSA. The final incubation mixture (100 $\mu$l) contained 0.135 M maleate buffer (pH 6.0), 3 mM EDTA, 1.4 mM PMSF, 0.21 $\mu$M

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angiotensinogen, 0.24 mGU renin [13], 0.44% BSA, 1% DMSO. At least 3 different concentrations of inhibitor which bracketed the concentration causing 50% inhibition ($IC_{50}$) were preincubated with renin for 5.0 min at 37°C, then substrate was added and the incubation was allowed to proceed for 10.0 min. The reaction was stopped by freezing the solution in a methanol-dry ice bath, and after thawing at 4°C an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percent inhibition of the reaction was determined and the $IC_{50}$ was calculated by regression analysis. The reaction time of 10.0 min was on the linear portion of the incubation time vs angiotensin I generation curve, and at the highest concentration tested none of the compounds cross-reacted with the antibody to angiotensin I. The presence of 1% DMSO in the final incubation mixture caused no statistically significant effect on the renin activity.

### 2.2. *Pepsin and cathepsin D assays*

Porcine pepsin (Sigma) and bovine cathepsin D (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 1.9 and 3.1, respectively, at 37°C, and measurement of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid [14].

### 2.3. *Chemical synthesis*

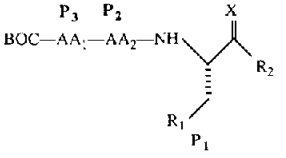
The compounds described in this study were synthesized by the following methods: Boc-L-amino aldehyde was condensed with perfluoroethyl [15] or perfluoropropyl lithium generated in situ by the addition of methyllithium (complexed with lithium bromide) to the corresponding perfluoroalkyl iodide at −78°C. The resulting Boc-fluoro alcohol was deprotected and coupled to Boc-Phe-His-OH or other dipeptides to give the precursor to the fluoro ketones. Oxidation of the fluoro alcohols to the final fluoro ketone products was performed with the oxidizing agent Dess-Martin periodinane [16]. Complete details of the chemical syntheses will be reported elsewhere.

## 3. RESULTS AND DISCUSSION

The small peptide analogs containing the perfluoroalkyl ketone functionality and their precursor alcohols that were prepared in this study, along with the corresponding $IC_{50}$ values against human renin, are shown in table 1. We have reasoned that the ketones, because of the strong electron-withdrawing effect of the perfluoroalkyl group, would exist predominantly in the hydrated form as shown in the following equation. This hydrated form of the ketones ($R_1$ = isopropyl or cyclohexyl;

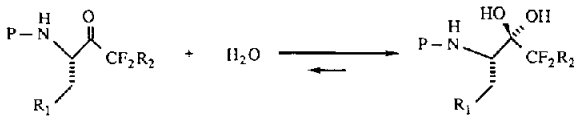
Table 1

Structure-activity relationship of perfluoroalkyl alcohols and ketones as inhibitors of human renin




| No. | $AA_1$ | $AA_2$ | X[a] | $R_1$ | $R_2$ | $IC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| 1 | Phe | Leu | H,OH | isopropyl | $CF_2CF_2CF_3$ | 1200 |
| 2 | α-Nal[b] | Leu | H,OH | isopropyl | $CF_2CF_2CF_3$ | 150 |
| 3 | Phe | Leu | H,OH | cyclohexyl | $CF_2CF_2CF_3$ | 52 |
| 4 | Phe | His | H,OH | cyclohexyl | $CF_2CF_2CF_3$ | 27 |
| 5 | Phe | Leu | H,OH | cyclohexyl | $CF_2CF_3$ | 150 |
| 6 | Phe | Leu | O | isopropyl | $CF_2CF_2CF_3$ | 120 |
| 7 | α-Nal | Leu | O | isopropyl | $CF_2CF_2CF_3$ | 23 |
| 8 | Phe | Leu | O | cyclohexyl | $CF_2CF_2CF_3$ | 3 |
| 9 | Phe | Leu | O | cyclohexyl | $CF_2CF_3$ | 13 |
| 10 | Phe | His | O | cyclohexyl | $CF_2CF_2CF_3$ | 7 |
| 11 | Phe | Leu | O | cyclohexyl | $CH_2CH_2CH_3$ | 870 |

[a] All hydroxy compounds are a mixture of α/β-OH
[b] α-Naphthylalanine



$R_2 = CF_3$ or $CF_2CF_3$; P = dipeptides) thus would mimic closely the tetrahedral transition state of the hydrolysis of the scissile $Leu^{10}$-$Val^{11}$ amide bond of the angiotensinogen sequence. As a result, they should show tighter binding to renin than their precursor alcohols. As demonstrated by the results shown in table 1, all of the fluoro ketones were more potent inhibitors ($\sim 10\times$) of human renin than the corresponding fluoro alcohols. Substitution of the more lipophilic amino acid Leu for the His at the $P_2$ site also maintained high inhibitory potency. The replacement of the isobutyl side chain of the Leu at the $P_1$ site with the more lipophilic cyclohexylmethyl [7] side chain increased the potency by about 20–40-fold. Shortening the terminal perfluoropropyl group to a perfluoroethyl group led to a decrease in potency. In order to assess the effect of the perfluoroalkyl group on the inhibition of renin, compound **11** was synthesized for a direct comparison with compound **8**. The only difference between **8** and **11** was that the perfluoropropyl group in **8** was replaced by an *n*-propyl group in **11**. Compound **8** was 290-times more potent than compound **11**, illustrating dramatically the proposed effect of the strong electron-withdrawing fluoroalkyl group on the degree of hydration of the ketone.

We tested the effect of compound **10** on the other aspartic proteinases pepsin and cathepsin D. At $1 \times 10^{-4}$ M, compound **10** exhibited no inhibition of either enzyme, thus demonstrating the marked specificity of compound **10** for renin.

The small size of the perfluoroalkyl ketones described in this study and their relative stability as compared to peptidic aldehydes may provide a possibility for the development of orally active renin inhibitors. The in vivo activities of these compounds are currently under investigation.

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