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Synthesis and molecular modeling of a lisinopril–tryptophan analogue inhibitor of angiotensin I-converting enzyme

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Abstract—With a view to developing a more C-domain-selective angiotensin I-converting enzyme (ACE)-inhibitor, a novel analogue of lisinopril has been synthesized which incorporates a bulky P'_2 tryptophan functionality. This inhibitor demonstrated a significantly increased specificity for the C-domain as compared with lisinopril. Molecular docking revealed hydrophobic and hydrogen-bonding interactions with residues of the C-domain S'_2 subsite.

Lisinopril, a widely used commercially available ACE inhibitor in the treatment of hypertension and myocardial infarction, is a tight-binding competitive inhibitor ($K_i = 2.7 \times 10^{-10}$).[1–5] However, side effects associated with ACE inhibitor use, such as angioedema and persistent cough,[6] have been attributed to a lack of adequate C-domain-selectivity and an inappropriate suppression of bradykinin hydrolysis by the ACE N-domain. Recently, Georgiadis and co-workers reported the synthesis of a highly C-domain-selective (~3000-fold) phosphinic acid ACE inhibitor, RXP A380.[7] One key feature of this C-domain-selectivity is a tryptophan moiety at the P'_2 position. With a view to developing more C-domain-selective ACE inhibitors with similar pharmacological profiles to that of lisinopril, we determined the effect of the introduction of a tryptophan moiety at the P'_2 position of lisinopril.

A lisinopril analogue, incorporating a tryptophan moiety at the P'_2 position, was synthesized as follows (Scheme 1): reductive amination of ethyl 2-oxo-4-phenyl butyrate **1** and *N*-ε-(*tert*-butoxycarbonyl)-L-lysine **2** using an ethanolic solution of $NaBH_3CN$[8] gave key intermediate **3** as a 55:45 mixture of diastereoisomers. Coupling of compound **3** with the L-tryptophan methyl ester (prepared by methylation of tryptophan in the presence of thionyl chloride and methanol as described previously[9,10]) was effected using EDC·HCl in the presence of HOBt and diisopropylethylamine to afford the diastereomeric pseudopeptide **4** in 74% yield.

Characterization of this diastereomeric mixture was achieved from the mass (EI-MS) and ^{1}H NMR spectroscopic data. The EI-MS data indicated a molecular ion peak at 637 corresponding to M^++H. The ^{1}H NMR spectrum showed two singlets at δ 3.71 and 3.73 ppm corresponding to the two methyl ester groups of the diastereomers. When the diastereomeric mixture **4** was stirred in 4 N HCl in EtOAc at room temperature for 24 h, followed by solvent removal and stirring in 0.5 N LiOH for a further 5 h, the desired product **5** was obtained in quantitative yield as a mixture of diastereoisomers (Scheme 1). Purification and separation of the 60:40 mixture by HPLC delivered the two diastereomers (**5a** and **5b**) (Fig. 1).

The two isomers (**5a** and **5b**) of the lisinopril–tryptophan analogues were tested for ACE inhibitory activity using purified testis ACE (C-domain) and somatic ACE N-domain together with *Z*-Phe-His-Leu as substrate.[11] The ACE inhibitory activities were determined as previously described[11] with some modifications. Recombinant truncated forms of the C- and N-domains[12,13] were used in the enzyme assays. Enzyme (4 μg/ml) was pre-incubated with inhibitor at concentrations ranging from 0 to 500 μM. Residual enzyme activity was determined from enzyme–inhibitor solution using *Z*-Phe-His-Leu at two substrate concentrations. The enzyme assay was

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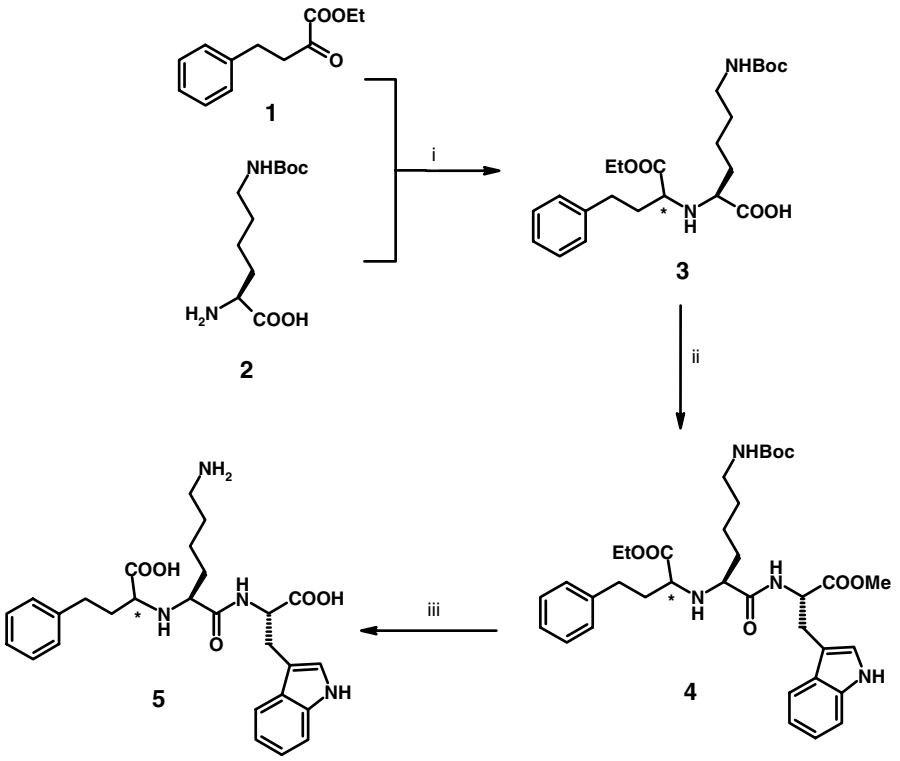

Scheme 1. Reagents and conditions: (i) ketone **1** (4.0 equiv), amino acid **2** (1.0 equiv), $NaBH_3CN$ (2.0 equiv), 50% EtOH/H_2O, rt, 12 h; (ii) L-tryptophan methyl ester, EDC·HCl, HOBt, *i*-Pr_2NEt (1.0 equiv), dry DMF, rt, 72 h; (iii) a—4 N HCl, EtOAc, rt, 24 h and b—0.5 N LiOH, THF–MeOH, rt, 5 h.

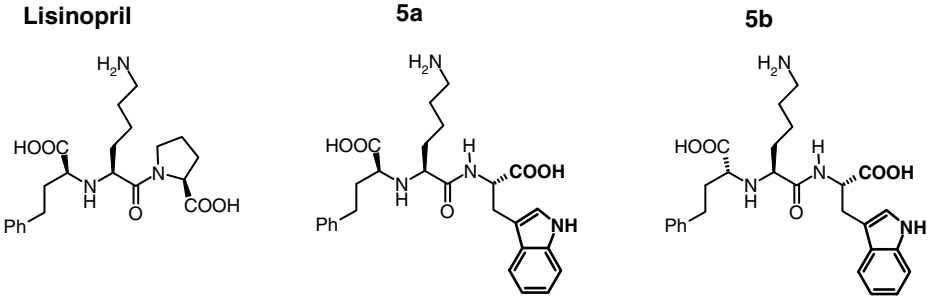

Figure 1. Structures of lisinopril and diastereomers **5a** and **5b**.

adapted so that it could be carried out in a 96-well plate and the fluorescence was measured at Ex = 360 nm; Em = 486 nm on a Varian Cary Eclipse plate reader. All assays were conducted in triplicate. Inhibition curves were plotted using GraphPad Prism 4.01 and K_i values were determined from Dixon plots of $1/V$ versus [I]. The ACE inhibitory activities of **5a** and **5b** are shown in Table 1.

The compounds all showed K_i values in the micromolar range as compared with the nanomolar K_i value of lisinopril. Incorporation of a tryptophan moiety at the P'_2 position of lisinopril (compounds **5a** and **5b**) resulted in a marked increase in C-domain-selectivity (25- to 100-fold) as compared with lisinopril (2.6-fold) (Table 1). This observation is in agreement with previously reported work by Georgiadis et al.[7] suggesting that a large P'_2 residue is an important feature for C-domain-selectivity.

To support these observations, the synthesized compounds **5a** and **5b** were subsequently modeled into the binding sites of the testis ACE (C-domain) crystal structure[14] and a homology model of sACE N-domain. Molecular modeling calculations were performed using the DISCOVER module of INSIGHT II (Accelrys Inc., Version 98.0) on a Silicon Graphics Octane 1 workstation. The starting structure was the X-ray crystal structure of testis ACE complexed with the inhibitor lisinopril[14] and the homology modeled structure of the N-domain. After removing the crystallographic water molecules and adding hydrogens, the CVFF and the ESFF (metal adapted) force-fields were used in all energy minimizations and dynamic runs. The conjugate gradient minimization algorithm was used after running 1000 initial steps, and then 3000 cycles of molecular dynamics followed by 3000 cycles of energy minimization in an NTV ensemble, at a temperature of 300 K.

Table 1. ACE inhibitory activity profiles of lisinopril and its derivatives **5a** and **5b**

Compound	Total PE (kcal/mol)		K_i		Selectivity K_i N/C
	C-dom	N-dom	C-dom	N-dom	
Lisinopril	−890.0	−743.7	51.0 nM	131.5 nM	2.6
5a	−869.6	−630.1	7.0 μM	NI	>100
5b	−830.6	−704.3	26.3 μM	NI	>25

NI: No inhibition up to a concentration of 500 μM.
Total potential energy (PE) is the sum of the energies calculated for all the bonded-atom interactions and for all the nonbonded-atom pairs.

All calculations were carried out in a dielectric constant of 1.00 and a cut-off distance of 9.50 Å. The structures of compounds **5a** and **5b** were generated with standard bond lengths and angles using the builder tool of INSIGHT II software (Accelrys Inc.) and then minimized. The initial position of the compounds in the C- and N-domain active sites of ACE was obtained by superimposing their important pharmacophoric groups on the corresponding atoms of lisinopril in the tACE-lisinopril complex. After removal of the reference inhibitor (lisinopril), the structure of the inhibitor-complex was refined by running energy minimizations and molecular dynamics. These compounds were found to occupy the S_1, S_2, and S_2' subsites, and an example of compound **5a** in the active site of testis ACE is shown in Figure 2. From the molecular docking experiments, the energy-minimized bound conformers of the inhibitors in the active site of the C- and N-domains were obtained. The inhibitors all exhibited negative total potential energies for the protein–ligand interaction. These were consistent with the determined biological binding affinities for the relevant domains and it was an indication of the relative stability of the enzyme–ligand complex (Table 1). The docking of compounds **5a** and **5b** into both domains showed consistency between the interactions of key ACE active site residues and the inhibitors, and also revealed that the synthesized compounds bound to the C- and N-domains in a similar fashion.

The residues forming the subsites of the C- and N-domain active site were defined as those located at a distance less than 6.00 Å from the important functionalities of the inhibitors. The active site residues for the C-domain were Gln281, Thr282, His353, Ala354, Ser355, Ala356, Val379, Val380, His383, Glu384, His387, Phe391, Glu411, Asp453, Phe457, Phe460, Lys+511, Phe512, His513, Ser516, Val518, Tyr520, Tyr523, and Phe527. The binding of compounds **5a** and **5b** in the C-domain active site resembled that of lisinopril, where the phenyl group of the P_1 phenylalanine interacted with the hydrophobic Val518 and the polar Ser355 residues of the S_1 subsite and the lysyl group was accommodated in the S_2' subsite. The distance between the phenylalanine-carbonyl and the Zn-atom in the active site of the C-domain was observed to be 2.73 Å for compound **5a** and 2.74 Å for compound **5b**. These values are within the range of a reasonable H-bonding interaction.

The S_2' subsite of the enzyme readily accommodated the tryptophan moiety of compounds **5a** and **5b**. This observation is in agreement with previous studies which have highlighted the importance of a deep S_2' pocket as a determinant of C-domain-selectivity.[7,15] The P_2' tryptophan moieties of compounds **5a** and **5b** demonstrated additional hydrophobic and hydrogen-bonding interactions with S_2' residues Thr282, Val379, Val380, and Asp453 of the C-domain active site (Fig. 2), and may thus explain the increased C-domain-selectivity of these compounds as compared with lisinopril.

Here, the aromatic ring of the P_2' tryptophan increases the overall hydrophobicity of the molecule and consequently the interaction with the hydrophobic Val379 and Val380 residues in the C-domain. These residues are replaced by the polar residues Ser357 and Thr358 in the N-domain. The other striking feature is the ability to establish a strong H-bonding interaction between the indole-NH and Asp453 in the C-domain which is replaced by a less polar Glu431 in the N-domain. This amino acid's side chain is longer than that of Asp, decreasing the size of the binding pocket. Interactions with the S_2' residues of the C- and N-domains are absent in the case of lisinopril which lacks a bulky P_2' functionality. From

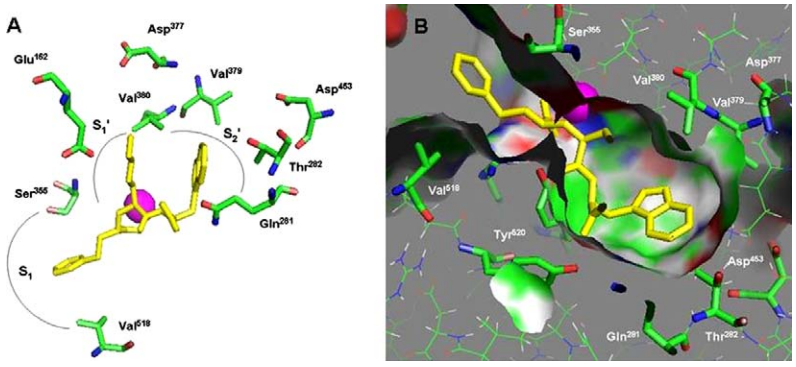

Figure 2. Interactions of Compound **5a** with S_1, S_1' and S_2' active site residues (A) and binding pockets (B) of tACE.

the molecular docking experiments and the observed ACE inhibitory activities, together with previous studies by Sieburth and co-workers,[16] the most potent compound **5a** possesses an *S*-configuration at the stereogenic center bearing the zinc-binding carboxylate group.

Docking experiments revealed that the total potential energies for the complex of compound **5a** with the C-domain and the N-domain were −869.6 and −630.1 kcal mol^{-1}, respectively. Whereas, for compound **5b** they were −830.6 and −704.3 kcal mol^{-1} for the C-domain and N-domain, respectively (Table 1). This is in agreement with the observed biological inhibiting binding affinity for compound **5a**, which was found to be >100-fold more C-domain-selective as compared with lisinopril (2.6-fold) and more C-domain-selective as compared with compound **5b**.

In summary, we have described the synthesis of lisinopril analogues incorporating a bulky tryptophan moiety at the P'_2 position. ACE inhibitory activities and molecular docking experiments have shown that this incorporation leads to a marked increase in C-domain-selectivity.

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