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Novel Angiotensin Converting Enzyme Inhibitors Containing α-Hydroxy Ketomethylene Dipeptide Isosteres †

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Abstract : Synthesis of novel angiotensin converting enzyme inhibitors has been accomplished using the α-hydroxy ketomethylene dipeptide isostere as a key constituent. (2*R*, 5*S*)-2-hydroxy-5-benzamido-4-oxo-6-phenyl hexanoyl (*S*)-(−)-indoline-2-carboxylic acid (7) showed potent inhibitory activity against angiotensin converting enzyme, IC_{50} = 0.09 μM.

Angiotensin converting enzyme (ACE) inhibitors hold great promise in the treatment of hypertension.[1] ACE is an important target for inhibition since it perform the last step in the biosynthesis of the octapeptide angiotensin II, which is a potent vasoconstrictor. In addition, ACE also destroys the potent vasodepressor bradykinin.[2] These combined actions of the converting enzyme may play a crucial role in blood pressure regulation.

Now, it becomes a well established approach to employ proteolytically stable, isosteric peptide analogs as enzyme inhibitors for providing desirable drug properties, such as oral stability, protease resistance and dual solubility in aqueous and lipid environments. Among various dipeptide isosteres,[3] ketomethylene and hydroxyethylene dipeptide isosteres have been frequently used for the synthesis of ACE inhibitors,[4] HIV protease inhibitors,[5] and substance P analogs.[6] Recently, we have introduced a couple of the novel dipeptide isosteres, 2-isoxazoline isostere [7] and α-hydroxy ketomethylene isostere.[8] We wish to report here the preliminery results of the novel ACE inhibitors containing the dipeptide isosteres as key elements.

In the first place, we designed potential ACE inhibitors based on the following strategies. i) Replacement of the scissile amide bond with the 2-isoxazoline isostere or α-hydroxy ketomethylene isostere, ii) Stereospecific generation of the additional stereogenic center in the isosteres by the asymmetric nitrile oxide cycloaddition, iii) Change in the amino acid of P_2' residue for greater affinity in S_2' subsite. From the above consideration, eight potential ACE inhibitors were proposed (Fig. 1).

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† This paper is dedicated to the memory of Dr. Hogil Kim, whose inspiration and enthusiasm will be greatly missed.

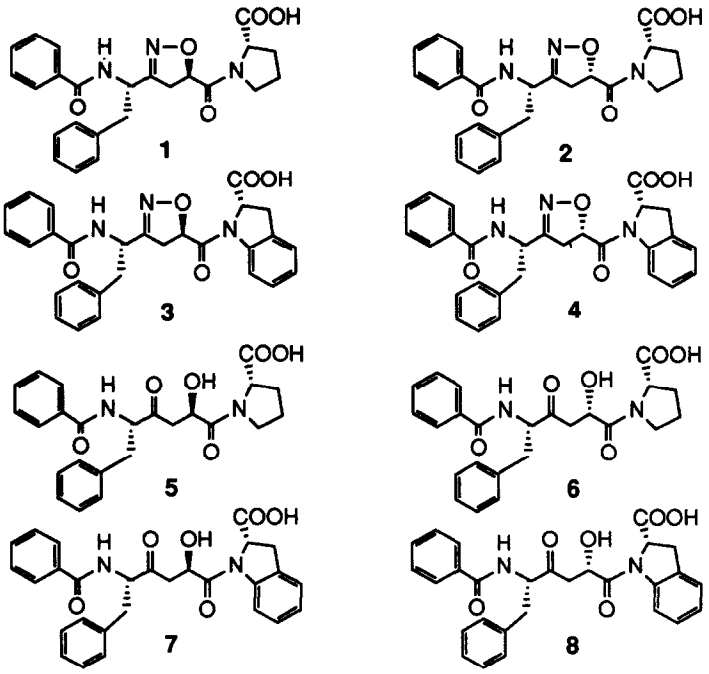
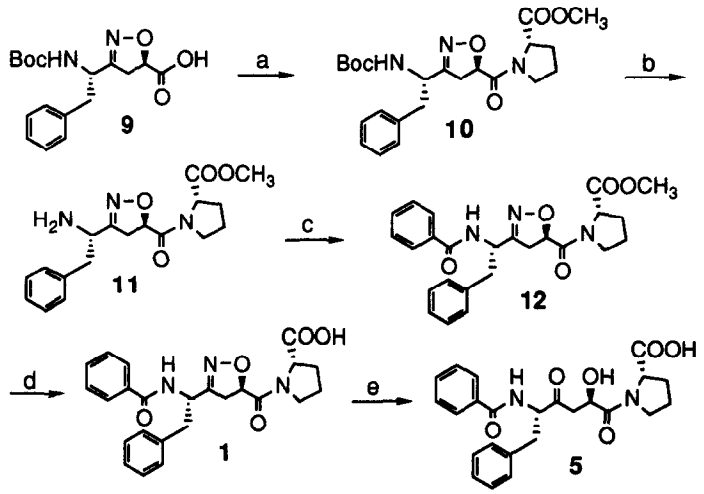


Fig. 1. Designed ACE Inhibitors

Synthesis of the target compounds are summarized in Scheme 1 and 2. The preparation of our starting material **9** has been already reported.[7,8] The reaction sequence for the target molecules **1** and **5** is quite straightforward (Scheme 1). For the amide bond formation with L-proline methyl ester, we used isobutylchlololformate as an activating reagent of carboxy group and employed N-methylmorpholine as a base.[9] Change of amino protection group from Boc to benzoyl and deprotection of carboxy group provided the desired product **1** in good yield. Reductive cleavage [10] of **1** afforded the α-hydroxy ketomethylene dipeptide isosteric inhibitor **5**. ACE inhibitors **3** and **7** which P_2' residues are replaced to (*S*)-(−)-indoline-2-carboxylic acid were also prepared in similar fashion (Scheme 2).

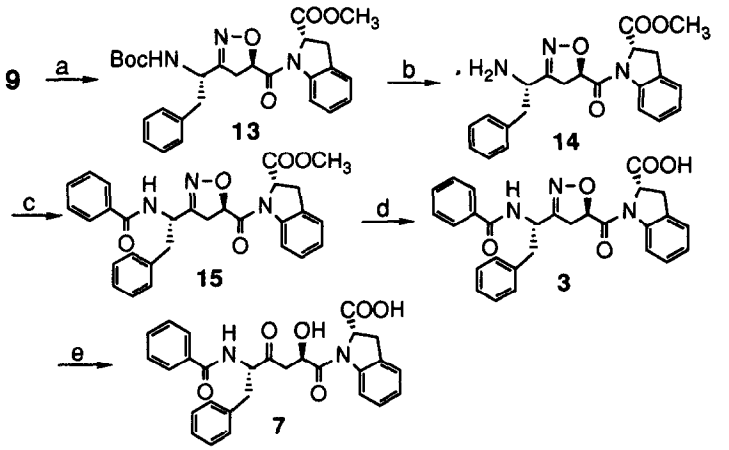
ACE inhibitor candidates **2**, **4**, **6**, and **8** which have (*S*) absolute configurations at 5 positions of 2-isoxazoline rings were synthesized from the starting material **16** [7,8] in the similar way as shown in the Scheme 1 and 2 (Scheme 3).

Scheme 1.



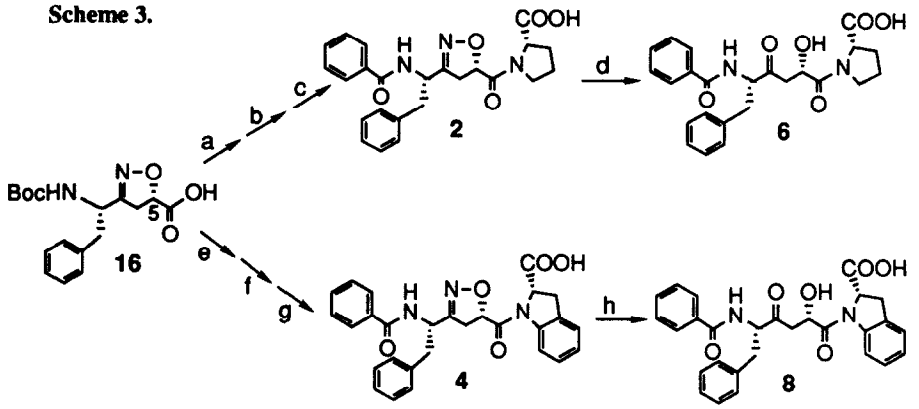
a: *i*-BuOCOCl, NMM, L-ProOMe, CH_2Cl_2, 65%. b: TFA, CH_2Cl_2 and c: benzoyl chloride, TEA, CH_2Cl_2, 60% (b and c). d: 2.0 N NaOH, MeOH, 90%. e: H_2, Ra-Ni, MeOH / H_2O (5/1), $B(OH)_3$, 74%.

Scheme 2.



a: *i*-BuOCOCl, NMM, (*S*)-IndOMe.HCl, 74%. b: TFA, CH_2Cl_2 and c: benzoyl chloride, TEA, CH_2Cl_2, 77% (b and c). d: 2.5 N NaOH, MeOH, 61%. e: H_2, Ra-Ni, MeOH / H_2O (5/1), $B(OH)_3$, 70%.

Scheme 3.



a : *i*-BuOCOCl, NMM, L-ProOMe, CH_2Cl_2, 60%. b : TFA, CH_2Cl_2, then benzoyl chloride, TEA, CH_2Cl_2, 85%. c: 2.0 N NaOH, MeOH, 60%. d: H_2, Ra-Ni, MeOH / H_2O (5/1), $B(OH)_3$, 70%. e : *i*-BuOCOCl, NMM, (*S*)-IndOMe.HCl, 87%. f : TFA, CH_2Cl_2, then benzoyl chloride, TEA, CH_2Cl_2, 87%. g : 1.5 N NaOH, MeOH, 60%. h : H_2, Ra-Ni, MeOH / H_2O (5/1), $B(OH)_3$, 70%.

All eight candidates for ACE inhibitors in hand, we next carried out the bioassay to check the inhibitory activity of each compound. Among several assay methods,[11] we investigated the inhibitory activity using Cushman's spectrophotometric assay[12] and employed captopril as a reference material. Before the inhibitory test, we studied the effect of incubation time and enzyme concentration on the activity of ACE. Based on the experimental results, we determined that the optimum incubation time was 1h and 5mU of ACE per assay should be enough. With detailed bioassay conditions, we investigated the inhibitory activity of compound **1**-**8** to the converting enzyme. Table shows the IC_{50} values of each compound.

Table. IC_{50} values of ACE inhibitors

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	NA [a]	**5**	5
2	NA [a]	**6**	100
3	>1mM	**7**	0.09
4	NA [a]	**8**	5
		captopril [b]	0.02

a : no activity in our experimental concentration range.
b: purchased from Sigma chemical company.

As shown in Table, there is no inhibitory activity in the case of compounds containing 2-isoxazoline heterocycles (compound **1-4**). On the other hand, compounds with α-hydroxy ketomethylene dipeptide isosteres (compound **5-8**) show the ACE inhibitory activities. Compounds **5** and **6** which have L-proline as P_2' residues reveal IC_{50} values in micromolar concentration and (*S, R,S*)-diastereomer **5** is more potent than (*S,S,S*)-diastereomer **6** by 20 times. Compounds **7** and **8** with (*S*)-(−)-indoline -2-carboxylic acid P_2' residues show better inhibitory activities than those of compounds **5** and **6**. Especially, (*S,R,S*)-diastereomer **7** reveals excellent IC_{50} value in nanomolar concentration. This result indicates that the binding of an inhibitor at S_2' subsite affects very much the affinity between ACE and an inhibitor. Our results for the ACE inhibitory activities of compounds **1-8** can be explained by the stereochemical importance of the absolute configuration at α-stereogenic center and the binding ability at hydrophobic S_2' subsite, [13] and the binding mode of one of our inhibitor, **7** to the enzyme might be suggested as shown in Fig. 2.

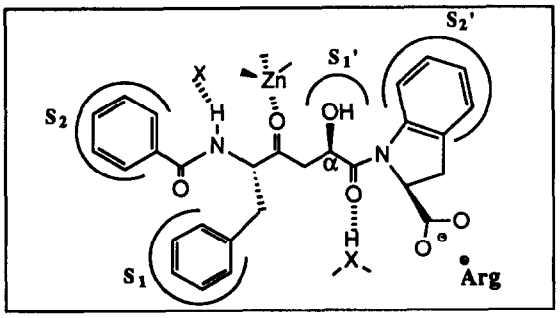


Fig. 2. Suggested Binding Mode to ACE

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