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NOVEL LOW MOLECULAR RENIN INHIBITORS WHICH SHOW GOOD ORAL BLOOD PRESSURE LOWERING EFFECTS IN MARMOSETS

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Abstract. A series of low molecular renin inhibitors (molecular weight < 600) were prepared and their biological activities were evaluated. The inhibitors containing indole-2-carbonyl moieties at the P3 position and 2-amino-3,5-*anti*-diol fragment at the P1-P1' position showed renin inhibitory activities with IC_{50} of 10^{-8} M order and good blood pressure lowering effects after oral administration to sodium-depleted marmosets.

The renin-angiotensin system (RAS) is one of the vasopressor systems in the body and an important blood pressure-humoral electrolyte adjusting system. Renin catalyzes the production of angiotensin-I at the first and rate-limiting step in the RAS, and angiotensinogen is the only known natural substrate for renin. Renin inhibitors are therefore expected to be new type of antihypertensive agents devoid of side effects associated with angiotensin converting enzyme (ACE) inhibitors due to their actions outside the RAS. Although many renin inhibitors with high *in vitro* potency have been reported, most of them are peptide-based compounds designed on the basis of angiotensinogen sequence and large molecules whose molecular weights are more than 600.[1] Consequently, these inhibitors tend to show low oral bioavailabilities and clinically useful renin inhibitors have not appeared yet. In an attempt to overcome the problem of poor oral efficacy, we focused on reduction of the molecular size and peptidic nature of inhibitors. During the course of our synthetic studies on renin inhibitors, we found a potent inhibitor, JTP-2724 containing (2*S*,3*S*,5*S*)-2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol (2-amino-3,5-*anti*-diol) fragment as a transition-state mimic at the P1-P1' position (Figure 1).[2] To explore low molecular renin

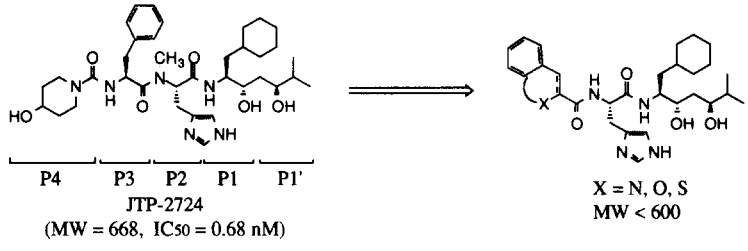
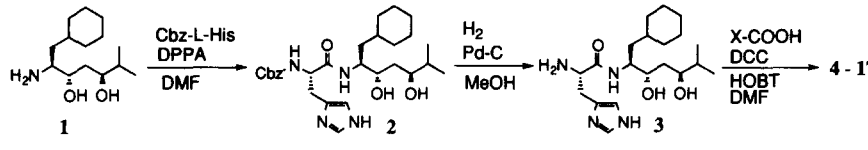


Figure 1

inhibitors, our attempts were directed at ruducing the molecular size of the P4-P3 position of JTP-2724 by replacing with conformationally restricted phenylalanine mimics as shown in Figure 1.[3] In this report, the biological activities of low molecular renin inhibitors synthesized on the basis of the above strategies are described, and the structure-activity relationship is discussed as well.

The synthetic pathway of the inhibitors listed in Table 1 is shown in Scheme 1. The 2-amino-3,5-*anti*-diol fragment **1** was prepared by the previously reported methods,[2] and condensed with Cbz-L-histidine using diphenylphosphoryl azide (DPPA) to give the coupled amide **2**. The Cbz group of **2** was removed by hydrogenation over Pd-C, and the resulting amine **3** was coupled with the corresponding carboxylic acids using standard DCC/HOBT conditions to give the inhibitors **4** - **17**.[4]

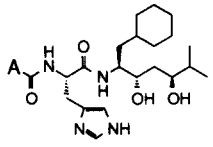
The inhibitory activities of compounds **4** - **17** against human plasma renin were summarized in Table 1.[5] As a residue of the P3 position, we first attempted indoline-2- and tetrahydroisoquinoline-3-carboxylic acid. These acids are regarded as phenylalanine mimics blocking rotation of the phenyl ring, and they have been employed in ACE inhibitors.[6] Compounds **4** and **5** containing these residues at the P3 position showed modelate renin inhibitory activities with IC_{50} of 10^{-7} M order. Then, we examined introduction of the corresponding conjugated system to the P3 position. As a result, compound **6** having indole-2-carbonyl moiety at the P3 position showed 10-fold higher activity than that of the indoline derivative **4**. However, replacement of the tetrahydroisoquinoline skeleton in compound **5** by an isoquinoline (compound **7**) having no proton in the moiety caused slight reduction in the inhibitory potency. On the other hand, compound **8** containing isoquinolin-1-one-3-carbonyl moiety which has NH group revealed good inhibitory activity nearly equal to that of the indole derivative **6**. When the NH group of indole in compound **6** was replaced by oxygen (**9**), sulfur (**10**) and acetyl-N (**11**), reductions of the activities were observed. From these results, it is suggested that the phenylalanine mimics containing both conjugated hetero aromatic ring and NH group are favorable for the renin inhibition. The NH groups of indole in **6** and isoquinolin-1-one in **8** probably contribute to the hydrogen bonding with renin as proton donors. Compared to the NH group of indole, the NH group of benzimidazole has somwhat higher acidity and is thought to be a better proton donor.[7] Therefore, the introduction of benzimidazole skeleton instead of indole was expected to enhance the binding affinity to renin. In fact, the inhibitory activity of the derivative **12** containing benzimidazole-2-carbonyl moiety at the P3 position was three fold higher than that of compound **6**. The pyrrole derivative **13** lacking phenyl ring and compound **14** having pyridine ring with basic character instead of phenyl ring showed much weaker activities compared to compound **6**. These results indicate that the phenyl ring of indole-2-carbonyl moiety in **6** contributes to the binding affinity to renin. Conjugation of the hetero aromatic rings with the amide carbonyls in **6**, **8**, **12** may restrict those two functional groups to a planar geometry, which might

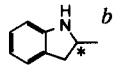
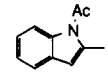
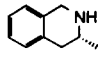
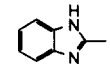
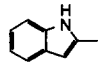
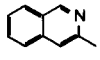
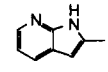
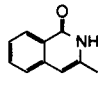
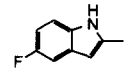
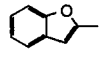
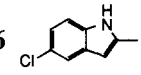
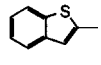
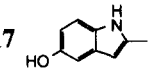


Scheme 1

play an important role for hydrophobic interaction of the phenyl ring with renin. In the substitutions at indole-5-position of compound **6**, the fluorine substitution (**15**) maintained the inhibitory activity, while the replacements by more bulky groups such as chlorine (**16**) and hydroxyl (**17**) caused disadvantages for the activity.

Table 1



Comp.	A	MW	IC_{50} (nM)[a]	Comp.	A	MW	IC_{50} (nM)[a]
4		525	880	**11**		565	340
5		539	420	**12**		524	13
6		523	45	**13**		473	3000
7		535	790	**14**		524	800
8		551	48	**15**		541	46
9		524	140	**16**		559	260
10		540	8200	**17**		539	600

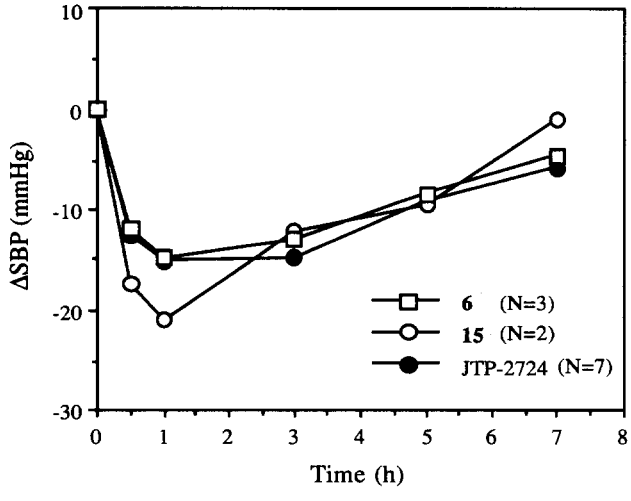
[a] Inhibitory activities against human plasma renin.
[b] IC_{50} value of one diastereomer showing higher activity is listed. The IC_{50} value of the other diastereomer was 4400 nM.

We selected four synthetic inhibitors (**6**, **8**, **12** and **15**) showing IC_{50} of 10^{-8} M order, and evaluated their actions in marmosets. After oral administration of 10 mg/kg of the inhibitor to salt-depleted, conscious marmosets, the reductions of systolic blood pressure (SBP) were measured.[8] Among the tested inhibitors, the compounds **6** and **15** containing indole and 5-fluoroindole, respectively, caused the considerable and long-lasting reductions of SBP as shown in Table 2. Although the benzimidazole derivative **12** was more active than **6** and

Table 2. Blood pressure lowering effects of the inhibitors in salt-depleted conscious marmosets (10 mg/kg, po)

No.	*N*	ΔSBP (mmHg)		
		0.5h	1h	3h
6	3	-12	-15	-13
8	2	-5	+1	+2
12	3	-9	-13	-7
15	2	-18	-21	-12

Figure 2



15 *in vitro*, it revealed weaker effect in marmosets. Furthermore, little blood pressure lowering effect was observed for the administration of compound **8** to marmosets. These results indicate that the oral efficacy of these inhibitors is greately influenced by the difference of chemical property at the P3 position and indole-2-carbonyl derivatives turned out to be good at oral efficacy. Figure 2 shows the blood pressure lowering effects of compounds **6** and **15** compared with our previously reported renin inhibitor, JTP-2724, which is a larger molecule than **6** and **15**. It is noteworthy that the oral blood pressure lowering effects of low molecular inhibitors **6** and **15** are equal or superior to that of JTP-2724, in spite of their weaker *in vitro* inhibitory activities than that of JTP-2724 (IC_{50} 0.68nM).

Inhibitory activities of compound **6** against other enzymes are shown in Table 3.[5)9)] The inhibitor **6** showed potent inhibitory activities against human, cynomolgus monkey and marmoset plasma renin, while much less activities against dog and rat plasma renin. Although pepsin and cathepsin D belong to aspartic proteinases as well as renin, they were not inhibited by **6** even at 100000 nM concentration. Furthermore, compound **6** showed no inhibitory activity against ACE. These results demonstrate that compound **6** has a high degree of specificity

for human renin, which is desirable for a useful therapeutic agent.

Table 3. Enzyme specificity of compound **6**.

Plasma renin	IC_{50}(nM)	Enzyme	IC_{50}(nM)
human	45	pepsin (porcine)	>100000 (5.6%)[a]
cynomolgus monkey	93	cathepsin D (bovine)	>100000 (2.4%)[a]
marmoset	42	ACE (human)	>100000 (6.0%)[a]
dog	3500		
rat	>100000 (41.1%)[a]		

[a] Inhibitory activities at 100000 nM.

In summary, a series of low molecular renin inhibitors (molecular weight < 600) containing 2-amino-3,5-*anti*-diol fragment at the P1-P1' position and replaced with various phenylalanine mimics at the P3 position were synthesized. Through this study, we found that indole-2-carbonyl, isoquinolin-1-one-3-carbonyl, benzimidazole-2-carbonyl, and 5-fluoroindole-2-carbonyl groups are useful as a phenylalanine mimic at the P3 position for *in vitro* renin inhibition. Among the synthesized low molecular inhibitors, compound **6** (JTP-3072) containing indole-2-carbonyl and compound **15** (JTP-4129) containing 5-fluoroindole-2-carbonyl at the P3 position revealed excellent activities in terms of both potency and duration of action on the blood pressure lowering effect in salt-depleted, conscious marmosets. The oral bioavailability and further pharmacological characterization of these compounds are under examination.

References and Notes

1) a) For review, see: Ocain, T. D.; Abou-Gharbia, M. *Drugs Fut.* **1991**, *16*, 37. b) Recently, some nonpeptide renin inhibitors have been reported. For example: Boyd, S. A.; Fung, A. K. L.; Baker, W. R.; Mantei, R. A.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Klinghofer, V.; Wessale, J. L.; Verburg, K. M.; Polakowski, J. S.; Adler, A. L.; Calzadilla, S. V.; Kover, P.; Yao, Z.; Hutchins, C. W.; Denissen, J. F.; Grabowski, B. A.; Cepa, S.; Hoffman, D. J.; Garren, K. W.; Kleinert, H. D. *J. Med. Chem.* **1994**, *37*, 2991.

2) a) Shibata, S.; Yamada, Y.; Ikemoto, Y.; Tada, H.; Shirakawa, E.; Ando, K.; Tsuji, H.; Uchida, I.; Nakamura, I.; Hayashi, Y.; Inui, J.; Ikegami, K. *The 111th Anual Meeting of the Pharmaceutical Society of Japan.* **1991**, Abstract, 2, P 201. b) Shibata, S.; Yamada, Y.; Tada, H.; Shirakawa, E.; Ando, K.; Kato, S.; Uchida, I. *The 111th Anual Meeting of the Pharmaceutical Society of Japan.* **1991**, Abstract, 2, P 90. c) Yamada, Y; Ando, K.; Ikemoto, Y.; Tada, H.; Shirakawa, E.; Shibata, S.; Nakamura, I.; Hayashi, Y.; Ikegami, K.; Uchida, I., submitted for publication in *Chem. Pharm. Bull.*

3) Renin inhibitors containing 1,2,3,4-tetrahydropyrrolo[3,4-*b*]indol-3-one or 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indol-1-one have been reported.: Kempf, D. J.; Condon, S. L. *J. Org. Chem.* **1990**, *55*, 1390.

4) a) Synthetic procedure of compound **6** : To a stirred solution of indole-2-carboxylic acid (140 mg, 0.867 mmol) in DMF (3 ml) was successively added HOBT (138 mg, 1.024 mmol) and DCC (179 mg, 0.867 mmol) at 0 °C. After being stirred for 2 h at 0 °C, a solution of compound **3** (300 mg, 0.788 mmol) in DMF (3 ml) was added to the mixture. The mixture was warmed to room temperature and stirred for 13 h. After filtration of the mixture, the filtrate was evaporated *in vacuo*. To the residue was added a combined solution of MeOH, water, and AcOH (94 : 3 : 3), and the mixture was heated at 60 °C for 30 min. After evaporation *in vacuo*, the residue was dissolved in $CHCl_3$, and the organic solution was washed twice with sat. $NaHCO_3$, dried over $MgSO_4$, then evaporated *in vacuo*. The residue was purified by silica gel chromatography with $CHCl_3$ and MeOH (20 : 1) as an eluent to give **6** (237 mg, 57 %) as a colorless amorphous solid.

Other compounds listed in Table 1 were prepared by essentially the same methods.

b) Physical data of typical compounds :

6: ^{1}H-NMR ($CDCl_3$) δ ; 0.6-1.8 (m, 16H), 0.86 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.5 Hz), 3.15 (m, 2H), 3.60 (m, 1H), 3.90 (m, 1H), 4.04 (m, 1H), 5.04 (m, 1H), 6.80 (s, 1H), 7.0-7.3 (m, 3H), 7.40 (d, 1H, J = 8.2 Hz), 7.49 (s, 1H), 7.62 (d, 1H, J = 7.8 Hz). SIMS *m/z* $[M+H]^+$ 524.

8: ^{1}H-NMR (CD_3OD) δ ; 0.8-2.0 (m, 16H), 0.93 (d, 6H, J = 6.6 Hz), 3.0-3.4 (m, 2H), 3.56 (m, 1H), 3.84 (m, 1H), 3.99 (m, 1H), 4.90 (m, 1H), 7.00 (s, 1H), 7.47 (s, 1H), 7.67 (m, 2H), 7.84 (m, 2H), 8.38 (d, 1H, J = 8.1 Hz). FABMS *m/z* $[M+H]^+$ 552.

12: ^{1}H-NMR (CD_3OD) δ ; 0.8-2.0 (m, 16H), 0.92 (d, 6H, J = 6.6 Hz), 3.1-3.4 (m, 2H), 3.55 (m, 1H), 3.84 (m, 1H), 4.00 (m, 1H), 4.92 (m, 1H), 7.01 (s, 1H), 7.37 (m, 2H), 7.64 (s, 1H), 7.69 (m, 2H). FABMS *m/z* $[M+H]^+$ 525.

15: ^{1}H-NMR ($CDCl_3$) δ ; 0.6-1.8 (m, 16H), 0.83 (d, 3H, J = 6.8 Hz), 0.91 (d, 3H, J = 6.4 Hz), 3.14 (m, 2H), 3.60 (m, 1H), 3.90 (m, 1H), 4.03 (m, 1H), 5.05 (m, 1H), 6.79 (s, 1H), 6.97 (m, 2H), 7.1-7.4 (m, 2H), 7.48 (s, 1H). FABMS *m/z* $[M+H]^+$ 542.

Other compounds listed in Table 1 were also characterized by ^{1}H-NMR and MS.

5) *In vitro* renin inhibitory assay : The renin inhibitory effect of compounds was evaluated for plasma renin activity (PRA) of human plasma. The assay was consisted of 200 μl of EDTA-plasma, 20 μl of citrate buffer, 10 μl of phenylmethylsulfonyl fluoride, and 10 μl of various concentrations of compound in DMSO. The reaction mixture was incubated at 37 °C for 60 min. After incubation, the PRA was estimated by measurement of the produced angiotensin I, which was quantified by radioimmunoassay using a commercial kit (RENIN RIABEAD, Dinabot Ltd., Japan). The percentage of inhibition was calculated at each concentration of point, and the concentration of renin inhibitors that inhibited PRA by 50 % (IC_{50}) was estimated. The species specificity of renin inhibitors was evaluated by comparison of the renin inhibitory effect on human, cynomolgus monkey, marmoset, dog, and rat plasmas.

6) a) Karanewsky, D. S.; Badia, M. C.; Cushman, D. W.; DeForrest, J. M.; Dejneka, T.; Lee, V. G.; Loots, M. J.; Petrillo, E. W. *J. Med. Chem.*, **1990**, *33*, 1459. b) Klutchko, S.; Blankley, C. J.; Fleming, R. W.; Hinkley, J. M.; Werner, A. E.; Nordin, I.; Holmes, A.; Hoefle, M. L.; Cohen, D. M.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1986**, *29*, 1953.

7) The reported pKa values of indole-NH and benzimidazole-NH are 16.97 and 13.2, respectively.: Gilchlist, T. L. "Heterocyclic Chemistry" **1985**, Pitman Publishing, London.

8) *In vivo* marmoset model : Conscious marmosets raised on a low salt diet (containing 0.02 % salt, 1/10 of the normal diet) for a week were orally administered with the inhibitor (10 mg/kg) dissolved in 0.1 M citric acid, at the ratio of 2 ml/kg. The blood pressure was measured with the lapse of time before and after the administration by the tail-cuff method.

9) Enzyme specificity : The enzyme specificity of renin inhibitors was evaluated by comparison of the inhibitory effect on renin, pepsin, cathepsin D, and ACE. Porcine pepsin (Sigma Chemical Co., USA) or bovine cathepsin D (Sigma Chemical Co.) was incubated at 37 °C for 10 or 30 min with bovine hemoglobin (Sigma Chemical Co.) as a substrate in the presence and absence of inhibitor. After incubation, proteins were precipitated with trichloroacetic acid and absorbance of the supernatant was measured at the wavelength of 280 nm. Inhibition of human serum ACE was estimated using a commercial assay kit (ACE color, Fujirebio Inc., Japan). Human serum was incubated at 37 °C for 20 min with the substrate mixture of *p*-hydroxybenzoyl-glycyl-L-histidyl-L-leucine and hippuricase in the presence and absence of inhibitor. The reaction mixture was incubated with $NaIO_4$ and absorbance of solution was measured at the wavelength of 505 nm.

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