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AN EFFICIENT SYNTHESIS OF (3*S*,5*S*)-5-[3,3-DIMETHYL-1-(*o*-TOLYL)-6-OXO-2*H*-PYRIDIN-4-YL]-PIPERIDINE-3-CARBOXAMIDE AS POTENT RENIN INHIBITOR

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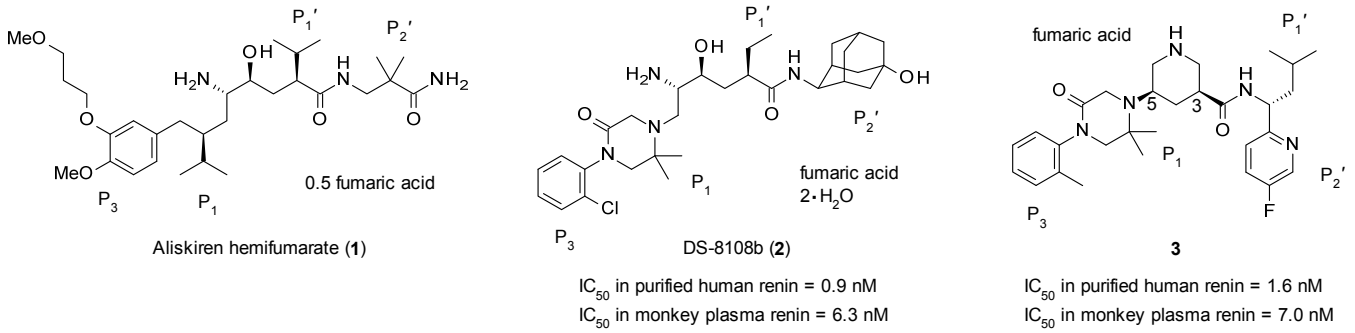
Abstract – We report synthesis and biological evaluation of (3*S*,5*S*)-5-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]piperidine-3-carboxamide as renin inhibitor. This effective synthetic route involves a zinc mediated Barbier reaction and an intramolecular Horner-Wadsworth-Emmons reaction of sterically hindered ketone as key reactions. The prepared compound **4** exhibited both potent renin inhibitory activity and significant *in vivo* efficacy in furosemide pretreated cynomolgus monkeys.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular disease, including chronic heart and kidney failures, myocardial infarction and stroke and is one of the leading causes of death in the developed world.[1] The renin-angiotensin-aldosterone system (RAAS) plays an important role in the regulation of blood pressure (BP) and fluid homeostasis.[2] The inhibition of either the formation or the action of angiotensin II (Ang II), the main product of the RAAS, represents a major therapeutic approach in the treatment of hypertension and the prevention of associated comorbidities.[3a,b] It has long been hypothesized that inhibition of renin, which is the rate-limiting enzyme in the RAAS cascade, may represent the most attractive therapeutic

strategy to block the RAAS.[4] However, only Aliskiren hemifumarate (**1**) has reached the market for the treatment of essential hypertension (Figure 1).[5,6]

In the preceding papers,[7,8] starting from our clinical candidate DS-8108b (**2**),[9,10] we discovered a 3,5-disubstituted piperidine derivative with 2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl group at the 5-position as a new type of renin inhibitor (Figure 1). The potent compound **3** obtained by the chemical modification of the P_1', P_2' and P_3 portion showed a significant BP lowering effect by oral administration in two hypertensive animal models, double transgenic rats[11] and furosemide pretreated cynomolgus monkeys. The renin inhibitory activity of **3** was almost the same level as that of DS-8108b (**2**), however, the potential for chemical modification of 5,5-dimethylpiperazin-2-one ring of the P_1 position remains due to the limitation on the synthesis and due to the requirement to keep intact our original partial structure. Regarding the P_1 portion, we previously reported the existence of hydrophobic interaction of *gem*-dimethyl group on 5,5-dimethylpiperazin-2-one ring with S_1 pocket of human renin as well as hydrogen bond interaction between the carboxamide oxygen on the ring and Tyr77 by X-ray crystallography.[9,12] However, the interaction between basic nitrogen on 5,5-dimethylpiperazin-2-one ring and the target enzyme was not observed. With consideration of these findings, we designed **4** having 3,3-dimethyl-1,2-dihydropyridine-6-one part as the P_1 portion (Scheme 1). Herein, we report an efficient synthesis of (3*S*,5*S*)-5-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]piperidine-3-carboxamide that demonstrates both potent renin inhibitory activity and significant *in vivo* efficacy in furosemide pretreated cynomolgus monkeys.



IC_{50} in purified human renin = 0.9 nM
IC_{50} in monkey plasma renin = 6.3 nM

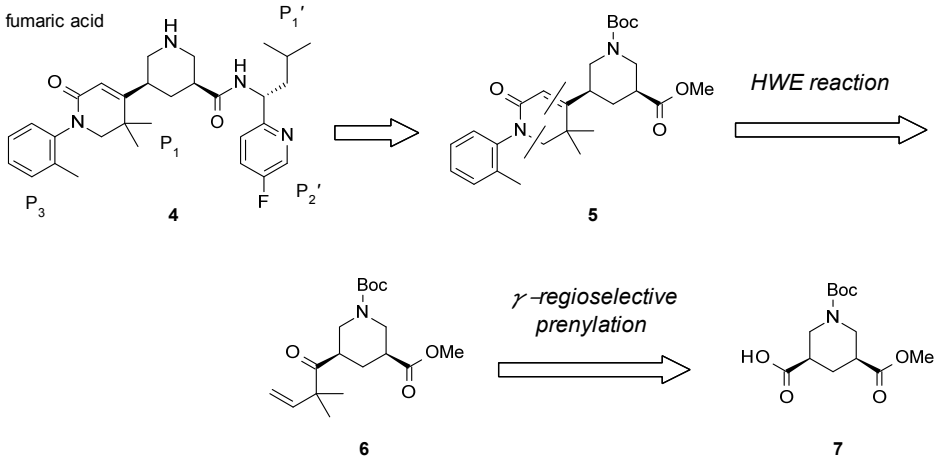
IC_{50} in purified human renin = 1.6 nM
IC_{50} in monkey plasma renin = 7.0 nM

Figure 1. Chemical structures of Aliskiren hemifumarate (**1**) and originally designed renin inhibitors, DS-8108b (**2**) and **3** containing 2,2-dimethyl-4-phenylpiperazin-5-one part

RESULTS AND DISCUSSION

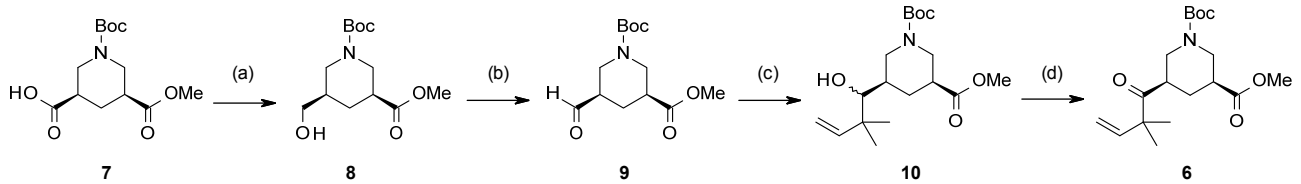
The retrosynthetic analysis of **4** is shown in Scheme 1. Compound **4** would be produced from **5**, which would be synthesized from ketone **6** by using the inter- or intramolecular Horner-Wadsworth-Emmons (HWE) reaction as the key step. Ketone **6** would be obtained through construction of quaternary carbon atom by γ-regioselective prenylation of aldehyde derivative prepared from chiral carboxylic acid **7**, which

is available on a large scale as described in previous paper.[8]



Scheme 1. Retrosynthetic analysis of **4**

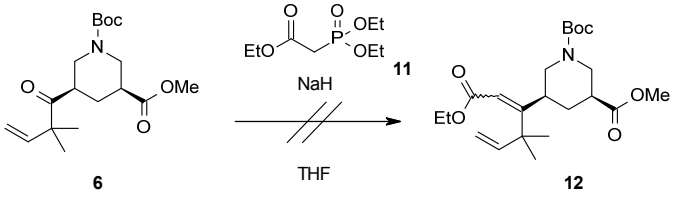
The synthetic pathway leading to the intermediate **6** is outlined in Scheme 2. Initially, known chiral carboxylic acid **7**[8] was reduced with borane-THF complex to give alcohol **8** in 91% yield. The following Swern oxidation of alcohol **8** yielded aldehyde **9**. Next, γ-regioselective prenylation of the aldehyde **9** by prenyl bromide was investigated. At first, a $CrCl_2$ mediated Nozaki-Hiyama reaction was attempted, however, the yield was moderate and the reproducibility was poor (45~68%, 2 steps). Then, a Barbier reaction using other metal was conducted alternatively. As a result, γ-regioselective prenylation proceeded smoothly by using zinc dust in the mixed solvent of NH_4Cl aq. and THF.[13] Finally, Swern oxidation of **10** led to the intermediate **6** (87%, 3 steps).



Scheme 2. Synthetic pathway leading to **6**. Reagents and conditions: (a) BH_3-THF, THF, −78 °C to rt, 1 h, 91%; (b) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, −78 °C to 0 °C, 2 h; (c) prenyl bromide, zinc dust, NH_4Cl aq., THF, rt, 1.5 h; (d) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, −78 °C to 0 °C, 2 h, 87% (3 steps)

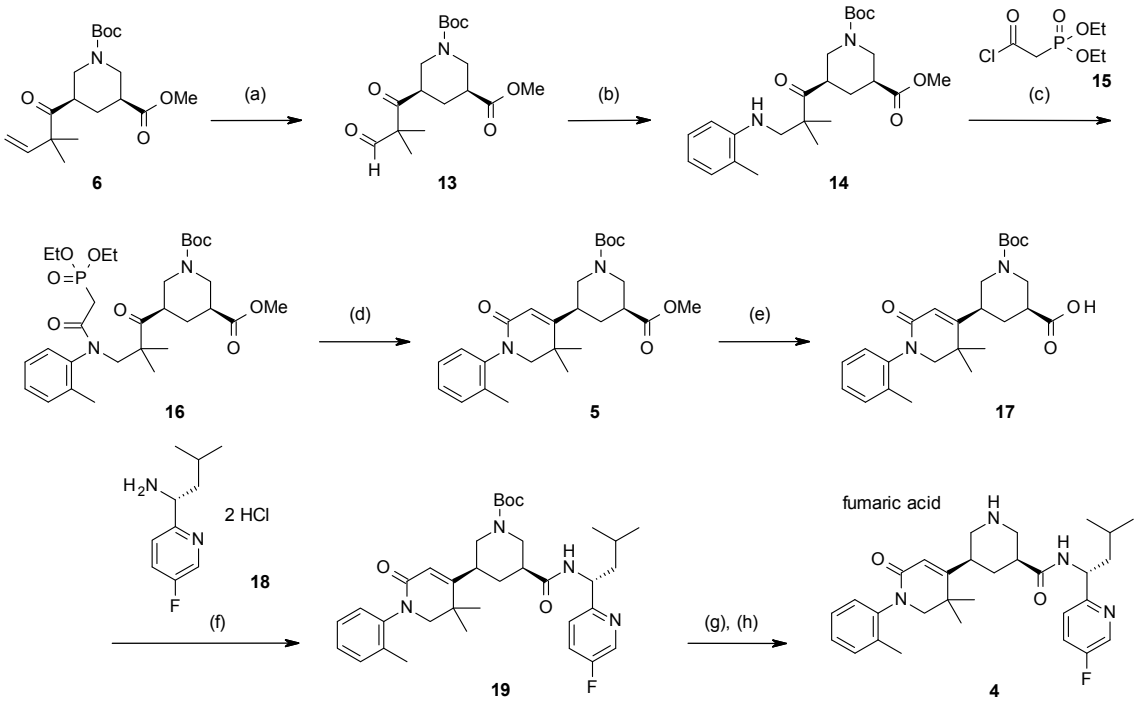
As a next step, an intermolecular HWE reaction of ketone **6** with **11** was conducted (Scheme 3). However, desired compound **12** was not obtained and most of the ketone **6** was recovered (89% recovery). This suggested that steric bulkiness around the carbonyl group of **6** was too high to accomplish the intermolecular HWE reaction. Thus, we focused on an alternative route involving the intramolecular HWE reaction which was reported to be effective for the substrates containing sterically hindered

carbonyl groups.[14]



Scheme 3. Intermolecular HWE reaction of **6** with **11**

The synthetic pathway leading to the target compound **4** is outlined in Scheme 4. Ozonolysis of the terminal vinyl group of **6** delivered aldehyde **13** in 81% yield. The following reductive amination between the aldehyde **13** and *o*-toluidine yielded aniline **14** (91%). The aniline **14** was acylated with freshly prepared CH_2Cl_2 solution of phosphorylacetyl chloride **15,**[15] providing the phosphono acetanilide **16** in 88% yield. Next, the key intramolecular HWE reaction of **16** was investigated. In our initial experiment, using NaH as a base produced **5** in 36% yield. After our efforts to improve the yield, compound **5** was obtained in 72% by using the KHMDS in the presence of 18-crown-6. Hydrolysis of ester **5** with $LiOH{\cdot}H_2O$ led carboxylic acid **17** in 92% yield. Amidation of **17** with chiral amine **18**[8] quantitatively proceeded to give **19**. Finaly, removal of the *N*-*tert*-butoxycarbonyl (Boc) group of **19** was followed by addition of fumaric acid to give the target compound **4** as fumarate salt (81%, 2 steps).



Scheme 4. Synthetic pathway leading to **4**. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C, 3 h, then PPh_3, 4 °C, 18 h, 81%; (b) *o*-toluidine, AcOH, toluene, 80 °C, 1 h, then $NaBH(OAc)_3$, rt, 1.5 h, 91%; (c) **15** in CH_2Cl_2, DMA, rt, 1 h, 88%; (d) 0.5 M KHMDS in toluene, 18-crown-6, THF, −78 °C to −20 °C, 3 h, 72%; (e) $LiOH{\cdot}H_2O$, THF, water, 0 °C, 1 h, 92%; (f) amine **18**, HBTU, *N*,*N*-diisopropylethylamine, DMF, 0 °C, 1 h, quant.; (g) TFA, CH_2Cl_2, rt, 40 min; (h) fumaric acid, MeOH, rt, 5 min, 81% (2 steps)

With **4** in hand, we carried out biological evaluation of **4**. Initially, renin inhibitory activity was measured (Table 1). Compound **4** showed high renin inhibitory activity. Especially, inhibitory activity against purified human renin (0.7 nM) was higher than that of **1** (1.5 nM, in-house data)[9] or **3** (1.6 nM). Next, to evaluate the *in vivo* efficacy of **4**, vehicle or 10 mg/kg of **4** were orally administered to cynomolgus monkeys pretreated with furosemide (Figure 2). Compound **4** showed a significant mean arterial blood pressure (MAP) reduction which was comparable to that of **3**. ($AUC_{0\text{-}24h}$ of BP lowering effect of **4** and **3** was −242 mmHg·h and −267 mmHg·h[8] respectively.)[16]

Table 1. *In vitro* renin inhibitory activities (IC_{50} and ratio) of **3** and **4**[a,b]

Compound	Purified human renin	Monkey plasma renin	
	IC_{50} (nM)	IC_{50} (nM)	Ratio[c]
3	1.6	7.0	0.9
4	0.7	2.7	0.8

[a] Compounds were obtained as fumarate salts. [b] Assay results of renin inhibitory activity are the average of at least two replicates. [c] Ratio = IC_{50} (nM) of compound / IC_{50} (nM) of **1**.

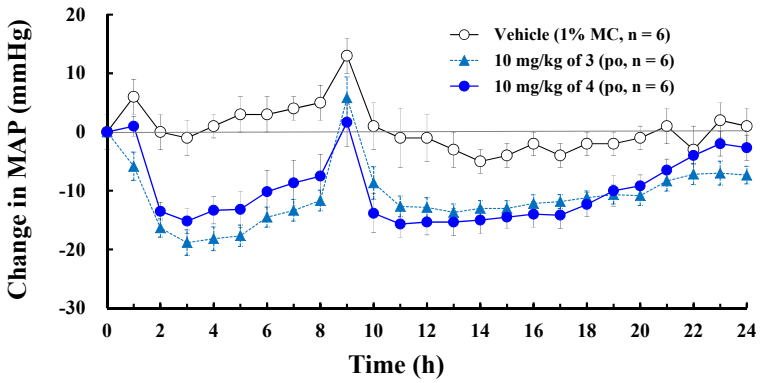


Figure 2. Effect of compound **3**[a] and **4** on MAP in cynomolgus monkeys pretreated with furosemide

[a] The data of compound **3** was cited from ref. 8.

In summary, we have synthesized and evaluated (3*S*,5*S*)-5-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]piperidine-3-carboxamide as novel renin inhibitor. The synthesis was achieved by using a zinc mediated Barbier reaction and an intramolecular HWE reaction of sterically hindered ketone as key steps. The prepared compound **4** exhibited potent renin inhibitory activity. In addition, **4** showed significant BP lowering effect by oral administration in furosemide pretreated cynomolgus monkeys. Thus, we selected

this newly designed 3,5-disubstituted piperidine derivative as appropriate candidate for further optimization and evaluation.

EXPERIMENTAL

Synthesis

Starting reagents were purchased from commercial suppliers and used without further purification unless otherwise specified. Flash column chromatography was performed on silica gel 60 N (spherical, neutral), 40-50 mesh, purchased from Kanto Chemical Co., Inc., or NH silica gel, 100-200 mesh, purchased from Fuji Silysia Chemical Ltd. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Unity 400 or 500 spectrometer, or a Bruker Avance III 500 spectrometer. Spectra were taken in the indicated solvent at ambient temperature, and chemical shifts are reported in parts per million (ppm (δ)) relative to the lock of the solvent used. Resonance patterns are recorded with the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained on a JEOL JMS-LCmate or an LC-MS system composed of Waters Xevo Q-Tof MS and Acquity UPLC systems. Optical rotations were measured on an Autopol V Plus. Infrared spectra were recorded in a KBr disc or ATR mode with a Jasco FT/IR-6100.

1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-hydroxymethylpiperidine-1,3-dicarboxylate (8)

BH_3-THF (133 mL, 1.09 M THF solution, 145 mmol) was added dropwise to a solution of **7**[8] (27.7 g, 96.4 mmol) in THF (500 mL) at −78 °C over a period of 25 min. After warming the reaction mixture to room temperature (rt), the mixture was stirred at the same temperature for 1 h. After cooling to 0 °C, saturated $NaHCO_3$ aq. was added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 3/1 to 1/3) to obtain **8** (24.1 g, 91%) as a colorless liquid. $[\alpha]_D^{25.0}$ 23.4 (*c* 1.06, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.38-4.26 (br m, 1H), 4.23-4.16 (br m, 1H), 3.69 (s, 3H), 3.57-3.50 (m, 2H), 2.77-2.72 (m, 1H), 2.53-2.46 (m, 1H), 2.42 (dd, *J* = 15.9, 8.5 Hz, 1H), 2.15 (d, *J* = 13.7 Hz, 1H), 1.76-1.70 (m, 1H), 1.53-1.48 (m, 1H), 1.45 (s, 9H), 1.37-1.30 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.3, 154.7, 80.1, 65.1, 51.8, 46.3, 45.8, 41.3, 38.1, 30.4, 28.4. IR: 3440, 2932, 2870, 1733, 1689, 1667, 1422, 1253, 1145, 881, 767 cm^{-1}. HRMS (ESI^{+}): *m/z* calcd for $C_{13}H_{23}NO_5$+H: 274.1654; found: 274.1655.

1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-(2,2-dimethylbut-3-enoyl)piperidine-1,3-dicarboxylate (6)

A solution of DMSO (14.4 mL, 203 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a solution of oxalyl chloride (8.70 mL, 101 mmol) in CH_2Cl_2 (300 mL) at −78 °C over a period of 20 min, and then the mixture was stirred at the same temperature for 20 min. To the reaction mixture, a solution of **8** (24.1 g, 88.2 mmol) in CH_2Cl_2 (150 mL) was added dropwise at −78 °C over a period of 25 min, and then the mixture was stirred at the same temperature for 20 min. To the reaction mixture, Et_3N (68.0 mL, 488 mmol) was added dropwise at −78 °C over a period of 15 min. After warming the reaction mixture to 0 °C, the mixture was stirred at the same temperature for 2 h. H_2O was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was washed with 1*N* HCl aq., saturated $NaHCO_3$ aq., and brine, and dried over anhydrous Na_2SO_4. After filtration, the filtrate was diluted with AcOEt. The solution was filtered through the silica gel pad. The filtrate was evaporated under reduced pressure to obtain crude **9** (23.3 g) as a colorless liquid. Saturated NH_4Cl aq. (60.0 mL) was added to a suspension of the crude **9** (23.3 g), prenyl bromide (14.4 mL, 125 mmol), and zinc dust (11.0 g, 168 mmol) in THF (500 mL) at 0 °C, and then the mixture was stirred at rt for 1.5 h. The reaction mixture was filtered through the celite pad. The filtrate was evaporated under reduced pressure until the volume of the solution was about 250 mL. The residue was extracted with AcOEt. Then, the organic layer was washed with 1*N* HCl aq., saturated $NaHCO_3$ aq., and brine, and dried over anhydrous Na_2SO_4. After filtration, the solution was filtered through the silica gel pad. The filtrate was evaporated under reduced pressure to obtain crude **10** (28.1 g) as a colorless solid. A solution of DMSO (14.0 mL, 197 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a solution of oxalyl chloride (8.50 mL, 99.1 mmol) in CH_2Cl_2 (300 mL) at −78 °C over a period of 20 min, and then the mixture was stirred at the same temperature for 20 min. To the reaction mixture, a solution of crude **10** (28.1 g) in CH_2Cl_2 (150 mL) was added dropwise at −78 °C over a period of 20 min, and then the mixture was stirred at the same temperature for 30 min. To the reaction mixture, Et_3N (63.0 mL, 452 mmol) was added dropwise at −78 °C over a period of 20 min. After warming the reaction mixture to 0 °C, the mixture was stirred at the same temperature for 2 h. H_2O was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was washed with 1*N* HCl aq., saturated $NaHCO_3$ aq., and brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 9/1 to 2/1) to obtain **6** (25.9 g, 87%, 3 steps) as a colorless solid. $[\alpha]_D^{25.0}$ −31.0 (*c* 1.00, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.91 (dd, *J* = 17.6, 10.7 Hz, 1H), 5.24 (d, *J* = 10.7 Hz, 1H), 5.22 (d, *J* = 17.6 Hz, 1H), 4.38-4.29 (m, 1H), 4.13-4.01 (m, 1H), 3.68 (s, 3H), 2.98-2.93 (m, 1H), 2.81-2.70 (m, 1H), 2.69-2.60 (m, 1H), 2.49-2.42 (m, 1H), 2.06-2.01 (m, 1H), 1.80-1.72 (m, 1H), 1.46 (s, 9H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 212.2, 173.0, 154.3, 141.1, 115.8, 80.2, 51.9, 51.6, 47.0, 45.3, 43.2, 40.4, 31.1, 28.4, 23.0, 22.9. IR: 2978, 1731, 1689, 1421, 1163, 1149, 963, 770 cm^{-1}. HRMS (ESI^+): *m/z* calcd for $C_{18}H_{29}NO_5$+H: 340.2124; found:

340.2153.

1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-(2,2-dimethyl-3-oxopropanoyl)piperidine-1,3-dicarboxylate (13)

Ozonized oxygen was bubbled through a solution of **6** (25.9 g, 76.3 mmol) in CH_2Cl_2 (380 mL) at −78 °C for 3 h. The end of ozonolysis was indicated by the blue color appearance in the reaction mixture. The ozone stream was then stopped and the solution was flushed with N_2 for 1.5 h to remove excess ozone. Triphenylphospine (26.0 g, 99.1 mmol) was then added at −78 °C and the reaction mixture was allowed to reach 4 °C and was stirred for additional 18 h. Silica gel (260 g) and Et_2O (380 mL) was added to the reaction mixture. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 19/1 to 3/2) to obtain **13** (21.1 g, 81%) as a colorless solid. $[\alpha]_D^{25.0}$ −49.8 (*c* 1.04, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 4.39-4.27 (m, 1H), 4.17-4.06 (m, 1H), 3.69 (s, 3H), 2.87-2.61 (m, 3H), 2.50-2.42 (m, 1H), 2.16-2.09 (m, 1H), 1.80-1.71 (m, 1H), 1.47 (s, 9H), 1.39 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 209.2, 200.5, 172.7, 154.2, 80.5, 61.0, 52.0, 46.6, 45.6, 44.8, 40.3, 30.4, 28.4, 18.9, 18.8. IR: 3411, 2979, 1730, 1693, 1425, 1254, 1147, 960, 892 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{27}NO_6$+H: 342.1917; found: 342.1918.

1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-[2,2-dimethyl-3-(2-methylanilino)propanoyl]piperidine-1,3-dicarboxylate (14)

To a solution of **13** (10.0 g, 29.3 mmol) in toluene (75.0 mL), *o*-toluidine (4.71 mL, 44.4 mmol) and AcOH (2.51 mL, 43.9 mmol) were added at rt, and the mixture was stirred at the same temperature for 15 min. Then, the mixture was stirred at 80 °C for 1 h. After cooling in an ice bath, $NaBH(OAc)_3$ (19.6 g, 92.5 mmol) and AcOH (5.28 mL, 92.2 mmol) were added to the reaction mixture. The mixture was further stirred at rt for 1.5 h. After cooling in an ice bath, saturated $NaHCO_3$ aq. was added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with 1*N* HCl aq., saturated $NaHCO_3$ aq., and brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 19/1 to 4/1) to obtain **14** (11.5 g, 91%) as a light yellow liquid. $[\alpha]_D^{25.0}$ −37.1 (*c* 1.01, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14-7.10 (m, 1H), 7.04 (d, *J* = 6.4 Hz, 1H), 6.69-6.64 (m, 2H), 4.40-4.30 (m, 1H), 4.16-4.04 (m, 1H), 3.67 (s, 3H), 3.30-3.22 (m, 2H), 3.07-2.99 (m, 1H), 2.80-2.64 (m, 2H), 2.49-2.42 (m, 1H), 2.10 (s, 3H), 2.08-2.05 (m, 1H), 2.04-2.02 (m, 1H), 1.80 (q, *J* = 12.6 Hz, 1H), 1.45-1.36 (m, 9H), 1.31 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 215.1, 172.9, 154.3, 146.1, 130.2, 127.1, 122.4, 117.3, 109.9, 80.3, 51.9, 51.7 49.5, 47.0, 45.4, 42.8, 40.5, 30.9, 28.3, 22.5, 22.4, 17.4. IR: 2972, 1735, 1689, 1419, 1251, 1144, 858, 745 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{24}H_{36}N_2O_5$+H:

433.2702; found: 433.2683.

1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-{3-[*N*-(2-diethoxyphosphorylacetyl)-2-methylanilino]-2,2-dimethylpropanoyl}piperidine-1,3-dicarboxylate (16)

Oxalyl chloride (3.35 mL, 38.5 mmol) was added to a solution of (diethoxyphosphinoyl)acetic acid (9.20 g, 46.9 mmol) in CH_2Cl_2 (120 mL) at 0 °C, and then the mixture was stirred at rt for 5 d. The solvent was evaporated under reduced pressure. After azeotropic drying with CH_2Cl_2, CH_2Cl_2 (38.0 mL) was added to the residue to obtain a crude **15** (*ca.* 1.00 M CH_2Cl_2 solution). The crude **15** (32.7 mL, *ca.* 1.00 M CH_2Cl_2 solution) was added to a solution of **14** (9.29 g, 21.5 mmol) in DMA (70.0 mL) at 0 °C for 3 h, and then the mixture was stirred at rt for 1 h. After cooling, saturated $NaHCO_3$ aq. was added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, AcOEt) to obtain **16** (11.6 g, 88%) as a colorless liquid. $[\alpha]_D^{25.0}$ −17.9 (*c* 1.00, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.18 (m, 4H), 4.46 (dd, *J* = 33.8, 13.9 Hz, 1H), 4.32-4.19 (m, 1H), 4.17-4.02 (m, 5H), 3.96-3.92 (m, 1H), 3.68-3.65 (m, 3H), 3.47-3.25 (m, 1H), 2.96-2.82 (m, 1H), 2.76-2.56 (m, 3H), 2.50-2.29 (m, 2H), 2.24-2.22 (m, 3H), 1.86-1.81 (m, 1H), 1.46-1.44 (m, 9H), 1.36-1.24 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 213.1, 213.0, 173.0, 172.9, 166.5, 166.4, 154.2, 141.5, 141.4, 135.5, 132.0, 131.8, 130.1, 129.8, 128.8, 128.7, 127.3, 127.0, 80.3, 62.5, 62.4, 62.3, 54.2, 54.0, 51.8, 49.8, 49.7, 45.4, 42.9, 40.5, 33.5, 32.4, 32.3, 30.5, 30.3, 28.6, 28.4, 23.8, 22.3, 22.1, 21.0, 17.8, 16.4, 16.3. IR: 2978, 1734, 1691, 1659, 1248, 1146, 1022, 957, 773 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{30}H_{47}N_2O_9P$+H: 611.3097; found: 611.3141.

1-*tert*-Butyl 3-methyl (3*S*,5*S*)-5-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]piperidine-1,3-dicarboxylate (5)

After azeotropic drying of **16** (11.4 g, 18.7 mmol) with toluene, 18-crown-6 (4.93 g, 18.7 mmol) and THF (350 mL) were added to the residue at rt, and then the mixture was stirred at −78 °C for 30 min. KHMDS (37.3 mL, *ca.* 0.5 M in toluene) was added to the reaction mixture at −78 °C, and then the mixture was stirred at the same temperature for 1 h. After warming the reaction mixture to −20 °C, the mixture was stirred at the same temperature for 3 h. Saturated NH_4Cl aq. and AcOEt were added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with saturated NH_4Cl aq. and brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 2/1) to obtain **5** (5.92 g, 72%) as a colorless solid. $[\alpha]_D^{25.0}$ −24.3 (*c* 1.00, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.19 (m, 3H), 7.14-7.10 (m, 1H), 5.85 (d, *J* = 4.3 Hz, 1H), 4.46-4.35 (br

m, 1H), 4.28-4.15 (br m, 1H), 3.71 (s, 3H), 3.51 (dd, *J* = 12.1, 8.2 Hz, 1H), 3.41 (dd, *J* = 12.1, 5.1 Hz, 1H), 2.83-2.75 (m, 1H), 2.59-2.49 (m, 2H), 2.34-2.18 (m, 5H), 1.75-1.64 (m, 1H), 1.48 (s, 9H), 1.30-1.24 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): 172.9, 163.7, 162.1, 154.3, 141.3, 135.7, 131.1, 127.6, 127.1, 126.3, 120.3, 80.3, 61.9, 52.0, 49.6, 45.5, 41.5, 36.8, 36.5, 34.5, 28.4, 24.4, 24.3, 18.2. IR: 2973, 1736, 1694, 1667, 1474, 1421, 1255, 1150, 874, 748 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{26}H_{36}N_2O_5$+H: 457.2702; found: 457.2702.

(3*S*,5*S*)-1-*tert*-Butoxycarbonyl-5-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]piperidine-3-carboxylic acid (17)

$LiOH·H_2O$ (1.09 g, 26.0 mmol) was added to a solution of **5** (5.92 g, 13.0 mmol) in THF (80.0 mL) and H_2O (40.0 mL) at 0 °C, and then the mixture was stirred at the same temperature for 1 h. 1 *N* HCl aq. (26.0 mL, 26.0 mmol) and CH_2Cl_2 were added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was washed with H_2O, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and then Et_2O was added to the residue. The resulting solid was collected by filtration to obtain **17** (5.27 g, 92%) as a colorless solid. $[\alpha]_D^{25.0}$ −31.1 (*c* 1.01, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.32-7.23 (m, 3H), 7.22-7.16 (m, 1H), 5.84 (s, 1H), 4.40-4.34 (m, 1H), 4.23-4.14 (m, 1H), 3.63-3.45 (m, 2H), 2.90-2.80 (m, 1H), 2.72-2.62 (m, 1H), 2.61-2.52 (m, 1H), 2.49-2.42 (m, 1H), 2.26-2.20 (m, 4H), 1.77-1.68 (m, 1H), 1.48 (s, 9H), 1.33-1.29 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD): 175.9, 166.2, 166.0, 156.1, 142.4, 136.9, 132.0, 128.9, 128.3, 127.5, 119.9, 81.6, 62.7, 51.4, 46.3, 42.4, 38.3, 37.7, 35.5, 28.7, 24.6, 24.5, 18.3. IR: 3418, 2979, 2928, 1717, 1686, 1649, 1425, 1268, 1150, 751 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{25}H_{34}N_2O_5$+H: 443.2546; found: 453.2545.

***tert*-Butyl (3*S*,5*S*)-3-[3,3-dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]-5-{[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (19)**

A solution of HBTU (0.62 g, 1.64 mmol) in DMF (4.00 mL) was added to a solution of **17** (0.600 g, 1.36 mmol), (1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutan-1-amine dihydrochloride **18**[8] (0.420 g, 1.65 mmol), and *N*,*N*-diisopropylethylamine (0.946 mL, 5.42 mmol) in DMF (12.0 mL) under ice-cooling, and then the reaction mixture was stirred at the same temperature for 1 h. Water was added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with 1 *N* HCl aq., saturated $NaHCO_3$ aq., and brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 1/1) to obtain **19** (0.823 g, quant.) as a colorless solid. $[\alpha]_D^{25.0}$ 17.9 (*c* 1.00, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (d, *J* = 2.7 Hz, 1H), 7.39-7.34 (m, 1H), 7.26-7.19 (m, 4H), 7.12-7.08 (m, 1H), 6.55-6.49 (m, 1H), 5.84 (d, *J* = 4.3 Hz, 1H), 5.13 (q, *J* = 7.8 Hz, 1H), 4.38-4.29 (m, 1H),

4.19-4.11 (m, 1H), 3.51-3.46 (m, 1H), 3.41-3.36 (m, 1H), 2.89-2.83 (m, 1H), 2.64-2.51 (m, 1H), 2.42-2.34 (m, 1H), 2.30-2.22 (m, 4H), 1.99-1.94 (m, 1H), 1.86-1.75 (m, 1H), 1.71-1.58 (m, 2H), 1.50-1.44 (m, 10H), 1.29-1.26 (m, 3H), 1.21 (d, J = 4.7 Hz, 3H), 0.96-0.92 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): 171.2, 163.7, 159.6, 157.6, 156.6, 154.4, 141.3, 137.6, 135.7, 131.1, 127.6, 127.1, 126.3, 123.5, 123.2, 120.3, 80.3, 61.8, 51.5, 50.6, 46.4, 45.7, 43.5, 36.9, 36.5, 34.5, 28.4, 24.9, 24.4, 22.7, 22.6, 18.2. IR: 3307, 2960, 1659, 1481, 1255, 1153, 849, 753, 558 cm^{-1}. HRMS (ESI$^+$): *m/z* calcd for $C_{35}H_{47}FN_4O_4$+H: 607.3660; found: 607.3696.

(3*S*,5*S*)-5-[3,3-Dimethyl-1-(*o*-tolyl)-6-oxo-2*H*-pyridin-4-yl]-*N*-[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (4)

Trifluoroacetic acid (5.00 mL) was added to a solution of **19** (0.823 g, 1.36 mmol) in CH_2Cl_2 (10.0 mL) at 0 °C, and the mixture was stirred at rt for 40 min. Saturated $NaHCO_3$ aq. was added to the reaction mixture under ice-cooling, followed by extraction with CH_2Cl_2. Then, the organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by NH silica gel column chromatography (eluent, CH_2Cl_2/MeOH = 20/1 to 7/3) to obtain the free base of **4** (0.610 g, 1.20 mmol). Fumaric acid (0.139 g, 1.20 mmol) was added to a solution of the free base of **4** (0.610 g, 1.20 mmol) in MeOH (1.00 mL) at room temperature, and the mixture was stirred at the same temperature for 5 min. The solvent was evaporated under reduced pressure, and then Et_2O was added to the residue. The resulting solid was collected by filtration to obtain **4** (0.690 g, 81%, 2 steps) as a colorless solid. $[\alpha]_D^{25.0}$ 35.9 (*c* 1.01, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (d, J = 2.3 Hz, 1H), 7.56 (td, J = 8.5, 3.0 Hz, 1H), 7.39 (dd, J = 8.8, 4.5 Hz, 1H), 7.30-7.23 (m, 3H), 7.18-7.14 (m, 1H), 6.70 (s, 2H), 5.85 (d, J = 2.3 Hz, 1H), 5.05 (dd, J = 9.0, 6.3 Hz, 1H), 3.59 (dd, J = 12.5, 11.3 Hz, 1H), 3.52-3.44 (m, 2H), 3.41-3.35 (m, 1H), 3.13-3.06 (m, 1H), 3.00-2.88 (m, 2H), 2.83-2.76 (m, 1H), 2.23-2.14 (m, 4H), 1.76-1.55 (m, 4H), 1.32-1.28 (m, 6H), 0.97 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD): 172.8, 171.5, 165.6, 164.5, 161.2, 159.2, 142.2, 138.2, 136.9, 136.3, 132.1, 129.0, 128.3, 127.5, 125.0, 123.8, 121.3, 62.6, 53.8, 45.8, 45.4, 41.5, 37.6, 35.2, 34.8, 26.2, 24.6, 24.5, 23.3, 22.3, 18.3. IR: 3293, 2960, 1658, 1482, 1388, 1252, 983, 751, 646. HRMS (ESI$^+$): *m/z* calcd for $C_{30}H_{39}FN_4O_2$+H: 507.3135; found: 507.3148.

Biological Assays

IC_{50} in buffer

The activity of renin inhibitors against purified enzyme was measured using the following protocol: All reactions were carried out in a flat bottom black opaque microtiter plate. Test compounds in DMSO (2 μL) were mixed with 100 μL of the assay buffer (50 mM Tris-HCl (pH 7.9), 100 mM NaCl) containing 5

μL of trypsin-activated recombinant human renin (final enzyme concentration of 50 μM), and the solution was pre-incubated at room temperature for 10 min. Next, 2 μM of the substrate (Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(DABCYL)-Arg) in 100 μL of the assay buffer was added, and the resulting mixture was incubated at 37 °C for 90 min. After completion of incubation, the concentration of generated angiotensin I was measured by fluorescence at 492 nm (excitation at 340 nm) using a multilabel reader (Perkin-Elmer Inc.). The slope of the linear portion of the plot of fluorescence increase as a function of time was then determined, and the rate was used to calculate % inhibition in relation to uninhibited control. The % inhibition values were plotted as a function of inhibitor concentration, and the IC_{50} value was determined by probit analysis. The IC_{50} value is defined as the concentration of a particular inhibitor that reduces the formation of product by 50% relative to a control sample containing no inhibitor.

IC_{50} in plasma

The activity of renin inhibitors *in vitro* in cynomolgus monkey plasma was measured by the decrease in plasma renin activity (PRA) levels observed in the presence of the compounds. Compounds and Aliskiren hemifumarate (**1**) were dissolved in DMSO and the final concentration of DMSO was 1%. Incubation mixtures were contained in the final volume of 20 μL of test compound solution, 200 μL of pooled mixed-gender human or cynomolgus monkey plasma stabilized with EDTA, 20 μL of pH adjusting solution, and 10 μL of Inhibitor A solution. The reaction mixture was incubated at 37 °C for 60 min. After incubation, angiotensin I in the reaction mixture was measured by competitive radioimmunoassay using a commercial available RIA kit, RENIN RIABEAD (Yamasa Co.). An uninhibited tube containing 1% DMSO and control tube incubated at 4 °C were used to derive the % inhibition for each concentration of inhibitors. The % inhibition values were plotted as the function of inhibitor concentration, and the IC_{50} value was determined from a fit of this data to a four parameter equation. The IC_{50} value is defined as above.

Animal Studies

Blood pressure study in cynomolgus monkeys pre-treated with furosemide

Arterial pressure was measured by a telemetry system in conscious, freely moving cynomolgus monkeys (n = 6). Pressure transmitters (TL11M2-D70-PCT, Data Sciences International Inc., USA) were implanted into the peritoneal cavity under aseptic conditions and anesthesia, and the sensor catheter was placed in the left femoral artery. Cynomolgus monkeys were allowed to recover for at least 1 week before any experiment. The animals were fasted from the morning on the dosing day. Feeding on the dosing day was conducted 8 hours after dosing or later. The animals were allowed free access to water the whole

time. Furosemide at 5 mg/kg/day was intramuscularly administered for 3 days before drug administration. Cynomolgus monkeys orally received **4** at dose 10 mg/kg, or vehicle (1% methylcellulose). Arterial pressure was continuously measured telemetrically from 3 h before administration to 24 h after administration with the data collection and real-time analysis system (Dataquest™ OpenART™, Data Sciences International, USA). The mean value for 1 h of MAP was calculated.

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