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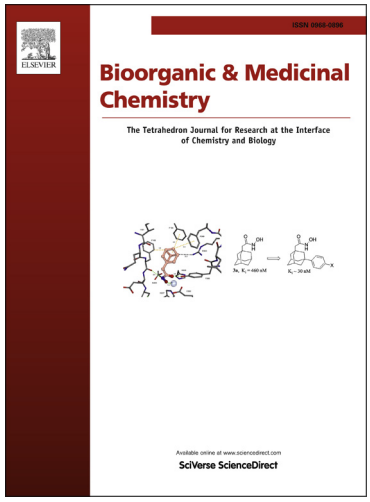

Synthesis and optimization of novel (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenyl-piperazin-1-yl)piperidine-3-carboxamides as orally active renin inhibitors

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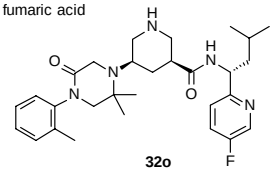
fumaric acid



32o

IC_{50} in purified human renin = 1.6 nM
IC_{50} in human plasma renin = 3.6 nM

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Synthesis and optimization of novel (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides as orally active renin inhibitors

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ABSTRACT

We report synthesis and optimization of a series of (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides as renin inhibitors. Chemical modification of P_1', P_2' and P_3 portions led to a promising 3,5-disubstituted piperidine **32o** showing high renin inhibitory activity and favorable oral exposure in both rats and cynomolgus monkeys with acceptable CYP and hERG current inhibition. Compound **32o** exhibited a significant blood pressure lowering effect by oral administration in two hypertensive animal models, double transgenic rats and furosemide pretreated cynomolgus monkeys.

1. Introduction

Hypertension is a major risk factor for cardiovascular disease, including chronic heart and kidney failure, 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. 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.[3]

During the 1980s, most of the renin inhibitors were based on the modified peptides that incorporated peptide hydrolysis transition state isosteres. However, because of their poor oral bioavailability, none of these inhibitors were ultimately deemed suitable for full development.[4] Beginning in the middle of the 1990s, several novel nonpeptidic renin inhibitors were reported and have entered human clinical trials, such as Aliskiren hemifumarate (**1**)[5], ACT-077825 (MK-8141) (**2**)[6], and VTP-27999 (**3**)[7] (Figure 1). However, only Aliskiren has reached the market for the treatment of essential hypertension.

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Figure 1. Chemical structures of Aliskiren hemifumarate (**1**), ACT-077825 (MK-8141) (**2**), and VTP-27999 (**3**).

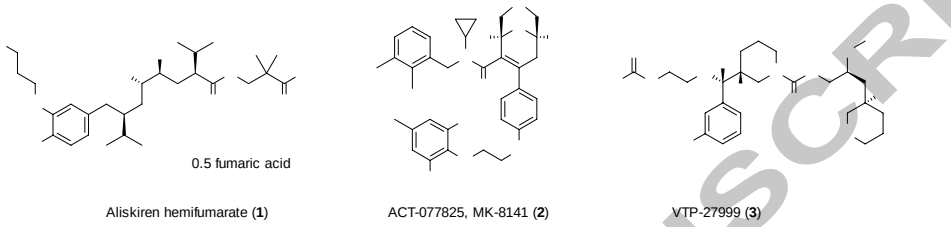
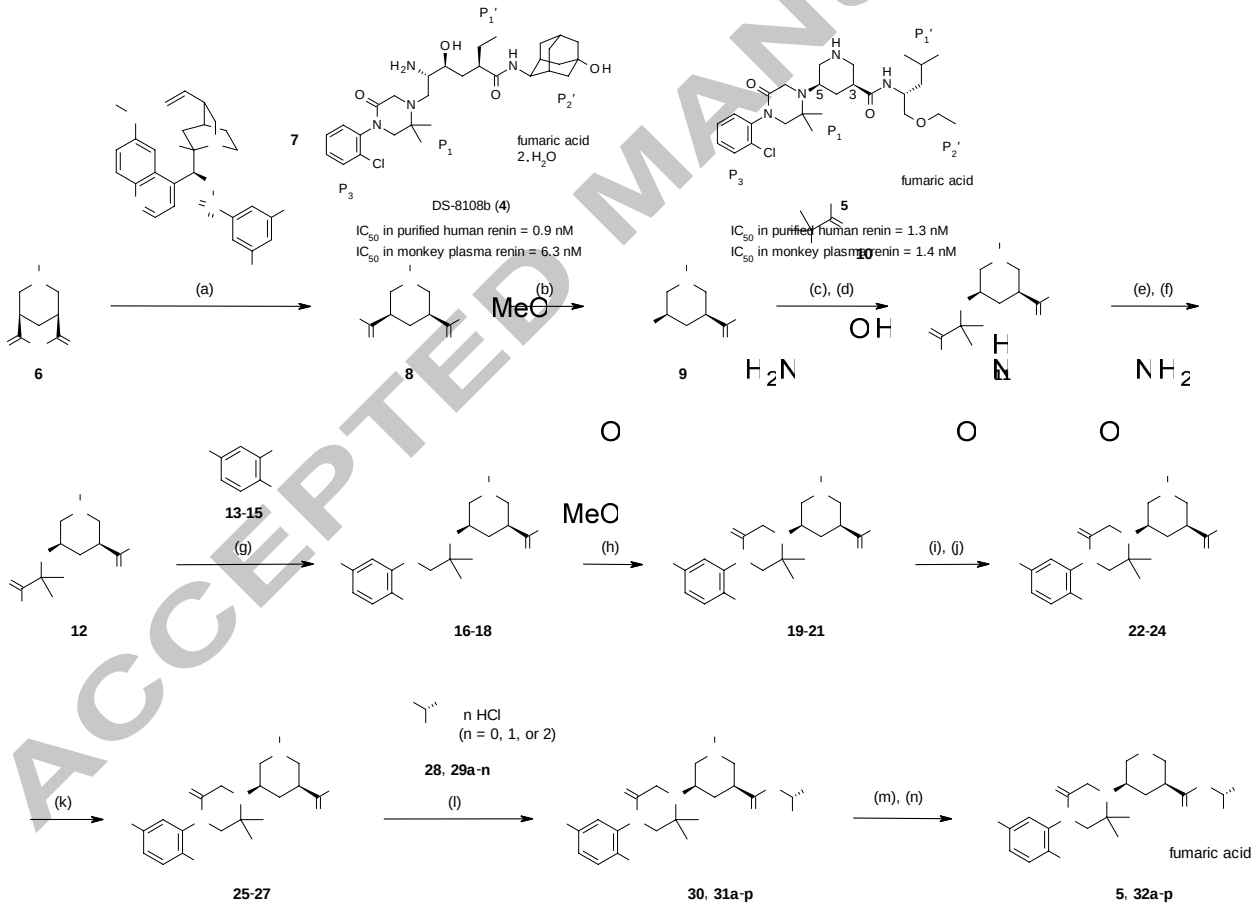
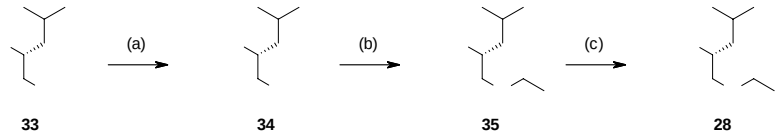


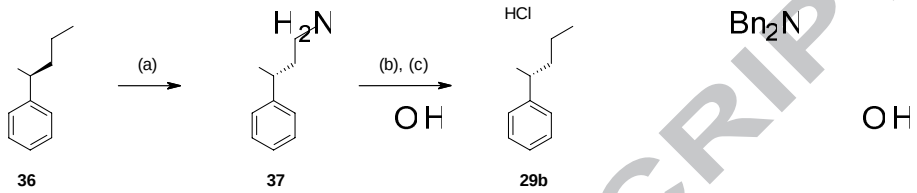
Figure 2. Chemical structures of originally designed renin inhibitors containing 2,2-dimethyl-4-phenylpiperazin-5-one.



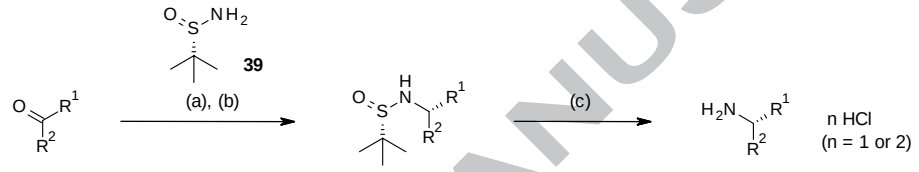
Scheme 1. Synthetic pathway leading to (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides **5** and **32a-p**. Reagents and conditions: (a) **7**, MeOH, THF, −20 °C, 1 day, 69%, 94% ee; (b) diphenylphosphoryl azide, Et_3N, toluene, benzyl alcohol, 90 °C, 5 h, 96%; (c) H_2, Pd on carbon, MeOH, rt, 1.5 h; (d) 2-bromo-2-methylpropanal **10**, DMF, water, rt, 1 day, 73% (2 steps); (e) 4*N*-HCl in 1,4-dioxane, rt, 0.5 h; (f) NsCl, $NaHCO_3$, 1,4-dioxane, water, 0 °C, 1.5 h, 86% (2 steps); (g) aniline **13-15**, AcOH, toluene, 130 °C, then $NaBH(OAc)_3$, rt; (h) bromoacetyl bromide, Et_3N, CH_2Cl_2, 0 °C, then 40 °C; (i) PhSH, Cs_2CO_3, CH_3CN, rt; (j) $(Boc)_2O$, $NaHCO_3$, AcOEt, water, rt; (k) LiOH-H_2O, THF, water, 0 °C; (l) amine **28** or **29a-n**, HBTU, *N*,*N*-diisopropylethylamine, DMF, 0 °C; (m) TFA, CH_2Cl_2, rt; (n) fumaric acid, MeOH, rt.



Scheme 2. Synthetic pathway leading to chiral amine **28**. Reagents and conditions: (a) BnBr, K_2CO_3, EtOH, rt, 4 days, 85%; (b) EtI, NaH, THF, rt, 18 h, 81%; (c) H_2, 20% $Pd(OH)_2$ on carbon, MeOH, rt, 6 h, 98%.



Scheme 3. Synthetic pathway leading to chiral amine **29b**. Reagents and conditions: (a) diphenylphosphoryl azide, DBU, toluene, rt, 2 days, 28%; (b) H_2, Pd on carbon, AcOEt, EtOH, rt, 3 h; (c) 4*N*-HCl in 1,4-dioxane, rt, 63% (2 steps).



Scheme 4. Synthetic pathway leading to chiral amines **29a** and **29d-n**. Reagents and conditions: (a) (*S*)-*t*-$BuSONH_2$ **39**, $Ti(OEt)_4$, THF, 70 °C; (b) L-selectride, THF, 0 °C; (c) 4*N*-HCl in 1,4-dioxane, MeOH, rt.

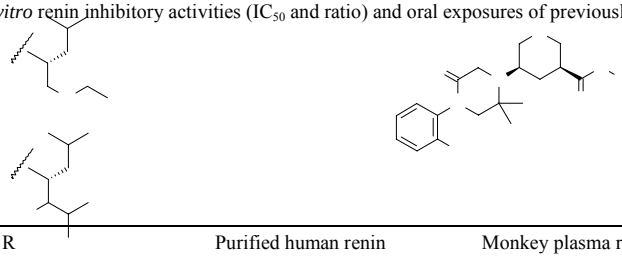
In the preceding paper,[8] starting from our clinical candidate DS-8108b (**4**)[9,10], we discovered a new type of renin inhibitor which is a unique 3,5-disubstituted piperidine derivative (Figure 2). Direct connection with 2,2-dimethyl-4-phenylpiperazin-5-one at the 5-position of the piperidine ring is essential to afford high renin inhibitory activities. Furthermore, introduction of the secondary amide part at the 3-position contributed to improve their renin inhibitory activities. The most potent compound (**5**) among the initialy optimized compounds demonstrated favorable oral bioavailability in rats (F = 45%) and a significant BP lowering effect by oral administration in hypertensive double transgenic rats (dTG rats) harboring both the human angiotensinogen and the human renin genes.[11] Encouraged by these results, we evaluated the oral efficacy of **5** in cynomolgus monkeys as a further characterization. Unfortunately, compound **5** did not show any oral efficacy in cynomolgus monkeys. As far as we know, only a few renin inhibitors have been reported to show oral efficacy in both rodent and non-rodent animal models.[12,13] We do not have enough information to explain which animal model is the most appropriate for predicting human efficacy. However, under this situation, we considered that obtaining the compounds showing *in vivo* efficacy in at least two different animal species would be necessary for acquiring a clinical candidate. Herein, we report synthesis and optimization of (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides to demonstrate favorable oral efficacy in both cynomolgus monkeys and rats.

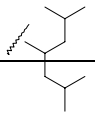
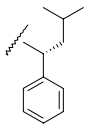
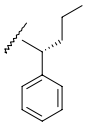
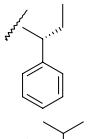
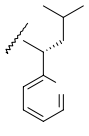
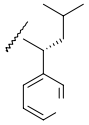
2. Chemistry

The synthetic pathway leading to (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides **5** and **32a-p** is outlined in Scheme 1. The synthetic methods for other amides **41** and **42** are described in the Supplementary Material. Chiral carboxylic acid **8** was successfully obtained by the enantioselective ring opening of known carboxylic acid anhydride **6**[14] with methanol in the presence of the chiral sulfonyl amide **7**[15] as an asymmetric catalyst. Then, Curtius rearrangement of the carboxylic acid (**8**) led to *N*-benzyloxycarbonyl (Cbz) protected amine **9**. Removal of the Cbz group of **9** gave the corresponding amine derivative. Conversion of the amine derivative into aldehyde **11** was achieved by using 2-bromo-2-methylpropanal **10**. Replacement of the *N*-*tert*-butoxycarbonyl (Boc) group with the *N*-2-nitrobenzenesulfonyl (Ns)[16] group, provided **12**. Compound **12** was converted into anilines **16-18** by reductive amination with various aniline derivatives **13-15**. Subsequent ring formation with bromoacetyl bromide yielded ketopiperazines **19-21**. Replacement of the *N*-Ns groups with *N*-Boc groups gave **22-24**. The condensation of various amines (**28** and **29a-n**) and carboxylic acids **25-27** obtained by the hydrolysis of **22-24** afforded the corresponding amide derivatives **30** and **31a-p**. Finally, removal of the *N*-Boc groups and then addition of fumaric acid gave **5** and **32a-p** as fumarate salts.

The synthetic pathway leading to chiral amine **28** used for the synthesis of **5** was outlined in Scheme 2. Compound **35** was prepared by *N*-benzyl (Bn) protection of amine **33** and then ethylation of the hydroxyl group. Subsequent removal of the two *N*-Bn groups gave chiral amine **28**. The synthetic route of chiral amine **29b** used for the synthesis of **32b** was outlined in Scheme 3. Alcohol **36** was converted into azide **37** with diphenylphosphoryl azide. Reduction of the azide group and then treatment with hydrogen chloride in 1,4-dioxane gave chiral amine **29b** as hydrochloride salt.[17] On the other hand, the synthetic route of chiral amines **29a** and **29d-n** used for the synthesis of **32a** and **32d-p** was outlined in Scheme 4. Sulfinimine formation of ketones **38a** and **38d-n** with chiral

Table 1. *In vitro* renin inhibitory activities (IC_{50} and ratio) and oral exposures of previously synthesized compounds[a,b]



Cmpd	R	Purified human renin	Monkey plasma renin		Cynomolgus monkey PK (1 mg/kg, po)[d]	
		IC_{50} (nM)	IC_{50} (nM)	Ratio[c]	$AUC_{po,0\text{-}4h}$ (ng*h/ml)	n[e]
5		1.3	1.4	0.5	14	4
41[f]		2.3	4.8	0.8	10	3
42		1.6	10	2.8	25	4
32a		2.1	9.0	1.0	51	3
32b		2.5	18	2.0	137	3
32c		2.1	6.6	1.8	60	3
32d		1.4	17	3.6	52	3
32e		2.1	4.2	0.9	1.6	3

[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**. [d] The data were obtained by cassette dosing. [e] Number of compounds dosed in cassette. [f] Compound **41** is a single diastereoisomer . Stereochemistry is not determined.

sulfinyl amine **39** in the presence of $Ti(OEt)_4$, and subsequent diastereoselective reduction of the sulfinimines afforded sulfinamides **40a** and **40d-n**.[18] Compounds **40a** and **40d-n** were obtained as the single diastereoisomer by the separation with silica gel column chromatography. Then, removal of the *N*-sulfinyl groups with hydrogen chloride in 1,4-dioxane was

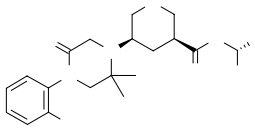
Table 2. Oral exposures of compounds **5** and **32c** in cynomolgus monkeys or rats[a]

Cmpd	Rat PK (10 mg/kg, po), $AUC_{0\text{-}24h}$ (ng*h/ml)	Cynomolgus monkey PK (3 mg/kg, po), $AUC_{0\text{-}24h}$ (ng*h/ml)
5	1940	60
32c	1500	730

[a] The data were obtained by individual dosing.

carried out as described in ref. 18. As a result, chiral amines **29a** and **29d-n** were obtained as hydrochloride salts.

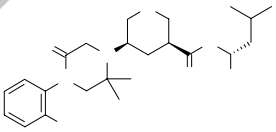
Table 3. *In vitro* renin inhibitory activities (IC_{50} and ratio) and oral exposures of compounds with modification of the P_2' phenyl ring [a,b]



Cmpd	R^1	R^2	Purified human renin	Monkey plasma renin		Cynomolgus monkey PK (1 mg/kg, po)[d]	
			IC_{50} (nM)	IC_{50} (nM)	Ratio[c]	$AUC_{po,0-4h}$ (ng*h/ml)	*n*[e]
32a	Isobutyl	Phenyl	2.1	9.0	1.0	51	3
32b	Propyl	Phenyl	2.5	18	2.0	137	3
32c	Ethyl	Phenyl	2.1	6.6	1.8	60	3
32f	Ethyl	2-Fluorophenyl	3.3	11	2.3	90	3
32g	Ethyl	3-Fluorophenyl	1.8	14	2.8	57	3
32h	Ethyl	4-Fluorophenyl	2.4	19	3.7	52	3
32i	Ethyl	3-Chlorophenyl	2.3	25	5.0	83	3
32j	Ethyl	4-Chlorophenyl	5.2	55	11	130	3

[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**. [d] The data were obtained by cassette dosing. [e] Number of compounds dosed in cassette.

Table 4. *In vitro* renin inhibitory activities (IC_{50} and ratio) and oral exposures of compounds with modification of the P_2' portion[a,b]



Cmpd	R^2	Purified human renin	Monkey plasma renin		Cynomolgus monkey PK (1 mg/kg, po)[d]	
		IC_{50} (nM)	IC_{50} (nM)	Ratio[c]	$AUC_{po,0-4h}$ (ng*h/ml)	*n*[e]
32d	2-Pyridyl	1.4	17	3.6	52	3
32k	5-Fluoro-2-pyridyl	1.0	9.7	2.1	53	3
32e	3-Pyridyl	2.1	4.2	0.9	1.6	3
32l	5-Fluoro-3-pyridyl	1.7	6.9	0.8	5.4	3
32m	5-Isoxazolyl	1.1	3.3	0.6	11	3
32n	2-Oxazolyl	1.9	8.3	1.4	15	3

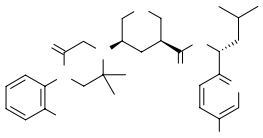
[a] Compounds were obtained as fumarate salts. [b] Assay results of renin inhibitory activity are the average of at least two replicates. [c] Ratio = IC_{50} (nM) of compound / IC_{50} (nM) of **1**. [d] The data were obtained by cassette dosing. [e] Number of compounds dosed in cassette.

3. Results and discussions

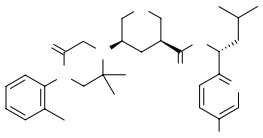
To identify the reason why compound **5** did not show any oral efficacy in cynomolgus monkeys, pharmacokinetics (PK) study of **5** in cynomolgus monkeys was performed. The $AUC_{po,0-24h}$ value of **5** ($AUC_{po,0-24h}$ = 60 ng*h/ml, 3 mg/kg, po) was revealed to be only about one-tenth of that of orally active DS-8108b (**4**) ($AUC_{po,0-24h}$ = 685 ng*h/ml, 3 mg/kg, po).[10] Based on this result, we speculated that the oral exposure of **5** did not reach a sufficient level to show oral efficacy in cynomolgus monkeys.

Thus, we attempted to explore 3,5-disubstituted piperidines showing appropriate oral exposure in cynomolgus monkeys. Same as in the case of discovering DS-8108b (**4**), the cassette dosing method (po, 0-4 h) was used to increase the throughput of PK screening.[10] Initially, we evaluated compounds **5**, **32a-e**, **41**, and **42** (Table 1). These compounds are potent renin inhibitors and have been described previously.[8] The $AUC_{po,0-4h}$ values of compounds containing the ether (**5**), hydroxyl (**41**), alkyl (**42**), or 3-pyridyl (**32e**) group were unfortunately low in cynomolgus monkeys. Meanwhile, compounds **32a-d** containing the phenyl or 2-pyridyl ring exhibited higher $AUC_{po,0-4h}$ values than **5**. Specifically, the P_2' phenyl derivatives (**32a-c**) showed high renin inhibitory activities against monkey plasma renin.

Table 5. *In vitro* renin inhibitory activities (IC_{50} and ratio), oral exposures, BP lowering effect, and CYP and hERG current inhibition of compounds with modification of the P_3 portion[a,b]



32k



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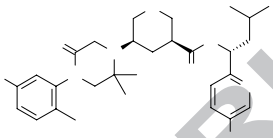

32p

Cmpd	Purified human renin	Monkey plasma renin		Cynomolgus monkey PK (10 mg/kg, po), $AUC_{po,0-24h}$ (ng*h/mL)[d]	BP lowering effect, AUC_{0-24h} (mmHg·h)[e]		CYP direct inhibition (% at 10 μM)		hERG current inhibition (% at 30 μM)[f]
	IC_{50} (nM)	IC_{50} (nM)	Ratio[c]		dTG rat (30 mg/kg, po)	Cynomolgus monkey (10 mg/kg, po)	3A4	2D6	
32k	1.0	9.7	2.1	2580	−535	−194	22	8.3	27
32o	1.6	7.0	0.9	3190	−581	−267	30	1.0	5.2
32p	1.3	16	2.1	NT[g]	−606	NT[g]	44	11	40

[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**. [d] The data were obtained by individual dosing. [e] Area under the change in MAP (mmHg) versus time curve. [f] hERG currents were measured using the patch clamp technique on IonWorks® Quattro™ system. [g] NT = not tested.

In order to verify our strategy, PK studies of **32c** in rats and cynomolgus monkeys were conducted by individual dosing (Table 2). The $AUC_{po,0-24h}$ value of **32c** in rats was almost similar to that of **5**. Moreover, as expected from the result of cassette dosing, compound **32c** exhibited 10-fold higher $AUC_{po,0-24h}$ value than **5** by individual dosing in cynomolgus monkeys.

Since 3,5-disubstituted piperidines with the phenyl ring at the P_2' position were suggested to have suitable properties in both renin inhibition and oral exposure, we started to evaluate the substituent effect on the P_2' phenyl ring as the next step (Table 3). The ethyl group was used as substituent at R^1 for this SAR study because the corresponding starting materials, ketones **38f-j**, were readily available from commercial sources unlike the case of R^1 isobutyl or propyl analogs. Introduction of the halogen group onto the phenyl ring did not affect the $AUC_{po,0-4h}$ values (**32f-j**), however, inhibitory activities against monkey plasma renin were deteriorated. Especially, compounds **32i** and **32j** containing the chloro group exhibited lower renin inhibitory activity than compounds **32g** and **32h** containing the fluoro group. Based on these SAR results, very limited space was suggested to be left for substitution on the P_2' phenyl ring.

For further characterization, we evaluated the *in vitro* safety profile of **32a**, which showed the highest inhibitory activity against monkey plasma renin among **32a-c**. However, direct inhibition of CYP 3A4 (49%) and CYP 2D6 (51%) at 10 μM, and hERG current inhibition (36%) at 30 μM were observed. These undesired interactions are known to be often lowered by increasing polarity of compounds.[19,20] Therefore, our attention was shifted to P_2' hetero-aromatic ring derivatives (Table 4). Compound **32k**, which has an additional fluoro group at the 5-position of the 2-pyridyl ring of **32d**, exhibited restored *in vitro* potency with sustained good oral exposure (**32k** *vs.* **32d**). On the other hand, introduction of the fluoro group to the 3-pyridyl ring of **32e** showed high *in vitro* potency, however did not have an attractive impact on the plasma drug concentration (**32l**). Unfortunately, other evaluated hetero-aromatic rings, the 5-isoxazolyl (**32m**) and 2-oxazolyl (**32n**) rings, showed low $AUC_{po,0-4h}$ values. In addition to the good *in vitro* potency and oral exposure, compound **32k** exhibited improvement in the inhibitory activity against CYP 3A4 (22%), CYP 2D6 (8.3%) at 10 μM, and hERG current (27%) at 30 μM compared to **32a**. Therefore, we selected the 5-fluoro-2-pyridyl ring as a favorable P_2' portion and decided to revisit the P_3 part modification.

Considering the previous SAR of the P_3 portion,[10,21] 2-chloro (**32k**), 2-methyl (**32o**), and 5-fluoro-2-methyl (**32p**) phenyl derivatives were prepared. Both *in vitro* and *in vivo* profiles of these compounds were evaluated and listed in Table 5. Compound **32k** showed suitable oral exposure in cynomolgus monkeys and reduction in mean arterial blood pressure (MAP) in cynomolgus monkeys as well as dTG rats with acceptable CYP and hERG current inhibition. Compound **32p** also showed BP lowering effect in dTG rats, however, strong hERG current inhibition was observed. Thus, further evaluation of **32p** was discontinued. Compound **32o** exhibited the most potent inhibitory activities against monkey plasma renin among three compounds, suitable oral exposure in cynomolgus monkeys and stronger BP lowering effects in both dTG rats and cynomolgus monkeys than **32k**. In addition, hERG current inhibition of **32o** was weaker than that of **32k**. From these results, we finally selected **32o** as the promising 3,5-disubstituted piperidine derivative for further evaluation.

The key characteristics of **32o** are summarized in Table 6. Compound **32o** showed high renin inhibitory activity in the

presence of human plasma as well as monkey plasma, and also showed favorable metabolic stability in human and monkey liver microsomes. With regard to the initial safety profile, effects on the cardiac action potential, CYP, and hERG current of **32o** were considered to be acceptable and the Ames test was negative. Regarding the PK profile, compound **32o** exhibited moderate oral bioavailability in both SD rats (20%) and cynomolgus monkeys (25%). The antihypertensive efficacy of **32o** was displayed in Figures 3 and 4. At first, vehicle or 30 mg/kg of **32o** was orally administered to dTG rats (Figure 3). Compound **32o** induced a significant reduction in MAP sustained over a period of 24 h. Next, vehicle, or 3 and 10 mg/kg of **32o** were orally administered to cynomolgus monkeys pretreated with furosemide (Figure 4). Compound **32o** showed a significant reduction in MAP in a dose-dependent manner sustained over a period of 24 h.

Table 6. *In vitro*, primary safety, and PK profiles of compound **32o**[a,b]

Purified human renin	IC_{50} (nM)	1.6
Human plasma renin	IC_{50} (nM)	3.6
	Ratio[c]	1.2
Monkey plasma renin	IC_{50} (nM)	7.0
	Ratio[c]	0.9
Molecular weight		510
Lipophilicity ($LogD_{7.4}$)		2.0
Metabolic stability (human, monkey, rat)[d]		100%, 84%, 89%
Plasma protein binding ratio (human, monkey, rat)[e]		38%, 55%, 63%
CYP 3A4, direct inhibition (10 μM)		30%
CYP 2D6, direct inhibition (10 μM)		1.0%
hERG current inhibition (30 μM)[f]		5.2%
Guinea pig right ventricular papillary muscle-cardiac action potential (30 μM)[g,h]	APD_{90}	−1.0% (Shortening)
	dV/dt max	−11.6% (Decrease)
Ames test (*Salmonella typhimurium* TA98, TA100)		Negative
SD rat, female (30 mg/kg p.o., 0.5% MC) (1 mg/kg i.v., Saline)	*F* (%)	20
	*C*max (ng/mL)	204
	$AUC_{po,0\text{-}24h}$ (ng*h/mL)	1100
	CL (L/h/kg)	4.8
	*V*ss (L/kg)	11
	$t_{1/2}$ (h)	5.4
Cynomolgus monkey, male (10 mg/kg p.o., 0.5% MC) (1 mg/kg i.v., Saline)	*F* (%)	25
	*C*max (ng/mL)	604
	$AUC_{po,0\text{-}24h}$ (ng*h/mL)	3190
	CL (L/h/kg)	0.57
	*V*ss (L/kg)	5.4
	$t_{1/2}$ (h)	7.9

[a] Compound **32o** was obtained as fumarate salt. [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**. [d] Percent remaining after 30 min in human, monkey, and rat liver microsomes. [e] The plasma protein binding ratio was determined by a micro-scale ultracentrifugation method (436,000g, 2 h 20 min, at 4 °C). [f] hERG currents were measured using the patch clamp technique on IonWorks® Quattro™ system. [g] Action potentials were measured using the glass microelectrode method. [h] % change from baseline.

4. Conclusion

We have synthesized and optimized a novel type of renin inhibitor based on a (3*S*,5*R*)-5-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)piperidine-3-carboxamides template. Chemical modification of P_1', P_2' and P_3 portions led to a promising 3,5-disubstituted piperidine **32o** showing high renin inhibitory activity and favorable oral exposure in both rats and cynomolgus monkeys with acceptable CYP and hERG current inhibition. Compound **32o** achieved the significant BP lowering effect over a period of 24 h in both cynomolgus monkeys pretreated with furosemide and dTG rat models by oral administration. This promising compound **32o** was selected for further evaluation.

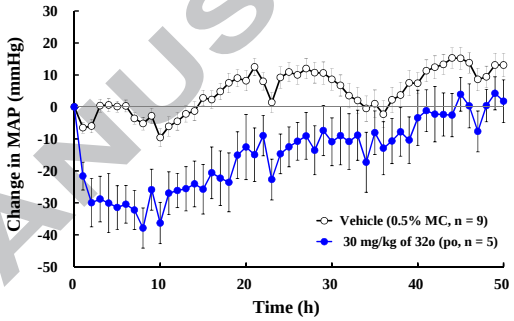

Figure 3. Effect of compound **32o** on MAP in dTG rats.

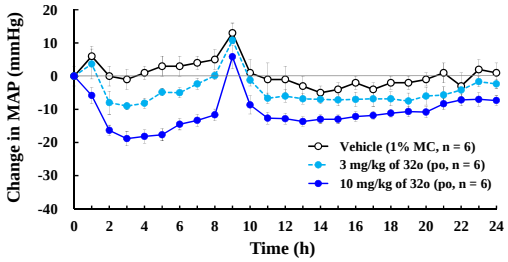

Figure 4. Effect of compound **32o** on MAP in cynomolgus monkeys pretreated with furosemide.

5. Experimental

5.1. 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. Elemental analyses for CHN and ClF were determined on a Microcorder JM10 and a Dionex ICS-1500, respectively. Infrared spectrum was recorded in a KBr disc with a Jasco FT/IR-6100.

5.1.1 (3*R*,5*S*)-1-*tert*-Butoxycarbonyl-5-methoxycarbonylpiperidine-3-carboxylic acid (8)

Methanol (43.0 mL, 105 mmol) was added to a solution of *tert*-butyl 2,4-dioxo-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate **6** (27.0 g, 105 mmol) and *N*-[(*S*)-(6-methoxy-4-quinolyl)-(5-vinylquinuclidin-2-yl)methyl]-3,5-bis(trifluoromethyl)benzenesulfonamide **7** (8.30 g, 13.8 mmol) in THF (1.00 L) at −20 °C, and the mixture was stirred at the same temperature for 1 day. 1*N* HCl aq. was added to the reaction mixture, followed by extraction with Et_2O-AcOEt. Then, the organic layer was washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 1/5 to 0/1) to obtain **8** (20.7 g, 69%, 94% ee) as a colorless solid. The ee value of **8** was determined by chiral HPLC analysis: column, Chiral Pack AD-H (4.6 x 250 mm); eluent, *n*-hexane/ethanol = 95/5; flow rate = 0.5 mL/min; t_R of (3*R*,5*S*)-isomer = 29.6 min; t_R of (3*S*,5*R*)-isomer = 33.3 min. $[\alpha]_D^{25.0}$ +5.77 (*c* 1.01, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.37 (br s, 2H), 3.71 (s, 3H), 2.72 (br s, 2H), 2.55-2.45 (m, 3H), 1.72 (q, *J* = 12.7 Hz, 1H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.8, 172.9, 154.5, 80.7, 52.0, 45.0, 40.7, 40.6, 30.0, 28.4. IR: 3197, 2982, 1739, 1670, 1435, 1252, 1147, 819, 627 cm^{-1}. HRMS (ESI$^-$): m/z calcd for $C_{13}H_{21}NO_6$−H: 286.1291; found: 286.1298.

5.1.2 1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-(benzyloxycarbonylamino)piperidine-1,3-dicarboxylate (9)

Et_3N (11.3 mL, 80.8 mmol) and diphenylphosphoryl azide (20.4 g, 74.1 mmol) were added to a solution of **8** (19.4 g, 67.4 mmol) in toluene (335 mL) at rt, and the mixture was stirred at 90 °C for 1.5 h. After cooling, benzyl alcohol was added to the reaction mixture, and mixture was stirred at 90 °C for 3.5 h. The reaction mixture was cooled to room temperature and poured into water, 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 = 4/1) to obtain **9** (25.5 g, 96%) as a colorless liquid. $[\alpha]_D^{25.0}$ −4.16 (*c* 1.01, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.26 (m, 5H), 5.11 (br s, 3H), 4.06-4.03 (m, 2H), 3.70-3.63 (m, 5H), 3.09-3.05 (m, 1H), 2.60 (br s, 1H), 2.31-2.27 (m, 1H), 1.61-1.56 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 155.5, 154.5, 128.4, 128.2, 127.6, 127.0, 80.3, 66.8, 65.3, 52.0, 46.4, 45.0, 40.0, 32.8, 28.3. IR: 3342, 2976, 1720, 1671, 1522, 1435, 1239, 1157, 1047, 737 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{20}H_{28}N_2O_6$+H: 393.2026; found: 393.2023.

5.1.3 1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-[(1,1-dimethyl-2-oxoethyl)amino]piperidine-1,3-dicarboxylate (11)

To a suspension of **9** (50.0 g, 127 mmol) in MeOH (300 mL), 10% Pd on carbon (50% wet, 5.00 g) were added. The suspension was stirred under H_2 atmosphere at room temperature for 1.5 h. H_2 in the reaction vessel was replaced by N_2, and then Pd on carbon was separated by filtration. The solvent was evaporated under reduced pressure to obtain crude 1-*tert*-butyl 3-methyl (3*S*,5*R*)-5-aminopiperidine-1,3-dicarboxylate as a colorless liquid. 2-Bromo-2-methylpropanal **10** (112 g, 792 mmol) was added to a solution of the crude 1-*tert*-butyl 3-methyl (3*S*,5*R*)-5-aminopiperidine-1,3-dicarboxylate in a mixed solvent of DMF (211 mL) and water (52.0 mL) at room temperature, and the mixture was stirred at the same temperature for 1 day. Saturated $NaHCO_3$ aqueous solution was added to the reaction mixture, followed by extraction with AcOEt. Then, the organic layer was washed with water, 10% NaCl aqueous solution, 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 **11** (31.8 g, 73%, 2 steps) as a light yellow liquid. $[\alpha]_D^{25.0}$ −9.47 (*c* 1.05, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.43 (s, 1H), 4.32-4.21 (m, 1H), 4.11-4.01 (m, 1H), 3.68 (s, 3H), 2.71-2.61 (m, 1H), 2.54-2.41 (m, 2H), 2.30 (dd, *J* = 12.7, 10.7 Hz, 1H), 2.20 (br d, *J* = 12.5 Hz, 1H), 1.45 (s, 9H), 1.40-1.33 (m, 1H), 1.22 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 203.8, 172.9, 154.6, 80.1, 61.3, 51.9, 50.8, 48.9, 44.9, 41.2, 36.5, 28.4, 22.7, 21.9. IR: 3334, 2974, 1726, 1679, 1425, 1326, 1252, 1172, 937, 899, 772, 661 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{16}H_{28}N_2O_5$+H: 329.2076; found: 329.2071.

5.1.4 Methyl (3*S*,5*R*)-5-[(1,1-dimethyl-2-oxoethyl)amino]-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (12)

4*N*-HCl in 1,4-dioxane (362 mL, 1.45 mmol) was added to **11** (31.8 g, 96.6 mmol) at room temperature, and the mixture was stirred at the same temperature for 0.5 h. The solvent was evaporated under reduced pressure to obtain crude methyl (3*S*,5*R*)-5-[(1,1-dimethyl-2-oxoethyl)amino]piperidine-3-carboxylate. $NaHCO_3$ (40.6 g, 483 mmol) and 2-nitrobenzenesulfonyl chloride (21.2 g, 95.6 mmol) were added to

a solution of the crude methyl (3*S*,5*R*)-5-[(1,1-dimethyl-2-oxoethyl)amino]piperidine-3-carboxylate in a mixed solvent of 1,4-dioxane (250 mL) and water (250 mL) at 0 °C, and the mixture was stirred at the same temperature for 1.5 h. Water 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 = 1/1 to 0/1) to obtain **12** (34.4 g, 86%, 2 steps) as a colorless solid. $[\alpha]_D^{25.0}$ +22.0 (*c* 1.04, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.44 (s, 1H), 8.01-7.99 (m, 1H), 7.74-7.69 (m, 2H), 7.66-7.63 (m, 1H), 4.01-3.98 (m, 1H), 3.83-3.80 (m, 1H), 3.69 (s, 3H), 2.79-2.64 (m, 3H), 2.38 (dd, *J* = 12.7, 10.7 Hz, 1H), 2.28 (br d, *J* = 12.7 Hz, 1H), 1.32 (q, *J* = 12.2 Hz, 1H), 1.24 (s, 3H), 1.18 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 203.7, 172.2, 148.0, 133.8, 132.1, 131.8, 130.9, 124.3, 61.4, 52.6, 52.1, 49.0, 46.6, 41.1, 35.9, 23.0, 21.9. IR: 3331, 2694, 1729, 1707, 1553, 1378, 1357, 1172, 1017, 782, 584 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{17}H_{23}N_3O_7S$+H: 414.1335; found: 414.1330.

5.1.5 Methyl (3*S*,5*R*)-5-{[2-(2-chloroanilino)-1,1-dimethylethyl]amino}-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (16)

To a single-neck flask upon which there was placed a Dean-Stark, **12** (17.1 g, 41.4 mmol), 2-chloroaniline **13** (7.87 g, 61.7 mmol), toluene (400 mL), and acetic acid (8.67 g, 144 mmol) were added. The mixture was stirred at 130 °C for 4 h. After cooling in an ice bath, $NaBH(OAc)_3$ (17.5 g, 82.6 mmol) was added to the reaction mixture. The mixture was further stirred at room temperature for 16 h. Saturated $NaHCO_3$ aqueous solution 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 = 4/1 to 7/3) to obtain **16** (16.0 g, 74%) as a light colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74 (d, *J* = 8.3 Hz, 1H), 7.64-7.58 (m, 2H), 7.52-7.48 (m, 1H), 7.25-7.23 (m, 1H), 7.17-7.14 (m, 1H), 6.65-6.62 (m, 2H), 4.85 (br s, 1H), 4.07 (br d, *J* = 9.3 Hz, 1H), 3.75-3.71 (m, 1H), 3.69 (s, 3H), 2.97 (d, *J* = 11.7 Hz, 1H), 2.94 (d, *J* = 11.7 Hz, 1H), 2.81-2.69 (m, 3H), 2.34 (dd, *J* = 12.5, 10.5 Hz, 1H), 2.21 (br d, *J* = 12.5 Hz, 1H), 1.32 (q, *J* = 12.2 Hz, 1H), 1.22 (s, 3H), 1.21 (s, 3H).

5.1.6 Methyl (3*S*,5*R*)-5-{[1,1-dimethyl-2-(2-methylanilino)ethyl]amino}-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (17)

The title compound was prepared from **12** and 2-methylaniline **14** in a manner similar to that described for **16** as a colorless solid (98%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.64-7.58 (m, 2H), 7.48-7.45 (m, 1H), 7.16-7.13 (m, 1H), 7.05 (br d, *J* = 7.3 Hz, 1H), 6.68-6.65 (m, 1H), 6.58 (br d, *J* = 8.3 Hz, 1H), 4.08-4.05 (m, 1H), 3.73-3.69 (m, 4H), 2.96 (d, *J* = 11.7 Hz, 1H), 2.91 (d, *J* = 11.7 Hz, 1H), 2.81-2.70 (m, 3H), 2.32 (dd, *J* = 12.2, 10.7 Hz, 1H), 2.21-2.17 (m, 1H), 2.12 (s, 3H), 1.31 (q, *J* = 12.0 Hz, 1H), 1.22 (s, 3H), 1.21 (s, 3H).

5.1.7 Methyl (3*S*,5*R*)-5-{[2-(5-fluoro-2-methylanilino)-1,1-dimethylethyl]amino}-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (18)

The title compound was prepared from **12** and 5-fluoro-2-methylaniline **15** in a manner similar to that described for **16** as a colorless solid (98%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.66-7.59 (m, 2H), 7.51 (dt, *J* = 7.8, 1.5 Hz, 1H), 6.95 (br t, *J* = 7.3 Hz, 1H), 6.32 (dt, *J* = 8.3, 2.4 Hz, 1H), 6.23 (dd, *J* = 11.2, 2.4 Hz, 1H), 4.26 (br s, 1H), 4.07-4.05 (m, 1H), 3.69-3.64 (m, 4H), 2.89-2.84 (m, 2H), 2.80-2.70 (m, 3H), 2.36-2.32 (m, 1H), 2.21-2.17 (m, 1H), 2.07 (s, 3H), 1.31 (q, *J* = 12.0 Hz, 1H), 1.22 (s, 3H), 1.20 (s, 3H).

5.1.8 Methyl (3*S*,5*R*)-5-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (19)

A solution of bromoacetyl bromide (24.4 g, 121 mmol) in CH_2Cl_2 (80 mL) was added dropwise to a solution of **16** (16.0 g, 30.5 mmol) and Et_3N (24.8 g, 245 mmol) in CH_2Cl_2 (320 mL) under ice-cooling over a period of 2.5 h. The reaction mixture was stirred at room temperature for 45 min, and then stirred at 40 °C for 3 h. After cooling, water was added to the reaction mixture, 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 silica gel column chromatography (eluent, *n*-hexane/AcOEt = 7/3 to 0/1) to obtain **19** (13.5 g, 78%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03-8.01 (m, 1H), 7.76-7.70 (m, 2H), 7.67-7.65 (m, 1H), 7.48-7.46 (m, 1H), 7.33-7.23 (m, 3H), 4.03 (br d, *J* = 11.2 Hz, 1H), 3.87-3.81 (m, 1H), 3.72 (s, 3H), 3.71-3.22 (m, 5H), 2.76-2.68 (m, 3H), 2.24-2.17 (m, 1H), 1.73-1.66 (m, 1H), 1.41-1.35 (m, 6H).

5.1.9 Methyl (3*S*,5*R*)-5-[2,2-dimethyl-4-(*o*-tolyl)-5-oxopiperazin-1-yl]-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (20)

The title compound was prepared from **17** in a manner similar to that described for **19** as a colorless solid (84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03-8.01 (m, 1H), 7.76-7.71 (m, 2H), 7.67-7.65 (m, 1H), 7.27-7.21 (m, 3H), 7.13-7.06 (m, 1H), 4.05-4.00 (m, 1H), 3.87-3.81 (m, 1H), 3.72 (s, 3H), 3.68-3.15 (m, 5H), 2.79-2.66 (m, 3H), 2.24-2.18 (m, 4H), 1.75-1.66 (m, 1H), 1.37-1.34 (m, 6H).

5.1.10 Methyl (3*S*,5*R*)-5-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-(2-nitrophenyl)sulfonylpiperidine-3-carboxylate (21)

The title compound was prepared from **18** in a manner similar to that described for **19** as a colorless solid (84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03-8.02 (m, 1H), 7.76-7.71 (m, 2H), 7.67-7.65 (m, 1H), 7.27-7.20 (m, 1H), 6.95 (dq, *J* = 8.3, 2.4 Hz, 1H), 6.87-6.81 (m, 1H), 4.05-3.99 (m, 1H), 3.87-3.80 (m, 1H), 3.72 (s, 3H), 3.68-3.14 (m, 5H), 2.80-2.66 (m, 3H), 2.32-2.17 (m, 4H), 1.74-1.66 (m, 1H), 1.37-1.34 (m, 6H).

5.1.11 1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-1,3-dicarboxylate (22)

Cesium carbonate (9.31 g, 28.6 mmol) was added to a solution of **19** (13.5 g, 23.9 mmol) and thiophenol (3.64 mL, 35.7 mmol) in CH_3CN (400 mL) under ice-cooling, and the mixture was stirred at room temperature for 2.5 h. After concentration under reduced pressure, water was added to the reaction mixture, 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 silica gel column chromatography (eluent, CH_2Cl_2/MeOH = 1/0 to 7/3) to afford methyl (3*S*,5*R*)-5-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-3-carboxylate (9.10 g, quant.). Di-*t*-butyl dicarbonate (6.00 g, 27.5 mmol) was added to a solution of methyl (3*S*,5*R*)-5-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-3-carboxylate (9.10 g, 23.9 mmol)

obtained above and $NaHCO_3$ (6.20 g, 73.8 mmol) in a mixed solvent of AcOEt (200 mL) and water (200 mL), and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was extracted with AcOEt, washed with brine, dried over anhydrous Na_2SO_4, and purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 7/3 to 1/9) and NH silica gel column chromatography (eluent, *n*-hexane/AcOEt = 1/0 to 1/1) to afford **22** (7.80 g, 68%) as colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (dd, *J* = 7.3, 1.5 Hz, 1H), 7.34-7.22 (m, 3H), 4.29 (br s, 1H), 4.12 (br s, 1H), 3.74-3.52 (m, 5H), 3.36-3.29 (m, 2H), 3.02-2.98 (m, 1H), 2.68-2.56 (m, 3H), 2.14 (br s, 1H), 1.84-1.73 (m, 1H), 1.48 (s, 9H), 1.44-1.31 (m, 6H).

5.1.12 1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-[2,2-dimethyl-4-(*o*-tolyl)-5-oxopiperazin-1-yl]piperidine-1,3-dicarboxylate (23)

The title compound was prepared from **20** in a manner similar to that described for **22** as a colorless solid (79%, 2steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27-7.21 (m, 3H), 7.12-7.08 (m, 1H), 4.30 (br s, 1H), 4.08 (br s, 1H), 3.72-3.52 (m, 5H), 3.39-3.35 (m, 1H), 3.21 (br d, *J* = 11.2 Hz, 1H), 3.01-2.97 (m, 1H), 2.70-2.54 (m, 3H), 2.24-2.23 (m, 3H), 2.12 (br d, *J* = 12.7 Hz, 1H), 1.83-1.74 (m, 1H), 1.48 (s, 9H), 1.36-1.32 (m, 6H).

5.1.13 1-*tert*-Butyl 3-methyl (3*S*,5*R*)-5-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-1,3-dicarboxylate (24)

The title compound was prepared from **21** in a manner similar to that described for **22** as a colorless liquid (72%, 2steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 (dd, *J* = 8.3, 6.8 Hz, 1H), 6.97-6.93 (m, 1H), 6.86-6.83 (m, 1H), 4.29 (br s, 1H), 4.09 (br s, 1H), 3.71-3.51 (m, 5H), 3.37-3.33 (m, 1H), 3.19 (br d, *J* = 11.7 Hz, 1H), 3.01-2.97 (m, 1H), 2.69-2.53 (m, 3H), 2.18-2.10 (m, 4H), 1.81-1.74 (m, 1H), 1.48 (s, 9H), 1.36-1.32 (m, 6H).

5.1.14 (3*S*,5*R*)-1-*tert*-Butoxycarbonyl-5-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-3-carboxylic acid (25)

A solution of **22** (7.80 g, 16.3 mmol) in a mixed solvent of THF (150 mL) and water (75.0 mL) was cooled in an ice bath, and then lithium hydroxide monohydrate (1.37 g, 32.6 mmol) were added. The mixture was stirred at the same temperature for 2 h. 1*N* HCl aqueous solution was added to the reaction mixture to acidify it (pH = 2-3). The reaction mixture was extracted with CH_2Cl_2, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure to obtain **25** (7.70 g, quant.) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.34-7.25 (m, 3H), 4.30 (br s, 1H), 4.12 (br s, 1H), 3.76 (br d, *J* = 17.6 Hz, 1H), 3.66 (br d, *J* = 17.6 Hz, 1H), 3.38-3.29 (m, 2H), 3.03-2.99 (m, 1H), 2.69-2.56 (m, 3H), 2.22-2.15 (m, 1H), 1.81-1.69 (m, 1H), 1.47 (s, 9H), 1.43-1.32 (m, 6H).

5.1.15 (3*S*,5*R*)-1-*tert*-Butoxycarbonyl-5-[2,2-dimethyl-4-(*o*-tolyl)-5-oxopiperazin-1-yl]piperidine-3-carboxylic acid (26)

The title compound was prepared from **23** in a manner similar to that described for **25** as a colorless solid (quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.23 (m, 3H), 7.12-7.08 (m, 1H), 4.30 (br s, 1H), 4.05 (br s, 1H), 3.79-3.63 (m, 2H), 3.44-3.18 (m, 2H), 3.00 (br s, 1H), 2.73-2.54 (m, 3H), 2.24-2.17 (m, 4H), 1.79-1.70 (m, 1H), 1.47 (s, 9H), 1.36-1.33 (m, 6H).

5.1.16 (3*S*,5*R*)-1-*tert*-Butoxycarbonyl-5-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]piperidine-3-carboxylic acid (27)

The title compound was prepared from **24** in a manner similar to that described for **25** as a colorless solid (quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.22 (dd, *J* = 8.3, 6.3 Hz, 1H), 6.98-6.94 (m, 1H), 6.87-6.83 (m, 1H), 4.29 (br s, 1H), 4.09 (br s, 1H), 3.78-3.61 (m, 2H), 3.42-3.18 (m, 2H), 3.01 (br s, 1H), 2.74-2.54 (m, 3H), 2.32-2.16 (m, 4H), 1.78-1.71 (m, 1H), 1.47 (s, 9H), 1.36-1.34 (m, 6H).

5.1.17 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(ethoxymethyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (30)

HBTU (180 mg, 0.475 mmol) was added to a solution of **25** (148 mg, 0.318 mmol) and (2*R*)-1-ethoxy-4-methylpentan-2-amine **28** (92.0 mg, 0.633 mmol) and *N*,*N*-diisopropylethylamine (0.166 mL, 0.953 mmol) in DMF (3.00 mL) under ice-cooling, and then the reaction mixture was stirred at room temperature for 18 h. Water 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 0/1) to obtain **30** (137 mg, 73%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48-7.46 (m, 1H), 7.33-7.22 (m, 3H), 5.64 (br d, *J* = 4.9 Hz, 1H), 4.22-4.10 (m, 2H), 3.77-3.70 (m, 1H), 3.62-3.31 (m, 8H), 2.98 (br s, 1H), 2.74-2.68 (m, 2H), 2.34 (br s, 1H), 1.99-1.91 (m, 2H), 1.58 (br s, 1H), 1.48 (s, 9H), 1.46-1.31 (m, 8H), 1.20 (t, *J* = 6.8 Hz, 3H), 0.93-0.91 (m, 6H).

5.1.18 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-3-methyl-1-phenylbutyl]carbamoyl}piperidine-1-carboxylate (31a)

The title compound was prepared from **25** and (1*R*)-3-methyl-1-phenylbutan-1-amine hydrochloride **29a** in a manner similar to that described for **30** as a colorless solid (74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (br d, *J* = 7.8 Hz, 1H), 7.35-7.23 (m, 8H), 5.92 (br s, 1H), 4.99-4.96 (m, 1H), 4.20-3.96 (m, 2H), 3.67-3.48 (m, 2H), 3.31-3.25 (m, 2H), 2.93-2.66 (m, 3H), 2.34 (br s, 1H), 1.88-1.26 (m, 20H), 0.94-0.90 (m, 6H).

5.1.19 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-phenylbutyl]carbamoyl}piperidine-1-carboxylate (31b)

The title compound was prepared from **25** and (1*R*)-1-phenylbutan-1-amine hydrochloride **29b** in a manner similar to that described for **30** as a colorless solid (85%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47-7.43 (m, 1H), 7.34-7.21 (m, 8H), 6.00 (br s, 1H), 4.91-4.84 (m, 1H), 4.10-3.93 (m, 2H), 3.69-3.62 (m, 1H), 3.58-3.47 (m, 1H), 3.32-3.25 (m, 2H), 2.94 (br s, 1H), 2.83-2.64 (m, 2H), 2.36 (br s, 1H), 1.96-1.68 (m, 6H), 1.46 (s, 9H), 1.35-1.21 (m, 6H), 0.90 (t, *J* = 7.6 Hz, 3H).

5.1.20 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-phenylpropyl]carbamoyl}piperidine-1-carboxylate (31c)

The title compound was prepared from **25** and (1*R*)-1-phenylpropan-1-amine **29c** in a manner similar to that described for **30** as a colorless solid (55%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.37-7.22 (m, 8H), 5.80 (br s, 1H), 4.88 (br q, *J* = 7.5 Hz, 1H), 4.26-4.00 (m, 2H), 3.74-3.50 (m, 2H), 3.34-3.29 (m, 2H), 2.95 (br s, 1H), 2.78-2.70 (m, 2H), 2.34 (br s, 1H), 1.93-1.78 (m, 4H), 1.48 (s, 9H), 1.39-1.27 (m, 6H), 0.89 (t, *J* = 7.6 Hz, 3H).

5.1.21 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-3-methyl-1-(2-pyridyl)butyl]carbamoyl}piperidine-1-carboxylate (31d)

The title compound was prepared from **25** and (1*R*)-3-methyl-1-(2-pyridyl)butan-1-amine dihydrochloride **29d** in a manner similar to that described for **30** as a colorless solid (67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (br d, *J* = 4.4 Hz, 1H), 7.45 (br t, *J* = 7.3 Hz, 1H), 7.46-7.45 (m, 1H), 7.32-7.18 (m, 5H), 6.60 (br d, *J* = 7.8 Hz, 1H), 5.12 (br q, *J* = 7.8 Hz, 1H), 4.26-4.00 (m, 2H), 3.71-3.50 (m, 2H), 3.29 (br s, 2H), 2.97 (br s, 1H), 2.78-2.70 (m, 2H), 2.40 (br s, 1H), 1.90 (br s, 2H), 1.70-1.27 (m, 18H), 0.95-0.93 (m, 6H).

5.1.22 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-3-methyl-1-(3-pyridyl)butyl]carbamoyl}piperidine-1-carboxylate (31e)

The title compound was prepared from **25** and (1*R*)-3-methyl-1-(3-pyridyl)butan-1-amine dihydrochloride **29e** in a manner similar to that described for **30** as a colorless solid (54%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58-8.44 (m, 2H), 7.60-7.54 (m, 1H), 7.47-7.40 (m, 1H), 7.32-7.18 (m, 4H), 6.23 (br s, 1H), 5.07-4.98 (m, 1H), 4.24-3.90 (m, 2H), 3.72-3.46 (m, 2H), 3.35-3.21 (m, 2H), 3.00-2.89 (m, 1H), 2.81-2.60 (m, 2H), 2.36 (br s, 1H), 1.97-1.79 (m, 2H), 1.74-1.18 (m, 18H), 0.95-0.86 (m, 6H).

5.1.23 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(2-fluorophenyl)propyl]carbamoyl}piperidine-1-carboxylate (31f)

The title compound was prepared from **25** and (1*R*)-1-(2-fluorophenyl)propan-1-amine hydrochloride **29f** in a manner similar to that described for **30** as a colorless solid (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.43 (m, 1H), 7.32-7.20 (m, 5H), 7.12-7.01 (m, 2H), 6.14 (br s, 1H), 5.02 (q, *J* = 8.0 Hz, 1H), 4.25-3.95 (m, 2H), 3.74-3.46 (m, 2H), 3.34-3.24 (m, 2H), 2.96 (br s, 1H), 2.76-2.68 (m, 2H), 2.38 (br s, 1H), 1.96-1.74 (m, 4H), 1.48 (s, 9H), 1.35-1.23 (m, 6H), 0.89-0.86 (m, 3H).

5.1.24 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(3-fluorophenyl)propyl]carbamoyl}piperidine-1-carboxylate (31g)

The title compound was prepared from **25** and (1*R*)-1-(3-fluorophenyl)propan-1-amine hydrochloride **29g** in a manner similar to that described for **30** as a colorless solid (64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (dd, *J* = 7.8, 2.2 Hz, 1H), 7.33-7.22 (m, 4H), 7.05 (d, *J* = 7.8 Hz, 1H), 6.98-6.93 (m, 2H), 6.24 (br s, 1H), 4.86 (q, *J* = 7.6 Hz, 1H), 4.25-3.98 (m, 2H), 3.73-3.50 (m, 2H), 3.34-3.27 (m, 2H), 2.96 (br s, 1H), 2.79-2.70 (m, 2H), 2.41 (br s, 1H), 1.99-1.86 (m, 2H), 1.81-1.74 (m, 2H), 1.49 (s, 9H), 1.36-1.23 (m, 6H), 0.89 (t, *J* = 7.6 Hz, 3H).

5.1.25 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(4-fluorophenyl)propyl]carbamoyl}piperidine-1-carboxylate (31h)

The title compound was prepared from **25** and (1*R*)-1-(4-fluorophenyl)propan-1-amine hydrochloride **29h** in a manner similar to that described for **30** as a colorless solid (65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.32-7.21 (m, 5H), 7.05-6.98 (m, 2H), 6.11 (br s, 1H), 4.84 (q, *J* = 7.6 Hz, 1H), 4.25-3.98 (m, 2H), 3.75-3.49 (m, 2H), 3.35-3.26 (m, 2H), 2.95 (br s, 1H), 2.78-2.70 (m, 2H), 2.37 (br s, 1H), 1.99-1.72 (m, 4H), 1.47 (s, 9H), 1.35-1.23 (m, 6H), 0.88 (t, *J* = 7.1 Hz, 3H).

5.1.26 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(3-chlorophenyl)propyl]carbamoyl}piperidine-1-carboxylate (31i)

The title compound was prepared from **25** and (1*R*)-1-(3-chlorophenyl)propan-1-amine hydrochloride **29i** in a manner similar to that described for **30** as a colorless solid (69%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.32-7.21 (m, 6H), 7.17-7.14 (m, 1H), 6.34 (br s, 1H), 4.83 (q, *J* = 7.6 Hz, 1H), 4.26-3.96 (m, 2H), 3.73-3.50 (m, 2H), 3.34-3.28 (m, 2H), 2.95 (br s, 1H), 2.80-2.68 (m, 2H), 2.41 (br s, 1H), 2.01-1.74 (m, 4H), 1.49 (s, 9H), 1.36-1.24 (m, 6H), 0.89 (t, *J* = 7.5 Hz, 3H).

5.1.27 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(4-chlorophenyl)propyl]carbamoyl}piperidine-1-carboxylate (31j)

The title compound was prepared from **25** and (1*R*)-1-(4-chlorophenyl)propan-1-amine hydrochloride **29j** in a manner similar to that described for **30** as a colorless solid (55%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.31-7.19 (m, 7H), 6.10 (br s, 1H), 4.83 (q, *J* = 7.6 Hz, 1H), 4.26-3.95 (m, 2H), 3.73-3.50 (m, 2H), 3.35-3.26 (m, 2H), 2.94 (br s, 1H), 2.81-2.65 (m, 2H), 2.37 (br s, 1H), 2.02-1.72 (m, 4H), 1.48 (s, 9H), 1.41-1.30 (m, 6H), 0.88 (t, *J* = 7.3 Hz, 3H).

5.1.28 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (31k)

The title compound was prepared from **25** and (1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutan-1-amine dihydrochloride **29k** in a manner similar to that described for **30** as a colorless solid (60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (br d, *J* = 2.9 Hz, 1H), 7.47-7.45 (m, 1H), 7.39-7.35 (m, 1H), 7.31-7.22 (m, 4H), 6.41 (br d, *J* = 8.3 Hz, 1H), 5.12 (br q, *J* = 8.3 Hz, 1H), 4.26-4.00 (m, 2H), 3.71-3.50 (m, 2H), 3.29 (br s, 2H), 2.97 (br s, 1H), 2.75-2.69 (m, 2H), 2.39 (br s, 1H), 1.92-1.86 (m, 2H), 1.69-1.27 (m, 18H), 0.95-0.92 (m, 6H).

5.1.29 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(5-fluoro-3-pyridyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (31l)

The title compound was prepared from **25** and (1*R*)-1-(5-fluoro-3-pyridyl)-3-methylbutan-1-amine dihydrochloride **29l** in a manner similar to that described for **30** as a colorless solid (46%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41-8.34 (m, 2H), 7.46 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.34-7.22 (m, 4H), 5.92 (br s, 1H), 5.06 (q, *J* = 8.0 Hz, 1H), 4.25-3.96 (m, 2H), 3.73-3.47 (m, 2H), 3.37-3.25 (m, 2H), 2.96 (br s, 1H), 2.85-2.64 (m, 2H), 2.38 (br s, 1H), 1.99-1.82 (m, 2H), 1.73-1.27 (m, 18H), 0.98-0.94 (m, 6H).

5.1.30 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-isoxazol-5-yl-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (31m)

The title compound was prepared from **25** and (1*R*)-1-isoxazol-5-yl-3-methylbutan-1-amine hydrochloride **29m** in a manner similar to that described for **30** as a colorless solid (80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.21-8.16 (m, 1H), 7.46 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.34-7.24 (m, 3H), 6.17-6.10 (m, 1H), 5.93 (br s, 1H), 5.35 (q, *J* = 8.0 Hz, 1H), 4.24-3.97 (m, 2H), 3.73-3.51 (m, 2H), 3.37-3.25 (m, 2H), 2.96 (br s, 1H), 2.81-2.66 (m, 2H), 2.36 (br s, 1H), 1.95-1.87 (m, 2H), 1.77-1.22 (m, 18H), 0.98-0.92 (m, 6H).

5.1.31 *tert*-Butyl (3*R*,5*S*)-3-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-3-methyl-1-oxazol-2-yl-butyl]carbamoyl}piperidine-1-carboxylate (31n)

The title compound was prepared from **25** and (1*R*)-3-methyl-1-oxazol-2-yl-butan-1-amine hydrochloride **29n** in a manner similar to that described for **30** as a colorless solid (37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62-7.59 (m, 1H), 7.46 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.34-7.24 (m, 3H), 7.09-7.04 (m, 1H), 6.16 (br s, 1H), 5.35-5.27 (m, 1H), 4.27-4.08 (m, 2H), 3.76-3.50 (m, 2H), 3.35-3.28 (m, 2H), 2.97 (br s, 1H), 2.79-2.68 (m, 2H), 2.42 (br s, 1H), 2.01-1.92 (m, 2H), 1.80-1.29 (m, 18H), 0.96-0.91 (m, 6H).

5.1.32 *tert*-Butyl (3*R*,5*S*)-3-[2,2-dimethyl-4-(*o*-tolyl)-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (31o)

The title compound was prepared from **26** and (1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutan-1-amine dihydrochloride **29k** in a manner similar to that described for **30** as a colorless solid (67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (br s, 1H), 7.39-7.35 (m, 1H), 7.26-7.21 (m, 4H), 7.11-7.05 (m, 1H), 6.49-6.48 (m, 1H), 5.13 (br q, *J* = 7.7 Hz, 1H), 4.26-4.00 (m, 2H), 3.71-3.49 (m, 2H), 3.35 (br d, *J* = 11.7 Hz, 1H), 3.19-3.16 (m, 1H), 2.96-2.69 (m, 3H), 2.40 (br s, 1H), 2.23 (s, 1.5H), 2.20 (s, 1.5H), 1.89 (br s, 2H), 1.69-1.28 (m, 18H), 0.95-0.92 (m, 6H).

5.1.33 *tert*-Butyl (3*R*,5*S*)-3-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-{[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]carbamoyl}piperidine-1-carboxylate (31p)

The title compound was prepared from **27** and (1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutan-1-amine dihydrochloride **29k** in a manner similar to that described for **30** as a colorless solid (61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (br s, 1H), 7.39-7.35 (m, 1H), 7.26-7.18 (m, 2H), 6.96-6.91 (m, 1H), 6.85-6.80 (m, 1H), 6.46 (br d, *J* = 8.2 Hz, 1H), 5.13 (br q, *J* = 7.8 Hz, 1H), 4.29-4.00 (m, 2H), 3.70-3.48 (m, 2H), 3.35 (br d, *J* = 10.5 Hz, 1H), 3.18-3.14 (m, 1H), 2.96-2.69 (m, 3H), 2.39 (br s, 1H), 2.18 (s, 1.5H), 2.14 (s, 1.5H), 1.88 (br s, 2H), 1.70-1.28 (m, 18H), 0.95-0.92 (m, 6H).

5.1.34 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(ethoxymethyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (5)

Trifluoroacetic acid (0.350 mL, 4.71 mmol) was added to a solution of **30** (135 mg, 0.228 mmol) in CH_2Cl_2 (0.700 mL) at room temperature, and the mixture was stirred at the same temperature for 15 min. Saturated $NaHCO_3$ aqueous solution 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 **5** (94.0 mg, 0.190 mmol). Fumaric acid (22.0 mg, 0.190 mmol) was added to a solution of the free base of **5** (94.0 mg, 0.190 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 **5** (97.0 mg, 70%, 2 steps) was obtained as a colorless solid by crystallization from CH_2Cl_2 and Et_2O. $[\alpha]_D^{25.0}$ +18.3 (*c* 1.05, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.54-7.52 (m, 1H), 7.43-7.32 (m, 3H), 6.69 (s, 2H), 4.12-4.08 (m, 1H), 3.69-2.94 (m, 13H), 2.84-2.78 (m, 1H), 2.16-2.10 (m, 1H), 1.98-1.89 (m, 1H), 1.67-1.58 (m, 1H), 1.54-1.32 (m, 8H), 1.17 (t, *J* = 7.0 Hz, 3H), 0.95-0.90 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD): δ 173.0, 171.4, 169.4, 140.1, 136.3, 133.3, 131.5, 130.9, 130.5, 129.5, 73.8, 67.5, 63.5, 54.8, 50.3, 47.7, 47.0, 45.6, 41.5, 32.6, 26.0, 25.1, 24.3, 23.7, 23.1, 22.3, 21.7, 15.6. IR: 2967, 2870, 1648, 1556, 1386, 1272, 1174, 984, 755 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{26}H_{41}ClN_4O_3$+H: 493.2945; found: 493.2963. Anal. $C_{26}H_{41}ClN_4O_3 \cdot C_4H_4O_4 \cdot H_2O$; calcd: C 57.45, H 7.55, N 8.93, Cl 5.65; found: C 57.43, H 7.50, N 8.82, Cl 5.54.

5.1.35 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-3-methyl-1-phenylbutyl]piperidine-3-carboxamide fumarate (32a)

The title compound was prepared from **31a** in a manner similar to that described for **5** as a colorless solid (54%, 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 7.52-7.22 (m, 9H), 6.70 (s, 2H), 4.94 (br dd, *J* = 9.3, 5.4 Hz, 1H), 3.63-3.22 (m, 7H), 3.05-2.88 (m, 3H), 2.06-2.00 (m, 1H), 1.87-1.69 (m, 2H), 1.61-1.54 (m, 2H), 1.38-1.35 (m, 6H), 0.97-0.91 (m, 6H). HRMS (ESI$^+$): m/z calcd for $C_{29}H_{39}ClN_4O_2$+H: 511.2840; found: 511.2850.

5.1.36 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-phenylbutyl]piperidine-3-carboxamide fumarate (32b)

The title compound was prepared from **31b** in a manner similar to that described for **5** as a colorless solid (63%, 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 7.53-7.50 (m, 1H), 7.48-7.23 (m, 8H), 6.70 (s, 2H), 4.87-4.82 (m, 1H), 3.62-3.21 (m, 7H), 3.05-2.86 (m, 3H), 2.08-2.00 (m, 1H), 1.94-1.93 (m, 1H), 1.87-1.70 (m, 4H), 1.42-1.31 (m, 6H), 0.94 (t, *J* = 7.3 Hz, 3H). HRMS (ESI$^+$): m/z calcd for $C_{28}H_{37}ClN_4O_2$+H: 497.2683; found: 497.2695.

5.1.37 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-phenylpropyl]piperidine-3-carboxamide fumarate (32c)

The title compound was prepared from **31c** in a manner similar to that described for **5** as a colorless solid (68%, 2 steps). $[\alpha]_D^{25.0}$ +19.5 (*c* 1.02, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 7.53-7.51 (m, 1H), 7.41-7.21 (m, 8H), 6.70 (s, 2H), 4.73 (br t, *J* = 7.3 Hz, 1H), 3.63-3.42 (m, 4H), 3.39-3.20 (m, 3H), 3.06-3.01 (m, 1H), 2.96 (br t, *J* = 12.2 Hz, 1H), 2.90-2.85 (m, 1H), 2.08-2.02 (m, 1H), 1.89-1.77 (m, 3H), 1.39-1.35 (m, 6H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 170.6, 168.0, 166.0, 143.5, 139.2, 135.0, 131.4, 129.9, 129.4, 129.2, 128.2, 128.1, 126.5, 126.2, 61.5, 54.0, 52.9, 49.1, 45.9, 43.9, 31.4, 29.2, 23.9, 23.1, 22.6, 21.1, 10.9. IR: 3318, 2968, 1648, 1551, 1493, 1334, 986, 757, 702 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{27}H_{35}ClN_4O_2$+H: 483.2527; found: 483.2541. Anal. $C_{27}H_{35}ClN_4O_2 \cdot C_4H_4O_4 \cdot H_2O$; calcd: C 60.33, H 6.70, N 9.08, Cl 5.74; found: C 60.61, H 6.57, N 9.13, Cl 5.75.

5.1.38 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-3-methyl-1-(2-pyridyl)butyl]piperidine-3-carboxamide fumarate (32d)

The title compound was prepared from **31d** in a manner similar to that described for **5** as a colorless solid (71%, 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.51 (br d, *J* = 4.9 Hz, 1H), 7.79 (br t, *J* = 7.6 Hz, 1H), 7.52-7.51 (m, 1H), 7.41-7.35 (m, 3H), 7.31-7.28 (m, 2H), 6.70 (s, 2H), 5.03 (dd, *J* = 9.3, 5.9 Hz, 1H), 3.64-3.44 (m, 4H), 3.39-3.22 (m, 3H), 3.06-3.01 (m, 1H), 2.98-2.89 (m, 2H), 2.17-2.11 (m, 1H), 1.87-1.78 (m, 1H), 1.76-1.58 (m, 3H), 1.41-1.36 (m, 6H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.3 Hz, 3H). HRMS (ESI$^+$): m/z calcd for $C_{28}H_{38}ClN_5O_2$+H: 512.2792; found: 512.2813.

5.1.39 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-3-methyl-1-(3-pyridyl)butyl]piperidine-3-carboxamide fumarate (32e)

The title compound was prepared from **31e** in a manner similar to that described for **5** as a colorless solid (66%, 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.51 (br s, 1H), 8.45 (br d, *J* =

5.1 Hz, 1H), 7.82-7.79 (m, 1H), 7.53-7.51 (m, 1H), 7.45-7.26 (m, 4H), 6.72 (s, 2H), 4.99 (dd, *J* = 9.4, 5.5 Hz, 1H), 3.63-3.22 (m, 7H), 3.09-2.85 (m, 3H), 2.10-2.01 (m, 1H), 1.87-1.75 (m, 2H), 1.64-1.57 (m, 2H), 1.39-1.36 (m, 6H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.94 (d, *J* = 6.3 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{28}H_{38}ClN_5O_2$+H: 512.2792; found: 512.2813.

5.1.40 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(2-fluorophenyl)propyl]piperidine-3-carboxamide fumarate (32f)

The title compound was prepared from **31f** in a manner similar to that described for **5** as a colorless solid (68%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 7.52 (br d, *J* = 7.8 Hz, 1H), 7.41-7.26 (m, 5H), 7.16 (dt, *J* = 7.3, 1.0 Hz, 1H), 7.08 (ddd, *J* = 10.7, 8.3, 1.0 Hz, 1H), 6.71 (s, 2H), 5.01 (br t, *J* = 7.6 Hz, 1H), 3.63-3.43 (m, 4H), 3.39-3.22 (m, 3H), 3.07-3.02 (m, 1H), 2.99-2.89 (m, 2H), 2.11-2.04 (m, 1H), 1.89-1.79 (m, 3H), 1.40-1.36 (m, 6H), 0.93 (t, *J* = 7.3 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{27}H_{34}ClFN_4O_2$+H: 501.2433; found: 501.2440.

5.1.41 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(3-fluorophenyl)propyl]piperidine-3-carboxamide fumarate (32g)

The title compound was prepared from **31g** in a manner similar to that described for **5** as a colorless solid (70%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 7.52-7.51 (m, 1H), 7.40-7.27 (m, 4H), 7.12 (br d, *J* = 7.8 Hz, 1H), 7.06-7.04 (m, 1H), 7.00-6.96 (m, 1H), 6.70 (s, 2H), 4.74 (br t, *J* = 7.3 Hz, 1H), 3.64-3.42 (m, 4H), 3.39-3.22 (m, 3H), 3.07-2.89 (m, 3H), 2.10-2.02 (m, 1H), 1.89-1.78 (m, 3H), 1.39-1.36 (m, 6H), 0.93 (t, *J* = 7.3 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{27}H_{34}ClFN_4O_2$+H: 501.2433; found: 501.2444.

5.1.42 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(4-fluorophenyl)propyl]piperidine-3-carboxamide fumarate (32h)

The title compound was prepared from **31h** in a manner similar to that described for **5** as a colorless solid (67%, 2 steps). ^1H NMR (400 MHz, CD_3OD): δ 7.53-7.51 (m, 1H), 7.38-7.28 (m, 5H), 7.09-7.04 (m, 2H), 6.70 (s, 2H), 4.73 (br t, *J* = 7.4 Hz, 1H), 3.64-3.22 (m, 7H), 3.07-2.86 (m, 3H), 2.08-2.00 (m, 1H), 1.89-1.77 (m, 3H), 1.38-1.36 (m, 6H), 0.91 (t, *J* = 7.4 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{27}H_{34}ClFN_4O_2$+H: 501.2433; found: 501.2435.

5.1.43 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(3-chlorophenyl)propyl]piperidine-3-carboxamide fumarate (32i)

The title compound was prepared from **31i** in a manner similar to that described for **5** as a colorless solid (50%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 7.53-7.50 (m, 1H), 7.39-7.23 (m, 7H), 6.70 (s, 2H), 4.71 (t, *J* = 7.6 Hz, 1H), 3.73-3.49 (m, 4H), 3.47-3.42 (m, 1H), 3.40-3.22 (m, 2H), 3.06-2.88 (m, 3H), 2.12-2.02 (m, 1H), 1.88-1.77 (m, 3H), 1.42-1.35 (m, 6H), 0.93 (t, *J* = 7.3 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{27}H_{34}Cl_2N_4O_2$+H: 517.2137; found: 517.2132.

5.1.44 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(4-chlorophenyl)propyl]piperidine-3-carboxamide fumarate (32j)

The title compound was prepared from **31j** in a manner similar to that described for **5** as a colorless solid (64%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 7.54-7.50 (m, 1H), 7.40-7.26 (m, 7H), 6.70 (s, 2H), 4.71 (t, *J* = 7.3 Hz, 1H), 3.64-3.22 (m, 7H), 3.07-2.86 (m, 3H), 2.09-2.01 (m, 1H), 1.89-1.77 (m, 3H), 1.41-1.34 (m, 6H), 0.92 (t, *J* = 7.5 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{27}H_{34}Cl_2N_4O_2$+H: 517.2137; found: 517.2144.

5.1.45 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (32k)

The title compound was prepared from **31k** in a manner similar to that described for **5** as a colorless solid (78%, 2 steps). $[\alpha]_D^{25.0}$ +16.6 (*c* 1.16, MeOH). ^1H NMR (500 MHz, CD_3OD): δ 8.41 (br d, *J* = 2.0 Hz, 1H), 7.58-7.50 (m, 2H), 7.42-7.35 (m, 3H), 7.33-7.30 (m, 1H), 6.71 (s, 2H), 5.05 (dd, *J* = 9.3, 6.4 Hz, 1H), 3.64-3.44 (m, 4H), 3.39-3.21 (m, 3H), 3.04 (br t, *J* = 11.7 Hz, 1H), 2.99-2.89 (m, 2H), 2.15-2.09 (m, 1H), 1.86-1.78 (m, 1H), 1.76-1.64 (m, 2H), 1.61-1.55 (m, 1H), 1.40-1.36 (m, 6H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD): δ 172.8, 171.5, 169.4, 161.2, 159.1, 140.1, 138.3, 136.3, 133.3, 131.5, 130.8, 130.4, 129.5, 125.0, 123.8, 63.4, 54.8, 53.7, 50.3, 47.7, 47.0, 45.5, 41.2, 32.6, 26.1, 25.1, 24.2, 23.3, 22.3, 21.8. IR: 3279, 2961, 1649, 1552, 1485, 1387, 1228, 983, 752, 633 cm^{-1}. HRMS (ESI^+): m/z calcd for $C_{28}H_{37}ClFN_5O_2$+H: 530.2698; found: 530.2700. Anal. $C_{28}H_{37}ClFN_5O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$; calcd: C 58.66, H 6.46, N 10.69, Cl 5.41, F 2.90; found: C 58.70, H 6.58, N 10.73, Cl 5.43, F 2.86.

5.1.46 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(5-fluoro-3-pyridyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (32l)

The title compound was prepared from **31l** in a manner similar to that described for **5** as a colorless solid (72%, 2 steps). ^1H NMR (400 MHz, CD_3OD): δ 8.39-8.37 (m, 2H), 7.62-7.59 (m, 1H), 7.51 (br d, *J* = 7.8 Hz, 1H), 7.41-7.35 (m, 2H), 7.31-7.27 (m, 1H), 6.70 (s, 2H), 5.02 (dd, *J* = 9.5, 5.6 Hz, 1H), 3.64-3.42 (m, 4H), 3.39-3.21 (m, 3H), 3.06-3.00 (m, 1H), 2.98-2.86 (m, 2H), 2.09-2.01 (m, 1H), 1.88-1.76 (m, 2H), 1.67-1.58 (m, 2H), 1.39-1.36 (m, 6H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{28}H_{37}ClFN_5O_2$+H: 530.2698; found: 530.2725.

5.1.47 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-isoxazol-5-yl-3-methylbutyl]piperidine-3-carboxamide fumarate (32m)

The title compound was prepared from **31m** in a manner similar to that described for **5** as a colorless solid (66%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 8.33-8.31 (m, 1H), 7.53-7.51 (m, 1H), 7.42-7.28 (m, 3H), 6.70 (s, 2H), 6.31-6.29 (m, 1H), 5.26 (dd, *J* = 9.3, 6.3 Hz, 1H), 3.68-3.23 (m, 7H), 3.08-2.86 (m, 3H), 2.14-2.08 (m, 1H), 1.95-1.87 (m, 1H), 1.82-1.74 (m, 2H), 1.68-1.61 (m, 1H), 1.41-1.33 (m, 6H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{26}H_{37}ClN_5O_3$+H: 502.2585; found: 502.2588.

5.1.48 (3*S*,5*R*)-5-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-3-methyl-1-oxazol-2-yl-butyl]piperidine-3-carboxamide fumarate (32n)

The title compound was prepared from **31n** in a manner similar to that described for **5** as a colorless solid (34%, 2 steps). ^1H NMR (500 MHz, CD_3OD): δ 7.88-7.86 (m, 1H), 7.53 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.41-7.35 (m, 2H), 7.33-7.30 (m, 1H), 7.14-7.12 (m, 1H), 6.70 (d, *J* = 2.9 Hz, 2H), 5.17 (dd, *J* = 8.5, 7.1 Hz, 1H), 3.69-3.22 (m, 7H), 3.08-2.95 (m, 2H), 2.92-2.84 (m, 1H), 2.23-2.15 (m, 1H), 1.94-1.85 (m, 1H), 1.82-1.77 (m, 2H), 1.65-1.60

(m, 1H), 1.43-1.36 (m, 6H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). HRMS (ESI$^+$): m/z calcd for $C_{26}H_{37}ClN_5O_3$+H: 502.2585; found: 502.2603.

5.1.49 (3*S*,5*R*)-5-[2,2-Dimethyl-4-(*o*-tolyl)-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (32o)

The title compound was prepared from **31o** in a manner similar to that described for **5** as a colorless solid (74%, 2 steps). $[\alpha]_D^{25.0}$ +15.3 (*c* 1.19, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 8.41 (br s, 1H), 7.59-7.54 (m, 1H), 7.41-7.38 (m, 1H), 7.30-7.24 (m, 3H), 7.12-7.09 (m, 1H), 6.70 (s, 2H), 5.06-5.03 (m, 1H), 3.64-3.46 (m, 4H), 3.41-3.19 (m, 3H), 3.04 (t, *J* = 12.2 Hz, 1H), 2.98-2.87 (m, 2H), 2.19 (s, 1.5H), 2.17 (s, 1.5H), 2.13-2.09 (m, 1H), 1.86-1.57 (m, 4H), 1.38-1.36 (m, 6H), 0.98-0.93 (m, 6H). ^{13}C NMR (125 MHz, DMSO-*d*6): δ 170.8, 168.1, 165.9, 158.9, 158.7, 156.9, 141.0, 136.6, 136.4, 135.1, 130.5, 127.4, 126.8, 123.6, 121.8, 61.9, 52.9, 51.7, 48.9, 45.9, 45.6, 43.9, 43.7, 31.3, 24.4, 24.0, 23.0, 22.7, 21.6, 21.0, 17.3. IR: 3296, 2960, 1643, 1485, 1387, 1228, 983, 749, 647 cm^{-1}. HRMS (ESI$^+$): m/z calcd for $C_{29}H_{40}FN_5O_2$+H: 510.3244; found: 510.3235. Anal. $C_{29}H_{40}FN_5O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$; calcd: C 62.44, H 7.15, N 11.03, F 2.99; found: C 62.47, H 7.41, N 10.97, F 2.88.

5.1.50 (3*S*,5*R*)-5-[4-(5-Fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-[(1*R*)-1-(5-fluoro-2-pyridyl)-3-methylbutyl]piperidine-3-carboxamide fumarate (32p)

The title compound was prepared from **31p** in a manner similar to that described for **5** as a colorless solid (72%, 2 steps). ^{1}H NMR (500 MHz, CD_3OD): δ 8.41 (br s, 1H), 7.59-7.52 (m, 1H), 7.43-7.38 (m, 1H), 7.31-7.28 (m, 1H), 7.04-7.00 (m, 1H), 6.96-6.93 (m, 1H), 6.69 (s, 2H), 5.04 (br dd, *J* = 8.4, 6.5 Hz, 1H), 3.64-3.18 (m, 7H), 3.02 (br t, *J* = 12.1 Hz, 1H), 2.98-2.90 (m, 2H), 2.16-2.07 (m, 4H), 1.89-1.56 (m, 4H), 1.37-1.35 (m, 6H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H). HRMS (ESI$^+$): m/z calcd for $C_{29}H_{39}F_2N_5O_2$+H: 528.3150; found: 528.3162.

5.1.51 (2*R*)-2-(Dibenzylamino)-4-methylpentan-1-ol (34)

K_2CO_3 (14.7 g, 107 mmol) was added to a solution of (*R*)-leucinol **33** (5.00 g, 42.7 mmol) and benzyl bromide (11.2 mL, 93.9 mmol) in ethanol (200 mL) at room temperature, and the mixture was stirred at the same temperature for 4 days. The reaction mixture was concentrated under reduced pressure and diluted with water, 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 = 10/1 to 1/1) to obtain **34** (10.8 g, 85%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.21 (m, 10H), 3.81 (d, *J* = 13.3 Hz, 2H), 3.48 (dd, *J* = 10.6, 5.1 Hz, 1H), 3.42 (d, *J* = 10.6 Hz, 1H), 3.37 (d, *J* = 13.3 Hz, 2H), 3.19 (br s, 1H), 2.84 (ddt, *J* = 9.8, 5.1, 2.4 Hz, 1H), 1.55-1.46 (m, 2H), 1.20-1.11 (m, 1H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.86 (d, *J* = 6.3 Hz, 3H).

5.1.52 (2*R*)-*N*,*N*-Dibenzyl-1-ethoxy-4-methylpentan-2-amine (35)

NaH (60%, dispersion in paraffin liquid) (349 mg, 8.74 mmol) was added to a solution of **34** (2.00 g, 6.72 mmol) and EtI (0.700 mL, 8.74 mmol) in THF (30.0 mL) at room temperature over a period of 10 min. The reaction mixture was stirred at room temperature for 18 h. Water 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 = 1/0 to 5/1) to obtain **35** (1.78 g, 81%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (br d, *J* = 7.4 Hz, 4H), 7.30-7.26 (m, 4H), 7.22-7.17 (m, 2H), 3.75 (d, *J* = 13.7 Hz, 2H), 3.66-3.61 (m, 3H), 3.45 (q, *J* = 7.0 Hz, 2H), 3.38 (dd, *J* = 9.8, 5.1 Hz, 1H), 2.89-2.83 (m, 1H), 1.84-1.74 (m, 1H), 1.46 (ddd, *J* = 13.7, 8.2, 5.4 Hz, 1H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.10 (ddd, *J* = 13.7, 8.2, 5.4 Hz, 1H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.59 (d, *J* = 6.3 Hz, 3H).

5.1.53 (2*R*)-1-Ethoxy-4-methylpentan-2-amine (28)

To a suspension of **35** (1.78 g, 5.47 mmol) in MeOH (25.0 mL), 20% $Pd(OH)_2$ on carbon (50% wet, 360 mg) were added. The suspension was stirred under 1 MPa H_2 at room temperature for 6 h. H_2 in the reaction vessel was replaced by N_2, and then $Pd(OH)_2$ on carbon was separated by filtration. The solvent was evaporated under reduced pressure to obtain **28** (799 mg, 98%) as a colorless liquid. $[\alpha]_D^{25.0}$ −11.8 (*c* 1.03, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.55-3.45 (m, 2H), 3.39 (dd, *J* = 9.0, 3.7 Hz, 1H), 3.12 (t, *J* = 8.6 Hz, 1H), 3.05-3.00 (m, 1H), 1.77-1.69 (m, 1H), 1.22-1.13 (m, 5H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). HRMS (ESI$^+$): m/z calcd for $C_8H_{19}NO$+H: 146.1545; found: 146.1552.

5.1.54 [(1*R*)-1-Azidobutyl]benzene (37)

DBU (2.40 mL, 16.1 mmol) and diphenylphosphoryl azide (3.5 mL, 16.3 mmol) were added to a solution of (1*S*)-1-phenylbutan-1-ol **36** (2.00 g, 13.3 mmol) in toluene (20 mL) at rt, and the mixture was stirred at the same temperature for 2 days. Water 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 = 1/0 to 9/1) to obtain **37** (0.650 g, 28%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.36 (m, 2H), 7.33-7.29 (m, 3H), 4.41 (t, *J* = 7.3 Hz, 1H), 1.87-1.79 (m, 1H), 1.76-1.68 (m, 1H), 1.45-1.38 (m, 1H), 1.35-1.27 (m, 1H), 0.93 (t, *J* = 7.4 Hz, 3H).

5.1.55 (1*R*)-1-Phenylbutan-1-amine hydrochloride (29b)

To a suspension of **37** (650 mg, 3.71 mmol) in AcOEt (6.00 mL) and EtOH (6.00 mL), 10% Pd on carbon (50% wet, 130 mg) were added. The suspension was stirred under H_2 atmosphere at room temperature for 3 h. H_2 in the reaction vessel was replaced by N_2, and then Pd on carbon was separated by filtration. The solvent was evaporated under reduced pressure. 4*N*-HCl in 1,4-dioxane (3.60 mL, 14.4 mmol) was added to the residue at room temperature, and the mixture was evaporated under reduced pressure. The residue was washed with AcOEt to obtain **29b** (430 mg, 63%, 2 steps) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.48-7.40 (m, 5H), 4.24 (dd, *J* = 9.3, 6.3 Hz, 1H), 1.98-1.91 (m, 2H), 1.35-1.28 (m, 1H), 1.26-1.18 (m, 1H), 0.95 (t, *J* = 7.6 Hz, 3H).

5.1.56 (S_S)-2-Methyl-*N*-[(1*R*)-3-methyl-1-phenylbutyl]propane-2-sulfinamide (40a)

$Ti(OEt)_4$ (6.82 g, 29.9 mol) was added to a solution of 3-methyl-1-phenylbutan-1-one **38a** (27.0 g, 105 mmol) and (*S*)-(−)-2-methylpropane-2-sulfinamide **39** (2.00 g, 16.5 mmol) in THF (36.0 mL) at rt, and the mixture was stirred at 70 °C for 5.5 h. After cooling, brine was added to the reaction mixture. The mixture was filtered, and then the solvent was extracted 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 =

1/0 to 7/3) to obtain (S_S)-2-methyl-*N*-[(1*E*)-3-methyl-1-phenylbutylidene]propane-2-sulfinamide (2.31 g, 47%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.82-7.80 (m, 2H), 7.48-7.40 (m, 3H), 3.35-3.31 (m, 1H), 3.08-3.05 (m, 1H), 2.10-2.02 (m, 1H), 1.32 (s, 9H), 0.98-0.96 (m, 6H). 1M L-selectride in THF (26.0 mL, 26.0 mmol) was added to a solution of (S_S)-2-methyl-*N*-[(1*E*)-3-methyl-1-phenylbutylidene]propane-2-sulfinamide (2.31 g, 8.70 mmol) in THF (20 mL) under ice-cooling over a period of 5 min, and the mixture was stirred at the same temperature for 1 h. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 1/0 to 1/1) to obtain **40a** (1.93 g, 83%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34-7.25 (m, 5H), 4.47-4.43 (m, 1H), 3.33 (br s, 1H), 1.72-1.63 (m, 2H), 1.48-1.40 (m, 1H), 1.16 (s, 9H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.4 Hz, 3H).

5.1.57 (S_S)-2-Methyl-*N*-[(1*R*)-3-methyl-1-(2-pyridyl)butyl]propane-2-sulfinamide (40d)

The title compound was prepared from 3-methyl-1-(2-pyridyl)butan-1-one **38d** in a manner similar to that described for **40a** as a colorless liquid (63%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58-8.57 (m, 1H), 7.65 (dt, *J* = 7.6, 1.8 Hz, 1H), 7.27-7.24 (m, 1H), 7.18 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H), 4.54-4.50 (m, 1H), 3.85 (d, *J* = 5.9 Hz, 1H), 1.85-1.76 (m, 2H), 1.56-1.51 (m, 1H), 1.15 (s, 9H), 0.95 (d, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 7.0 Hz, 3H).

5.1.58 (S_S)-2-Methyl-*N*-[(1*R*)-3-methyl-1-(3-pyridyl)butyl]propane-2-sulfinamide (40e)

The title compound was prepared from 3-methyl-1-(3-pyridyl)butan-1-one **38e** in a manner similar to that described for **40a** as a colorless liquid (45%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57-8.54 (m, 2H), 7.64-7.61 (m, 1H), 7.30-7.27 (m, 1H), 4.50 (dt, *J* = 7.4, 2.3 Hz, 1H), 3.40 (d, *J* = 1.5 Hz, 1H), 1.70 (t, *J* = 7.3 Hz, 2H), 1.48-1.43 (m, 1H), 1.17 (s, 9H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H).

5.1.59 (S_S)-*N*-[(1*R*)-1-(2-Fluorophenyl)propyl]-2-methylpropane-2-sulfinamide (40f)

The title compound was prepared from 1-(2-fluorophenyl)propan-1-one **38f** in a manner similar to that described for **40a** as a colorless liquid (49%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31-7.23 (m, 2H), 7.14-7.10 (m, 1H), 7.05-7.01 (m, 1H), 4.62-4.58 (m, 1H), 3.44 (d, *J* = 4.4 Hz, 1H), 1.99-1.84 (m, 2H), 1.17 (s, 9H), 0.89 (t, *J* = 7.6 Hz, 3H).

5.1.60 (S_S)-*N*-[(1*R*)-1-(3-Fluorophenyl)propyl]-2-methylpropane-2-sulfinamide (40g)

The title compound was prepared from 1-(3-fluorophenyl)propan-1-one **38g** in a manner similar to that described for **40a** as a colorless liquid (60%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.28 (m, 1H), 7.09-7.05 (m, 1H), 7.02-6.94 (m, 2H), 4.32-4.28 (m, 1H), 3.38 (br s, 1H), 1.88-1.74 (m, 2H), 1.19 (s, 9H), 0.85 (t, *J* = 7.4 Hz, 3H).

5.1.61 (S_S)-*N*-[(1*R*)-1-(4-Fluorophenyl)propyl]-2-methylpropane-2-sulfinamide (40h)

The title compound was prepared from 1-(4-fluorophenyl)propan-1-one **38h** in a manner similar to that described for **40a** as a colorless liquid (70%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.23 (m, 2H), 7.05-7.00 (m, 2H), 4.31-4.25 (m, 1H), 3.38 (br s, 1H), 1.87-1.72 (m, 2H), 1.18 (s, 9H), 0.83 (t, *J* = 7.3 Hz, 3H).

5.1.62 (S_S)-*N*-[(1*R*)-1-(3-Chlorophenyl)propyl]-2-methylpropane-2-sulfinamide (40i)

The title compound was prepared from 1-(3-chlorophenyl)propan-1-one **38i** in a manner similar to that described for **40a** as a colorless liquid (57%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29-7.24 (m, 3H), 7.19-7.16 (m, 1H), 4.29-4.26 (m, 1H), 3.38 (br s, 1H), 1.87-1.74 (m, 2H), 1.20 (s, 9H), 0.85 (t, *J* = 7.4 Hz, 3H).

5.1.63 (S_S)-*N*-[(1*R*)-1-(4-Chlorophenyl)propyl]-2-methylpropane-2-sulfinamide (40j)

The title compound was prepared from 1-(4-chlorophenyl)propan-1-one **38j** in a manner similar to that described for **40a** as a colorless liquid (60%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.29 (m, 2H), 7.24-7.21 (m, 2H), 4.30-4.26 (m, 1H), 3.37 (br s, 1H), 1.86-1.72 (m, 2H), 1.18 (s, 9H), 0.84 (t, *J* = 7.3 Hz, 3H).

5.1.64 (S_S)-*N*-[(1*R*)-1-(5-Fluoro-2-pyridyl)-3-methylbutyl]-2-methylpropane-2-sulfinamide (40k)

The title compound was prepared from 1-(5-fluoro-2-pyridyl)-3-methylbutan-1-one **38k**[22] in a manner similar to that described for **40a** as a colorless liquid (53%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, *J* = 2.7 Hz, 1H), 7.36 (dt, *J* = 8.6, 2.7 Hz, 1H), 7.29-7.26 (m, 1H), 4.55 (dt, *J* = 7.4, 5.5 Hz, 1H), 3.73 (br d, *J* = 5.5 Hz, 1H), 1.84-1.72 (m, 2H), 1.54-1.44 (m, 1H), 1.15 (s, 9H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H).

5.1.65 (S_S)-*N*-[(1*R*)-1-(5-Fluoro-3-pyridyl)-3-methylbutyl]-2-methylpropane-2-sulfinamide (40l)

The title compound was prepared from 1-(5-fluoro-3-pyridyl)-3-methylbutan-1-one **38l** in a manner similar to that described for **40a** as a colorless liquid (41%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41-8.40 (m, 2H), 7.40-7.37 (m, 1H), 4.54 (dt, *J* = 7.2, 1.7 Hz, 1H), 3.56 (d, *J* = 2.0 Hz, 1H), 1.76-1.63 (m, 2H), 1.53-1.42 (m, 1H), 1.19 (s, 9H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H).

5.1.66 (S_S)-*N*-[(1*R*)-1-Isoxazol-5-yl-3-methylbutyl]-2-methylpropane-2-sulfinamide (40m)

The title compound was prepared from 1-isoxazol-5-yl-3-methylbutan-1-one **38m**[22] in a manner similar to that described for **40a** as a colorless liquid (69%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18 (d, *J* = 1.9 Hz, 1H), 6.18 (d, *J* = 1.9 Hz, 1H), 4.70 (dt, *J* = 7.6, 5.0 Hz, 1H), 3.38 (d, *J* = 4.9 Hz, 1H), 1.90-1.77 (m, 2H), 1.64-1.59 (m, 1H), 1.19 (s, 9H), 0.98-0.94 (m, 6H).

5.1.67 (S_S)-2-Methyl-*N*-[(1*R*)-3-methyl-1-oxazol-2-yl-butyl]propane-2-sulfinamide (40n)

The title compound was prepared from 3-methyl-1-oxazol-2-yl-butan-1-one **38n** in a manner similar to that described for **40a** as a colorless liquid (58%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.08 (s, 1H), 4.68-4.62 (m, 1H), 3.53 (d, *J* = 5.9 Hz, 1H), 1.97-1.82 (m, 2H), 1.63-1.57 (m, 1H), 1.17 (s, 9H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H).

5.1.68 (1*R*)-3-Methyl-1-phenylbutan-1-amine hydrochloride (29a)

4*N*-HCl in 1,4-dioxane (3.60 mL, 14.4 mmol) was added to a solution of **40a** (1.93 g, 7.22 mmol) in methanol (3.60 mL) at room temperature, and the mixture was stirred at the same temperature for 40 min. Et_2O was added to the reaction mixture, and then the precipitate was collected by filtration to obtain **29a** (1.30 g, 90%) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.49-7.41 (m, 5H), 4.31 (dd, *J* = 10.0, 5.6 Hz, 1H), 1.93 (ddd, *J*

= 15.6, 10.3, 5.4 Hz, 1H), 1.81-1.75 (m, 1H), 1.44-1.36 (m, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H).

5.1.69 (1*R*)-3-Methyl-1-(2-pyridyl)butan-1-amine dihydrochloride (29d)

The title compound was prepared from **40d** in a manner similar to that described for **29a** as a colorless solid (89%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.81-8.79 (m, 1H), 8.28 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.86 (br d, *J* = 7.8 Hz, 1H), 7.75 (ddd, *J* = 7.8, 5.4, 1.0 Hz, 1H), 4.66 (t, *J* = 7.3 Hz, 1H), 1.99-1.87 (m, 2H), 1.57-1.49 (m, 1H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H).

5.1.70 (1*R*)-3-Methyl-1-(3-pyridyl)butan-1-amine dihydrochloride (29e)

The title compound was prepared from **40e** in a manner similar to that described for **29a** as a colorless solid (84%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.16-9.15 (m, 1H), 8.99-8.98 (m, 1H), 8.87-8.84 (m, 1H), 8.24 (dd, *J* = 8.3, 5.9 Hz, 1H), 4.76 (dd, *J* = 8.8, 6.8 Hz, 1H), 2.07-1.96 (m, 2H), 1.54-1.46 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H).

5.1.71 (1*R*)-1-(2-Fluorophenyl)propan-1-amine hydrochloride (29f)

The title compound was prepared from **40f** in a manner similar to that described for **29a** as a colorless solid (89%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.51-7.45 (m, 2H), 7.33-7.29 (m, 1H), 7.25-7.21 (m, 1H), 4.47 (dd, *J* = 9.3, 5.9 Hz, 1H), 2.12-1.94 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H).

5.1.72 (1*R*)-1-(3-Fluorophenyl)propan-1-amine hydrochloride (29g)

The title compound was prepared from **40g** in a manner similar to that described for **29a** as a colorless solid (80%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.52-7.47 (m, 1H), 7.27-7.16 (m, 3H), 4.21 (dd, *J* = 9.3, 5.9 Hz, 1H), 2.08-1.90 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H).

5.1.73 (1*R*)-1-(4-Fluorophenyl)propan-1-amine hydrochloride (29h)

The title compound was prepared from **40h** in a manner similar to that described for **29a** as a colorless solid (quant.). ^{1}H NMR (400 MHz, CD_3OD): δ 7.50-7.44 (m, 2H), 7.23-7.18 (m, 2H), 4.19 (dd, *J* = 9.2, 5.7 Hz, 1H), 2.09-1.88 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H).

5.1.74 (1*R*)-1-(3-Chlorophenyl)propan-1-amine hydrochloride (29i)

The title compound was prepared from **40i** in a manner similar to that described for **29a** as a colorless solid (90%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.50-7.44 (m, 3H), 7.38-7.36 (m, 1H), 4.19 (dd, *J* = 9.0, 6.1 Hz, 1H), 2.06-1.91 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H).

5.1.75 (1*R*)-1-(4-Chlorophenyl)propan-1-amine hydrochloride (29j)

The title compound was prepared from **40j** in a manner similar to that described for **29a** as a colorless solid (59%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.49-7.47 (m, 2H), 7.45-7.42 (m, 2H), 4.19 (dd, *J* = 9.3, 5.9 Hz, 1H), 2.09-1.89 (m, 2H), 0.89 (t, *J* = 7.6 Hz, 3H).

5.1.76 (1*R*)-1-(5-Fluoro-2-pyridyl)-3-methylbutan-1-amine dihydrochloride (29k)

The title compound was prepared from **40k** in a manner similar to that described for **29a** as a colorless solid (quant.). $[\alpha]_D^{25.0}$ −4.39 (*c* 1.00, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 8.56 (d, *J* = 2.9 Hz, 1H), 7.67 (dt, *J* = 8.6, 2.9 Hz, 1H), 7.53 (dd, *J* = 8.8, 4.4 Hz, 1H), 4.51-4.48 (m, 1H), 1.86 (ddd, *J* = 13.7, 8.3, 6.4 Hz, 1H), 1.76 (ddd, *J* = 13.7, 7.8, 6.4 Hz, 1H), 1.51-1.43 (m, 1H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H). HRMS (ESI^+): m/z calcd for $C_{10}H_{15}FN_2$+H: 183.1298; found: 183.1294.

5.1.77 (1*R*)-1-(5-Fluoro-3-pyridyl)-3-methylbutan-1-amine dihydrochloride (29l)

The title compound was prepared from **40l** in a manner similar to that described for **29a** as a colorless solid (88%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.71 (d, *J* = 2.0 Hz, 1H), 8.67 (br s, 1H), 8.03 (dt, *J* = 8.8, 2.0 Hz, 1H), 4.58 (dd, *J* = 9.3, 6.4 Hz, 1H), 2.00-1.94 (m, 1H), 1.91-1.86 (m, 1H), 1.49-1.41 (m, 1H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H).

5.1.78 (1*R*)-1-Isoxazol-5-yl-3-methylbutan-1-amine hydrochloride (29m)

The title compound was prepared from **40m** in a manner similar to that described for **29a** as a colorless solid (87%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.48 (d, *J* = 1.5 Hz, 1H), 6.63 (d, *J* = 1.5 Hz, 1H), 4.75 (dd, *J* = 9.8, 5.9 Hz, 1H), 2.04-1.97 (m, 1H), 1.86-1.80 (m, 1H), 1.56-1.49 (m, 1H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

5.1.79 (1*R*)-3-Methyl-1-oxazol-2-yl-butan-1-amine hydrochloride (29n)

The title compound was prepared from **40n** in a manner similar to that described for **29a** as a colorless solid (quant.). ^{1}H NMR (500 MHz, CD_3OD): δ 8.01 (s, 1H), 7.26 (s, 1H), 4.62 (dd, *J* = 9.3, 5.9 Hz, 1H), 2.05-1.98 (m, 1H), 1.83-1.77 (m, 1H), 1.63-1.55 (m, 1H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H).

5.2. Biological Assays

5.2.1. 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 L of the assay buffer (50 mM Tris-HCl (pH 7.9), 100 mM NaCl) containing 5 L 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 (PerkinElmer 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.

5.2.2. 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. 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.

5.3. Animal Studies

5.3.1. Blood pressure study in dTG rats

Double transgenic rats (dTGRs) were bred at PhoenixBio Co., Ltd. from parents who were homozygous for hREN or hAOGEN.[13c] Male and female hypertensive dTGRs (150-200 g) >8 weeks of age were instrumented with telemetric devices (TA11PA-C40, Data Sciences International Inc.) using a sterile technique. Baseline values were recorded for 24 h prior to administration. Following measurement of baseline values, rats were dosed by oral gavage with vehicle (0.5% methylcellulose, n = 9) or compound **32o** at 30 mg/kg (n = 5) after which animals were monitored for 48 h post-dose. Blood pressure signals were digitized and analyzed using Dataquest Art Software (Data Sciences International Inc.) for the derivation of MAP. These MAP values are reported as 1 h average values and also as changes from the corresponding time-matched baseline values.

5.3.2. 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 **32o** at doses of 3 and 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.

5.3.3. Pharmacokinetics

The pharmacokinetics of compounds **5**, **32c**, **32k**, and **32o** were determined in fasting SD rats and cynomolgus monkeys. Compounds **5**, **32c**, **32k**, and **32o** were administered intravenously as a saline solution or by oral gavage in 0.5% methylcellulose. Following dosing, blood samples were taken over 24 h with a minimum of 7 time points, and plasma concentrations were measured by HPLC/MS/MS. PK parameters were calculated from non-compartmental analysis of the plasma concentration-time curves.

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Supplementary Material

Experimental details and characterization data for compounds **38k**, **38m**, **41**, and **42** are available on-line.

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22. The synthetic methods for ketones **38k** and **38m** are described in the Supplementary Material.