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Lead optimization of 5-amino-6-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)-4-hydroxyhexanamides to reduce a cardiac safety issue: Discovery of DS-8108b, an orally active renin inhibitor

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ABSTRACT

With the aim to address an undesired cardiac issue observed with our related compound in the recently disclosed novel series of renin inhibitors, further chemical modifications of this series were performed. Extensive structure–activity relationships studies as well as in vivo cardiac studies using the electrophysiology rat model led to the discovery of clinical candidate *trans*-adamantan-1-ol analogue **56** (DS-8108b) as a potent renin inhibitor with reduced potential cardiac risk. Oral administration of single doses of 3 and 10 mg/kg of **56** in cynomolgus monkeys pre-treated with furosemide led to significant reduction of mean arterial blood pressure for more than 12 h.

1. Introduction

The renin–angiotensin–aldosterone system (RAAS) is well established as an endocrine system in regulating blood pressure and extracellular fluid volume.[1] In the RAAS, the aspartyl protease renin is the first and rate-limiting enzyme, which cleaves angiotensinogen to produce angiotensin I (Ang I). Ang I is further converted by angiotensin converting enzyme (ACE) to Ang II, which acts on the angiotensin receptor type 1 (AT_1), leading to pharmacological effects such as vasoconstriction, inflammation and fibrosis.[2] Direct renin inhibitors are expected to provide better kidney and heart protection while offering superior blood pressure lowering, but also cause fewer mechanism-based adverse events than existing ACE inhibitors and AT_1 blockers.[3] Significant research efforts and resources from the pharmaceutical industry have been invested toward the discovery of orally active renin inhibitors.[4] During the past few decades, most of the scaffolds were based on peptidic or peptidomimetic that were designed to mimic the N-terminal sequence of angiotensinogen, however, these scaffolds exhibited poor pharmacokinetic properties and low oral bioavailabilities.[5] Continued efforts for research on these scaffolds led to the discovery of aliskiren hemifumarate (**1**) (Fig. 1).[6] After disclosure of non-peptidic **1**, novel renin inhibitors on diverse scaffolds such as ACT-077825 (MK-8141) (**2**)[7] and VTP-27999 (**3**)[8] were reported (Fig. 1). Although these have advanced to human clinical trials, only aliskiren has been launched to the market for the treatment of hypertension to date.[9]

Recently, we have reported the discovery and initial optimization of a novel series of renin inhibitors, (2*S*,4*S*,5*S*)-5-amino-6-(2,2-dimethyl-5-oxo-4-phenylpiperazin-1-yl)-4-hydroxy-2-isopropyl hexanamides as exemplified by compound **4** (Fig. 2).[10] Compound **4** exhibited 2.0 and 25 nM inhibitory activities in purified human and monkey plasma renin, 18.5% oral bioavailability in cynomolgus

Abbreviations: HR, heart rate; PR, PR interval; QRS, QRS width; QTc, corrected QT interval.

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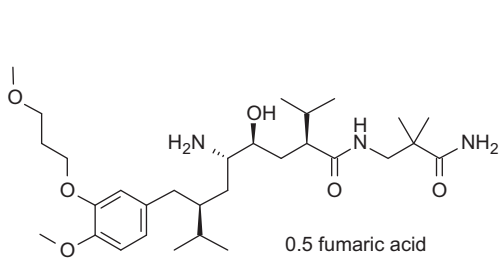
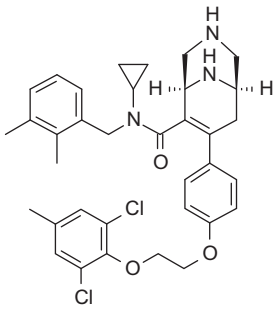
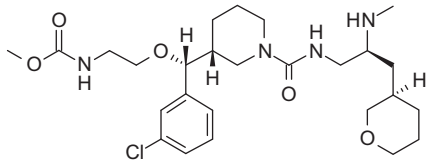


Aliskiren hemifumarate (**1**)

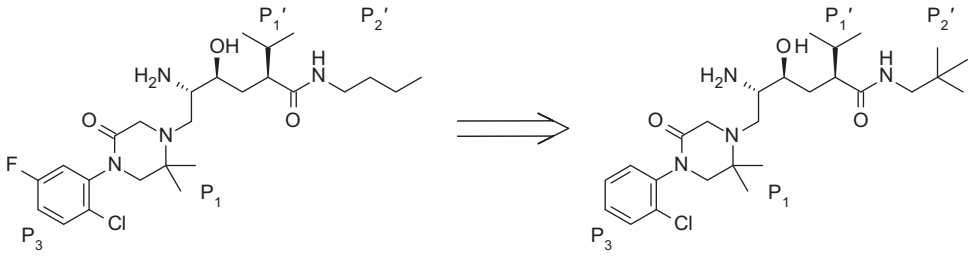


ACT-077825, MK-8141 (**2**)



VTP-27999 (**3**)

Figure 1. Representative non-peptidic renin inhibitors.



4
IC_{50} in purified human renin: 2.0 nM
IC_{50} in monkey plasma renin: 25 nM
Bioavailability: 18.5%

5
IC_{50} in purified human renin: 1.0 nM
IC_{50} in monkey plasma renin: 3.0 nM
Bioavailability: 26.4%

Figure 2. Structures of compounds **4** and **5**.

monkeys, and specificity over other proteases without interacting to the S_3^{sp} pocket of renin.[11] During the course of further modification of **4**, we identified neopentyl derivative **5**, which demonstrated excellent in vitro potencies in both purified human and monkey plasma assays, while maintaining the bioavailability similar to **4** (Fig. 2). Oral administration of **5** at a 3 mg/kg dose in cynomolgus monkeys pre-treated with furosemide showed significant reduction in plasma renin activity (PRA) and mean arterial blood pressure (MAP) (data not shown). Encouraged by these results, **5** was advanced to safety profiling, but unfortunately a serious cardiac issue was revealed. After administration of a single oral dose of 50 mg/kg of **5** to telemetered cynomolgus monkeys, ventricular tachycardia (VT) and fibrillation (VF) were observed. Concurrently, compound **5** was found to reduce the maximum rate of rise and shorten the action potential duration (APD) at 90% repolarizations from 10 μM in guinea pig papillary muscle. Although **5** did not affect hERG and $hCa_v1.2$ currents at 100 μM, it was found to show 27.2% inhibition against $hNa_v1.5$ current at 10 μM.[12] Compound **5** also showed 87% inhibition against Site 2 Na channel binding assay in rats. Based on these data, VT/VF observed in monkeys was supposed to be mainly derived from the action of **5** on the Na channel in the myocardium, however, we could not exclude the possibility of other channels' contributions. Therefore, we decided to use in vivo and ex vivo cardiac studies for assessing VT/VF concern of this series. In this paper, we report our efforts to address this issue and the discovery of compound **56** (DS-8108b).[13]

2. Strategy to obtain compounds with reduced arrhythmogenicity

Under the circumstance described above, we used a more time and resource sparing rodent alternative to large animal models.[14] As a result, we figured that the electrophysiology (EP) rat model was applicable for assessing a given compound's potential to induce QRS complex abnormality mainly based on the action of the Na channel. Iv bolus injections of **5** at 3 and 10 mg/kg doses caused QRS complex abnormalities in a dose-dependent manner in electrocardiogram (ECG) and finally resulted in deaths (Fig. 3). We considered that these ECG changes were related to VT/VF observed in monkeys, and decided to initiate rat in vivo screening. In this screening, the QRS complex abnormality was qualitatively classified to the three levels of ECG change; no (Fig. 3A), moderate (Fig. 3B) or severe (Fig. 3C) in the order of seriousness. After the ECG screening in rats, selected compounds were evaluated in an

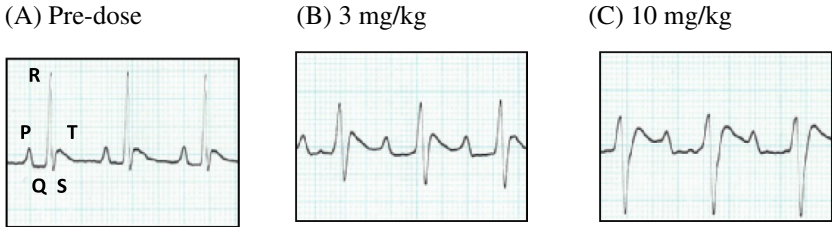


Figure 3. ECG after iv bolus injection of compound **5** in rats: (A) pre-dose, no change; (B) 3 mg/kg, moderate change; (C) 10 mg/kg, severe change.

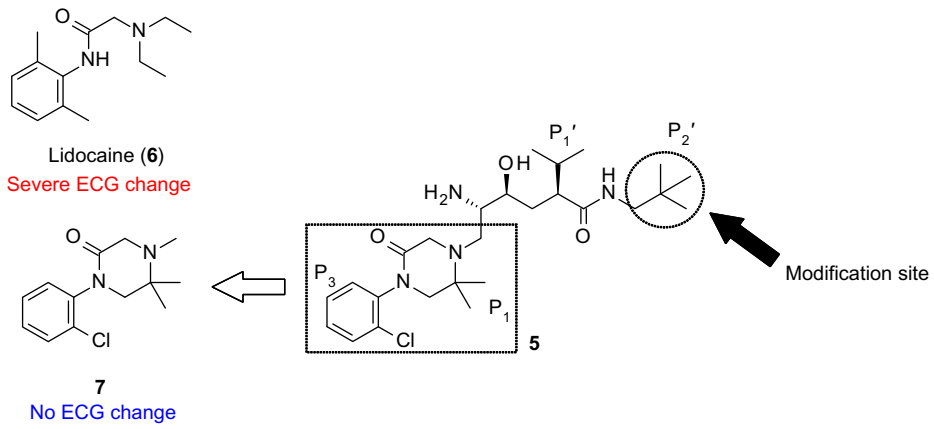


Figure 4. Structures of **6** and **7**, and modification site.

isolated Langendorff-perfused rabbit heart or APD in guinea pig papillary muscle to confirm the reduction of arrhythmogenicity at the ex vivo level. Final assessment of VT/VF concern was planned to be evaluated in telemetered monkeys in addition to rabbits and dogs.

Compounds **1** and **5** utilize the same transition-state mimetic scaffold and interact with S_3, S_1, S_1' and S_2' regions of renin. Since cardiac concern of aliskiren has not been reported in humans, we surmised that at least one of the structurally different portions between **1** and **5**, P_3–P_1 or P_2' portion, was associated with arrhythmogenicity observed with **5**. Initially, the structural similarity of Na channel blocker lidocaine (**6**) suggested to us that the P_3–P_1 portion of **5** might be attributed to the action on the Na channel (Fig. 4). Lidocaine caused QRS complex abnormality based on Na channel inhibition in the EP rat model (n = 2, 10 mg/kg, bolus injection), whereas compound **7** did not affect the ECG signal. From these results, we assumed that the contribution of the P_3–P_1 portion to the action on the Na channel would be low. Hence, we decided to focus on the modification of the P_2' portion to reduce arrhythmogenicity of this series.

3. Chemistry

All derivatives evaluated in this report are prepared from three key components depicted in Figure 5, except compound **26** (see Supplementary data). First component, 4-phenylketopiperazines **8** were prepared by the same procedure reported previously.[10] Second component, amines **9** (R^4-NH_2) were either obtained commercially or prepared according to the literature methods.[9a,15] Third component, *N*-2-nitrobenzenesulfonyl (*N*-Ns) protected alcohols **10** were prepared as shown in Scheme 1.[13,16] Diastereoselective alkylation of **11** with benzyl (2*E*)-4-bromobut-2-en-1-yl ether[17] proceeded smoothly, and the chiral auxiliaries were removed with $LiOH$–H_2O_2 to afford the chiral carboxylic acids **12**. Shi's asymmetric epoxidation[18] of **12** and subsequent intramolecular lactonization produced alcohols **13** directly. Mesylation of **13** with MsCl and Et_3N, followed by S_N2 substitution of the methanesulfonyloxy groups with the azide groups were accomplished by treating with NaN_3 in DMPU to obtain azides **14**. Then, hydrogenation of **14** in the presence of HCl, and the subsequent reaction with NsCl[19], followed by crystallization gave *N*-Ns-alcohols **10a**–**c** as a single diastereomer. Using these three components, derivatives are prepared as shown in Scheme 2. *N*-Ns-aziridines **15** were formed by the Mitsunobu reaction of **10** with DEAD and Ph_3P. The ring opening of aziridines **15** with ketopierazines **8** afforded lactones **16** in excellent yields. After replacement of the *N*-Ns groups with the *N*-Boc protecting group, sequential N-terminal amide bond formations with amines **9** under neat conditions in the presence of 2-hydroxypyridine as a catalyst[20], removal of the *N*-Boc protecting groups with TFA, and treatment with fumaric acid afforded the desired compounds as fumarate salts. In the case of aromatic amines **9**, the amide formation with **17** proceeded in the presence of Me_2AlCl.[21]

Preparation of derivatives, which were difficult to synthesize via direct lactone-opening with amine under the established conditions, is outlined in Scheme 3. After conversion of lactones **17a** and **17b** to the methyl esters by the hydrolysis with NaOH, followed by the treatment with $TMSCHN_2$, reaction with 2,2-dimethoxypropane and PPTS gave *N*,*O*-acetals **18**. Acetals **18** were hydrolyzed with NaOH, and then coupling of amines **9** with resulting carboxylic acids using HATU as an activating reagent afforded carboxamides **19**. Deprotection of the *N*,*O*-acetal and *N*-Boc protecting groups was achieved smoothly by reaction with 10% HCl/MeOH. Finally, treatment with fumaric acid afforded desired derivatives as fumarate salts.

4. Results and discussion

4.1. Structure–activity relationships of P_2' portion

A broad range of derivatives bearing simple alkyl (**4**, **5**, **20**–**26**), functionalized alkyl (**27**–**34**), or aromatic (**35**–**39**) group, were

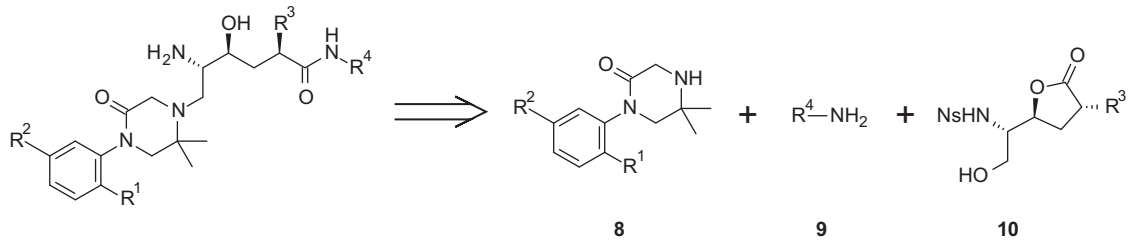
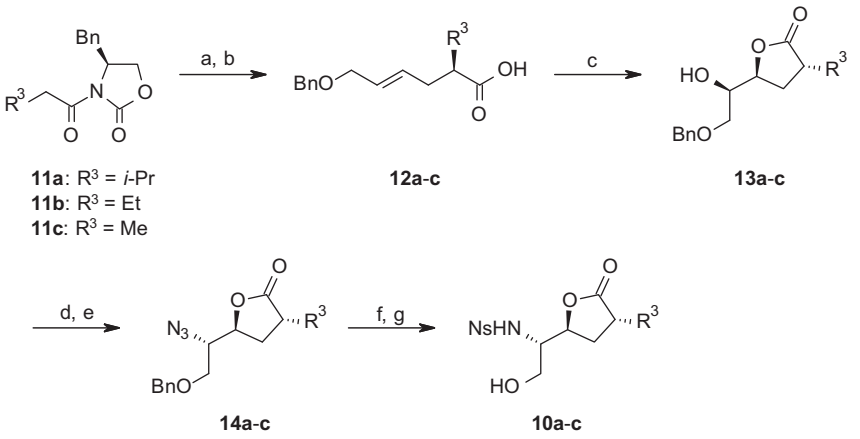
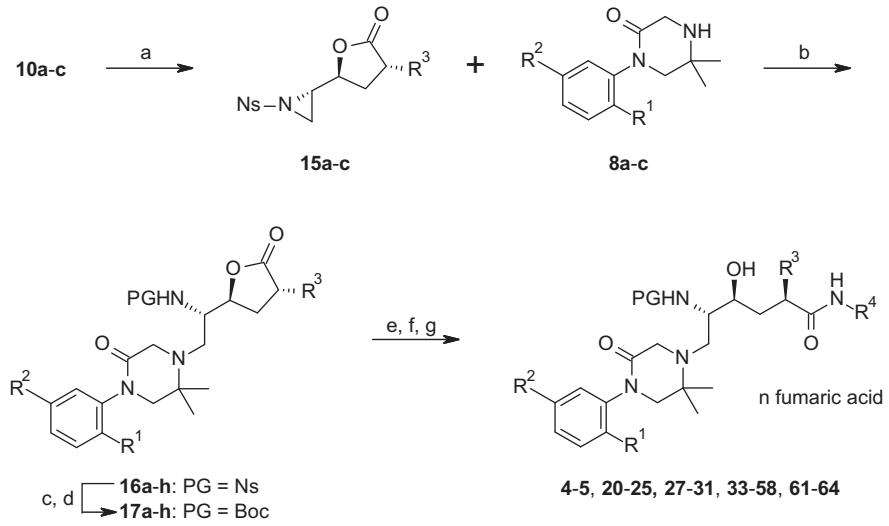


Figure 5. Key components for the preparation of derivatives.

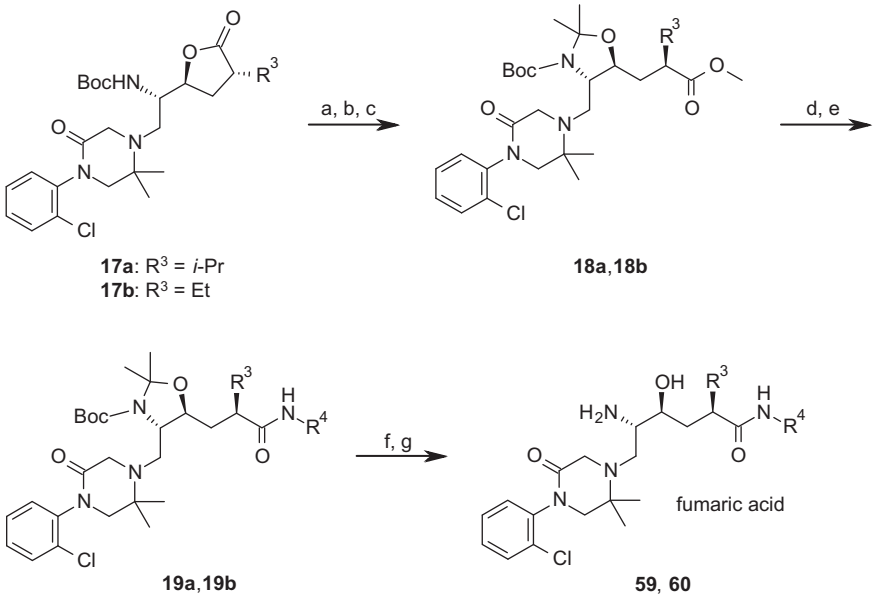


Scheme 1. Reagents and conditions: (a) $NaN(TMS)_2$, (*E*)-$BnOCH_2CH{=}CHCH_2Br$, THF, −78 to −40 °C; (b) LiOH, 30% H_2O_2, THF–H_2O (3:1), 0 °C to rt; (c) 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiulo-2,6-pyranose, oxone, K_2CO_3, aq. $Na_2(EDTA)_2$, $Na_2B_4O_7{\cdot}10H_2O$, dimethoxymethane-MeCN (2:1), 0 °C; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C; (e) NaN_3, DMPU, 60 °C; (f) cat. Pd–C, H_2, HCl, EtOH, rt; (g) (i) NsCl, Et_3N, THF–H_2O (10:1), rt; (ii) crystallization in diisopropylether–AcOEt.



Scheme 2. Reagents and conditions: (a) DEAD, Ph_3P, THF, 0 °C; (b) toluene, 110 °C; (c) PhSH, Cs_2CO_3, DMF, rt; (d) Boc_2O, Et_3N, CH_2Cl_2, rt; (e) amines **9**, cat. 2-hydroxypyridine, 80–120 °C, or Me_2AlCl, aromatic amines **9**, CH_2Cl_2, rt; (f) TFA, CH_2Cl_2, rt; (g) fumaric acid, MeOH, rt.

examined to investigate the impact on renin inhibition and the ECG signal. Table 1 summarizes the result of in vitro IC_{50} data against purified human and monkey plasma renin, and classification of ECG changes in rats. The ratios of IC_{50} values between the tested compounds and **1** in a same monkey plasma batch are also shown since the IC_{50} values varied from batch to batch with in-house in vitro assay. The values of the ratio lower than 1.0 indicated that inhibitory activities of those compounds are superior to that of **1**. Higher renin inhibitory activities than lead compound **4** were detected for most of the simple alkyl derivatives, in which neopentyl analogues (**5**, **23–25**) were the most potent compounds. Of functionalized alkyl analogues, alcohol analogues (**28**, **29**, **33**) showed improved renin inhibitory activities in both purified human and monkey plasma assays compared to **4**. Other functionalized alkyl analogues such as amide (**30**), methoxy carbonyl (**31**), and ether (**34**) exhibited almost the same level of monkey plasma renin inhibitory activities as **4**, but a marked decrease in renin inhibition was observed in carboxylic acid analogue **32**. Meanwhile, in the case of aromatic analogues,



Scheme 3. Reagents and conditions: (a) NaOH, THF–MeOH–H_2O, rt; (b) $TMSCHN_2$, toluene–MeOH, rt; (c) 2,2-dimethoxypropane, PPTS, DMF, 65 °C; (d) NaOH, THF–MeOH–H_2O, 65 °C; (e) amines **9**, HATU, Et_3N, DMF, rt −40 °C; (f) 10% HCl–MeOH, rt; (g) fumaric acid, MeOH, rt.

installation of the 2-pyridyl group (**36**) into P_2' portion improved renin inhibitory activity, but phenyl (**35**), 3- and 4-pyridyl analogues (**37**–**39**) showed similar or decreased renin inhibitory activities in monkey plasma compared to **4**. Next, our attention was turned to the cardiac effects of these compounds in the EP rat model. Simple alkyl analogues (**20–23**, **25**, **26**) were found to show a similar undesired effect on ECG signals as **5** regardless of the P_1' and P_2' structures. Intriguingly, P_2' geminal methyl analogue **26** provided the same effect on ECG as **5** despite the weak renin inhibitory activity, suggesting that the observed effect on the ECG signal did not correlate with the intension of renin inhibition. Thus, we decided to stop further investigation of P_2' simple alkyl analogues because of the unveiled high risk of arrhythmogenicity. By contrast, functionalized alkyl analogues (**27**–**30**, **33**, **34**) showed a tendency to improve the effect on ECG, except methoxycarbonyl analogue **31**. In particular, alcohol analogues (**27**, **29**, **33**) and amide analogue **30**, which possessed the same P_2' residue as **1**, did not cause ECG changes. Similarly, aromatic analogues (**35**, **38**, **39**) exhibited improved effects on ECG compared to P_2' simple alkyl analogues. Based on these results and the comparison of the *c*log*P* value,[22] it is suggested that not only the lipophilicity of the molecules but also the structural feature of the P_2' part plays an important role for determining the effect on ECG in rats.

4.2. Modification of P_2' portion of aromatic analogues

Functionalized alkyl derivatives (**29**, **30**, **33**) realized potent renin inhibition as well as free from ECG change, but poor PK profiles in cynomolgus monkeys emerged as the next issue; for example AUC_{0-24} of **33** (75 ng h/mL) versus AUC_{0-24} of **5** (460 ng h/mL). Meanwhile, regarding aromatic analogues (**35**–**39**), milder ECG changes compared to simple alkyl derivatives in spite of their higher lipophilicity than functionalized alkyl derivatives are worth further exploration (Table 2). Fluorination at the 4-position on the P_2' phenyl group of **35** (**40**, **41**) showed high renin inhibitory activities, whereas substitution of the fluoro group of **41** with the methyl or methoxy group (**42**, **43**) and installation of the second fluoro group at the 2- or 3-position on the P_2' phenyl group of **40** (**44**, **45**) weakened renin inhibitions. Meanwhile, introduction of the fluoro group at the 5-position onto the 2-pyridine ring of **36** exhibited potent inhibitory activities in both purified human and monkey plasma renin assays (**36** vs **46**, **47**), but with further modifications (**48**–**51**), better compounds could not be found compared to **46** and **47**. ECG screening in rats revealed that 2-pyridyl analogues showed improved ECG signals from the severe to moderate state, but none of the compounds listed in Table 3 could accomplish the elimination of ECG change. Thus, we decided to stop further efforts on this series.

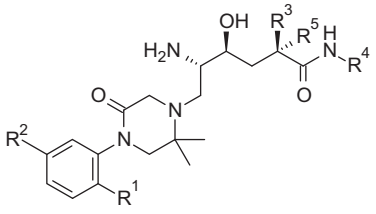
4.3. Cardiac study on isolated Langendorff-perfused rabbit heart

To investigate whether the improvement in in vivo rat screening would be reflected on the reduction of arrhythmogenicity at the ex vivo level, cardiac study on isolated Langendorff-perfused rabbit heart was conducted using compounds **29** and **46**, which showed no and moderate ECG changes with potent renin inhibitory activities, respectively (Table 3). As a result, compound **46** was found to prolong QRS width, PR, and QTc intervals significantly at 10 μM, and caused VT/VF at 30 μM similar to **5**. On the other hand, undesired signals were not observed with **29** compared to **5** and **46**. Taken these results together, we concluded that only compounds free from ECG change might have a chance to reduce arrhythmogenicity at the ex vivo level.

4.4. Optimization of alcohol and amide analogues

Encouraged by the result of the cardiac study on an isolated Langendorff-perfused rabbit heart, we focused our attention on another approach; adjustment of lipophilicity of alcohol and amide analogues (**29**, **30**, **33**) to obtain compounds with good PK profiles (Table 4). Exposure levels of designed compounds were mainly measured by cassette-dosing assay in cynomolgus monkeys. At first, increase of the bulkiness and the lipophilicity of the geminal methyl group of **29** improved human renin inhibitory activities (**52**–**54**). However, these modifications affected the ECG signals and the exposure levels were low. Next, with an aim of improving oral exposure as well as keeping its desirable attributes of cyclohexanol analogue **33**, adamantanol analogues (**55**–**59**) were designed and evaluated. Introduction of the adamantanol groups (**55**–**58**) improved human renin inhibitory activities compared to

Table 1
SAR of modification of P_2' portion[a]



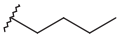
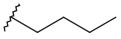
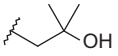
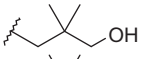
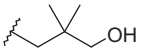
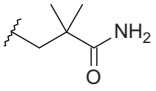
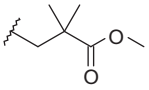
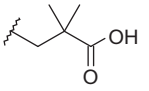
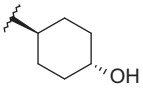
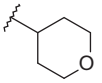
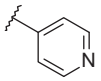
Compound	R^1	R^2	R^3	R^4	R^5	Purified human renin	Monkey plasma renin		ECG change in rats[c]	clog P[d]
						IC_{50} (nM)	IC_{50} (nM)	Ratio[b]		
4	Cl	F	*i*-Pr		H	2.0	25	1.2	NT[e]	5.33
20	Cl	H	*i*-Pr		H	1.0	9.0	0.8	C	5.19
21	Cl	H	*i*-Pr		H	1.4	4.0	0.7	C	5.06
22	Cl	H	*i*-Pr		H	2.9	33	2.7	C	4.44
5	Cl	H	*i*-Pr		H	1.0	3.0	0.5	C	5.46
23	Cl	H	Et		H	0.8	2.3	0.3	C	5.06
24	Cl	H	Me		H	1.0	2.1	0.5	NT	4.53
25	Me	F	Me		H	1.1	3.4	0.6	C	4.46
26	Cl	F	Me		Me	97	738	132	C	4.67
27	Cl	H	*i*-Pr		H	2.4	15	1.9	A	3.98
28	Cl	H	*i*-Pr		H	1.7	9.6	0.8	B	4.34
29	Cl	H	Et		H	1.6	5.2	0.8	A	3.94
30	Cl	H	*i*-Pr		H	1.4	7.0	1.2	A	3.72
31	Cl	H	*i*-Pr		H	1.8	23	1.6	C	4.88
32	Me	F	*i*-Pr		H	16	39	16	NT	2.06
33	Cl	H	*i*-Pr		H	1.4	4.3	0.8	A	3.55
34	Cl	H	*i*-Pr		H	2.0	9.8	1.4	B	3.23
35	Cl	H	*i*-Pr		H	1.4	9.2	1.3	B	5.60
36	Cl	H	*i*-Pr		H	1.8	3.4	0.7	C	4.93
37	Cl	H	*i*-Pr		H	2.1	19	1.9	NT	4.93
38	Me	F	*i*-Pr		H	2.4	33	3.3	B	4.86

Table 1 (*continued*)

Compound	R^1	R^2	R^3	R^4	R^5	Purified human renin	Monkey plasma renin		ECG change in rats[c]	*c*log*P*[d]
						IC_{50} (nM)	IC_{50} (nM)	Ratio[b]		
39	Cl	H	*i*-Pr		H	1.6	15	1.5	B	4.93

[a] Compound **32** was obtained as a succinate salt. Other compounds were obtained as fumarate salts. Assay results are the average of at least two replicates.
[b] Relative activity of inhibition of monkey plasma renin: IC_{50} of compound/IC_{50} of **1**.
[c] ECG change (n = 2, 10 mg/kg, iv bolus injection): A = no change; B = moderate change; C = severe change. See Figure 3.
[d] See Ref. 22.
[e] NT = not tested.

33 except 3-hydroxyadamantane (**59**). In particular, replacement of the P_1' isopropyl group of **55** with ethyl group (**56**) exhibited sub-nanomolar inhibition in purified human renin assay. However, twofold decrease of renin inhibitory activity in monkey plasma was observed when the P_1' ethyl group of **56** was changed to the methyl group (**57**). Regarding the ECG evaluation, only *trans*-adamantan-1-ol analogues (**55**–**57**) did not affect the signal. As *cis*-adamantan-1-ol analogue **58** having almost the same *c*log*P* value as **56** affected the ECG signal, we assumed that the structural feature of *trans*-adamantan-1-ol portion would play an important role to prevent an undesired effect on ECG in rats. Although oral exposure levels of **55**, **58**, and **59** were poor in cynomolgus monkeys, pleasingly, compound **56** exhibited drastically improved exposure levels in both cassette- and single-dosing assays (Tables 4 and 5). Further chemical modification of the terminal hydroxyl group to carboxamide group (**60**) showed potent renin inhibition, but the exposure was poor (Table 5). Having obtained potent compound **56** with good exposure, the PRA suppressive effect of **56** was evaluated in cynomolgus monkeys pre-treated with furosemide. As a result, compound **56** exhibited excellent suppressive effects in a dose-dependent manner at a dose of 1, 3 and 10 mg/kg (Fig. 6).[13] Since *trans*-adamantan-1-ol analogue **56** was obtained, our final approach was to select the best combination of P_3 and P_1' portions for further evaluation (Table 6). All compounds (**61**–**64**) exhibited high in vitro potencies in monkey plasma without any effect on ECG. As a result of evaluation of PRA suppressive effect of the *trans*-adamantan-1-ol analogues (**61**–**64**) in ex vivo assay, compound **56** showed the most potent efficacy within this series (data not shown). Oral administration of single doses of 1, 3 and 10 mg/kg of **56** in cynomolgus monkeys pre-treated with furosemide reduced MAP dose-dependently, and the reduction was sustained over a period of 12 h (Fig. 7).[13] This reduction in blood pressure was almost paralleled by the suppression on ex vivo PRA within that time, indicating that the pharmacological effect was mechanistically related to renin inhibition.

4.5. Cardiac assessments of 56

Finally, we evaluated the cardiac safety of **56**. In agreement with the result of the EP rat model, **56** did not cause significant prolongation of QRS width, PR, or QTc intervals in the ex vivo Langendorff rabbit heart, and in in vivo rabbits, dogs, and monkeys (Table 7). In addition, compound **56** showed no effects on the Site 2 Na channel (10 μM),[23] $hNa_v1.5$ current or hERG current (up to 100 μM). These data support that the potential cardiac risk of **56** is considered to be low. Compound **56** also demonstrated low potential of drug-drug interactions risk based on the combination of our single time- and concentration-dependent CYP3A4 inhibition assay (77.9% remaining at 100 μM)[24] and estimated therapeutic blood concentration (data not shown). Based on its overall in vitro/in vivo profile, compound **56** (DS-8108b) was selected as a clinical candidate.

5. Conclusion

We have described our efforts to address arrhythmogenicity observed with the related compound in the recently disclosed novel series of renin inhibitors. Utilizing the EP rat model as an in vivo first screening, optimizations of the P_3, P_1', and P_2' portions of this series led to the identification of clinical candidate *trans*-adamantan-1-ol analogue **56** (DS-8108b), as a potent renin inhibitor with reduced cardiac potential risk. Oral administration of single doses of 3 and 10 mg/kg of **56** in cynomolgus monkey pre-treated with furosemide elicited sustained reductions mean arterial blood pressure in a dose-dependent manner.

6. Experimental

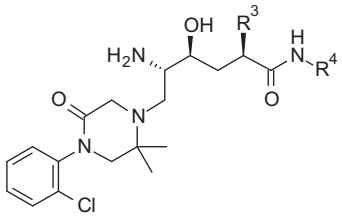
6.1. Synthesis

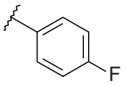
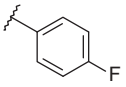
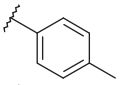
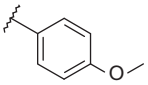
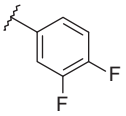
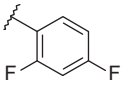
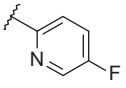
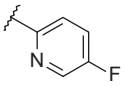
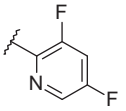
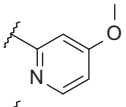
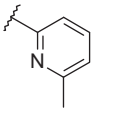
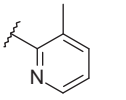
Starting reagents were purchased from commercial suppliers and used without further purification unless otherwise specified. Flash column chromatography was performed on silica gel 60 N (spherical, neutral), 40–50 mesh, purchased from Kanto Chemical Co., Inc., or NH silica gel, 100–200 mesh, purchased 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 Cl 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.

6.1.1. (2*R*,4*E*)-6-(Benzyloxy)-2-methylhex-4-enoic acid (12c)

A solution of sodium bis(trimethylsilyl)amide in *n*-hexane (1.03 M, 164 mL, 169 mmol) was added to a solution of **11c** (32.9 g, 141 mmol) in THF (330 mL) under N_2 atmosphere and at −78 °C over 45 min, and the mixture was stirred at the same temperature for 30 min. Then, a solution of benzyl (2*E*)-4-bromobut-2-en-1-yl ether (35.5 g, 148 mmol) in THF (80 mL) was added to the above solution over 30 min, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was raised to −40 °C and further stirred for 4 h. Saturated NH_4Cl aqueous solution (100 mL) was added to the reaction mixture, and the mixture was further stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and diluted with water (500 mL), followed by extraction with AcOEt. Then, the organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under

Table 2
SAR of modifications of P_1' and P_2' portions of aromatic analogues[a]



Compound	R^3	R^4	Purified human renin	Monkey plasma renin		ECG change in rats[c]	*c*log*P*[d]
			IC_{50} (nM)	IC_{50} (nM)	Ratio[b]		
40	*i*-Pr		1.4	7.2	1.2	B	6.00
41	Et		1.6	3.4	0.9	B	5.60
42	Et		3.2	28	4.5	B	5.70
43	Et		1.4	11	2.1	B	5.28
44	*i*-Pr		1.9	21	4.5	B	6.17
45	*i*-Pr		2.0	14	3.0	B	5.64
46	*i*-Pr		0.9	3.4	0.5	B	5.19
47	Et		1.0	2.1	0.6	B	4.79
48	*i*-Pr		1.7	3.3	0.7	B	4.77
49	Et		6.7	33	13	NT[e]	4.97
50	*i*-Pr		5.6	47	7.3	B	5.43
51	Et		3.8	15	2.9	B	4.38

[a] Compounds were obtained as fumarate salts. Assay results are the average of at least two replicates.
[b] Relative activity of inhibition of monkey plasma renin: IC_{50} of compound/IC_{50} of **1**.
[c] ECG change (*n* = 2, 10 mg/kg, iv bolus injection): B = moderate change. See Figure 3.
[d] See Ref. 22.
[e] NT = not tested.

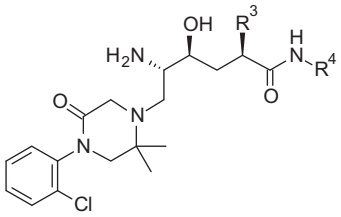
reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 7:1–2:1) to obtain (4*S*)-4-benzyl-3-[(2*R*,4*E*)-6-(benzyloxy)-2-methylhex-4-enoyl]-1,3-oxazolidin-2-one (37.9 g, 69%, 99% ee) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.14 (m, 10H), 5.75 (dt, 1H, *J* = 15.6, 6.3 Hz), 5.69 (dt, 1H, *J* = 15.6, 5.4 Hz), 4.71–4.63 (m, 1H), 4.49 (s, 2H), 4.22–4.11 (m, 2H), 3.98 (d, 2H, *J* = 5.5 Hz), 3.92–3.81 (m, 1H), 3.28 (dd, 1H, *J* = 13.3, 3.1 Hz), 2.67 (dd, 1H, *J* = 13.3, 10.2 Hz), 2.58–2.49 (m, 1H), 2.30–2.21 (m, 1H), 1.19 (d, 3H, *J* = 6.7 Hz). A solution of (4*S*)-4-benzyl-3-[(2*R*,4*E*)-

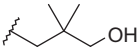
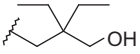
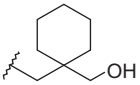
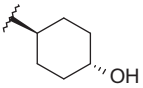
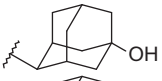
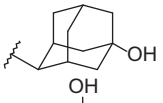
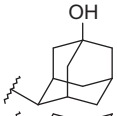
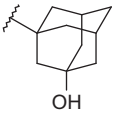
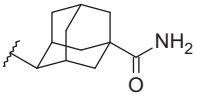
Table 3
Summary data of compounds on the Langendorff rabbit heart

Compound	HR (%)[a]		PR (%)[a]		QRS (%)[a]		QTc (%)[a]	
	10 μM	30 μM	10 μM	30 μM	10 μM	30 μM	10 μM	30 μM
5	−4.6	VT/VF	68.9	VT/VF	15.6	VT/VF	−1.5	VT/VF
46	−3.7	VT/VF	64.1	VT/VF	74.7	VT/VF	13.5	VT/VF
29	−3.5	−4.3	−7.8	3.4	3.8	1.7	−2.1	−5.4

[a] % Change from baseline.

Table 4
SAR of modifications of P_1' and P_2' portions[a]



Compound	R^3	R^4	Purified human renin	Monkey plasma renin		ECG change in rats[c]	AUC_{0-4} in monkeys[d]		clog*P*[f]
			IC_{50} (nM)	IC_{50} (nM)	Ratio[b]		(ng h/mL)	*n*[e]	
29	Et		1.6	5.2	0.8	A	NT[g]	––	3.94
52	Et		1.0	3.0	0.8	C	NT	––	5.00
53	Et		1.1	3.0	0.7	B	5	3	4.70
54	Et		1.1	3.4	0.6	B	4	3	5.26
33	*i*-Pr		1.4	4.3	0.8	A	11	4	3.55
55	*i*-Pr		1.3	2.2	0.9	A	17	3	4.17
56	Et		0.9	6.3	0.8	A	67	3	3.78
57	Me		1.1	7.3	1.6	A	NT	––	3.25
58	Et		1.1	1.8	0.7	B	7	3	3.76
59	*i*-Pr		2.0	6.5	0.7	B	2	3	5.04
60	Et		1.3	1.2	0.5	NT	NT	––	3.43

[a] Compounds were obtained as fumarate salts. Assay results are the average of at least two replicates.
[b] Relative activity of inhibition of monkey plasma renin: IC_{50} of compound/IC_{50} of **1**.
[c] ECG change (n = 2, 10 mg/kg, iv bolus injection): A = no change; B = moderate change; C = severe change. See Figure 3.
[d] Cassette-dosing assay at a 1 mg/kg oral dose in cynomolgus monkeys.
[e] Number of compounds dosed in cassette.
[f] See Ref. 22.
[g] NT = not tested.

Table 5
Mean PK parameters in cynomolgus monkeys after the single administration

Compound	AUC_{0-24}[a] (ng h/mL)	C_{max}[a] (ng/mL)	$T_{1/2}$[a] (h)	V_{ss}[b] (L/kg)	CL[b] (mL/min/kg)	F (%)
5	460	111	15.3	23.5	28.4	26.4
33	75	19	NT[c]	NT	NT	NT
56[d]	685	102	5.1	1.12	3.59	4.1
60	90	19	NT	NT	NT	NT

[a] 3 mg/kg PO.
[b] 1 mg/kg iv.
[c] NT = not tested.
[d] See Ref. 13.

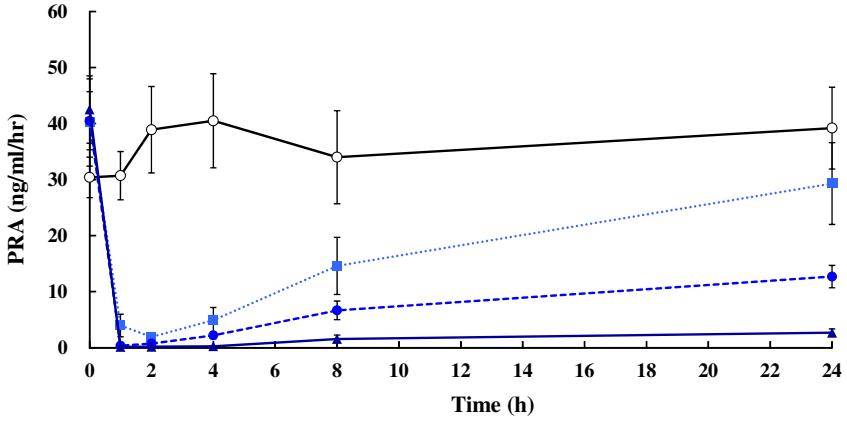
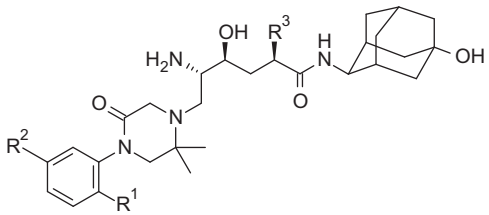


Figure 6. Ex vivo PRA suppressive effects of **56**. Vehicle (1% MC solution, ○), 1 mg/kg (■), 3 mg/kg (●) and 10 mg/kg (▲) of **56** were orally administered to cynomolgus monkeys pre-treated with furosemide. PRA was measured 1, 2, 4, 8, and 24 h after oral administration. See Ref. 13.

Table 6
Optimizations of P_3 and P_1' portions of *trans*-adamantan-1-ol analogues[a]



Compound	R^1	R^2	R^3	Purified human renin	Monkey plasma renin		ECG change in rats[c]	$c\log P$[d]
				IC_{50} (nM)	IC_{50} (nM)	Ratio[b]		
56	Cl	H	Et	0.9	6.3	0.8	A	3.78
61	Cl	F	*i*-Pr	1.9	7.8	0.7	A	4.32
62	Cl	F	Et	1.4	3.6	0.7	A	3.92
63	Me	F	*i*-Pr	1.7	10	0.9	A	4.10
64	Me	F	Et	1.6	4.6	0.9	A	3.70

[a] Compounds were obtained as fumarate salts. Assay results are the average of at least two replicates.
[b] Relative activity of inhibition of monkey plasma renin: IC_{50} of compound/IC_{50} of **1**.
[c] ECG change (n = 2, 10 mg/kg, iv bolus injection): A = no change. See Figure 3.
[d] See Ref. 22.

6-(benzyloxy)-2-methylhex-4-enoyl]-1,3-oxazolidin-2-one (18.7 g, 47.5 mmol) in a mixed solvent of THF (700 mL) and water (230 mL) was cooled in an ice bath, and then 30% hydrogen peroxide aqueous solution (30.0 mL) and lithium hydroxide monohydrate (4.15 g, 95.3 mmol) were added. The mixture was stirred at the same temperature for 30 min, and then raised to room temperature and further stirred for 16 h. After cooling in an ice bath, 1.5 M sodium thiosulfate aqueous solution (250 mL) was added to the reaction mixture. The mixture was further stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, diluted with water (500 mL) and washed with AcOEt. Then, the aqueous layer was made acidic with sodium dihydrogenphosphate (30.0 g), followed by extraction with AcOEt. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure to obtain crude **12c** (11.0 g) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.25 (m, 5H), 5.73–5.62 (m, 2H), 4.49 (s, 2H), 4.03–3.93 (m, 2H), 2.61–2.51 (m, 1H), 2.50–2.40 (m, 1H), 2.28–2.17 (m, 1H), 1.19 (d, 3H, J = 7.0 Hz).

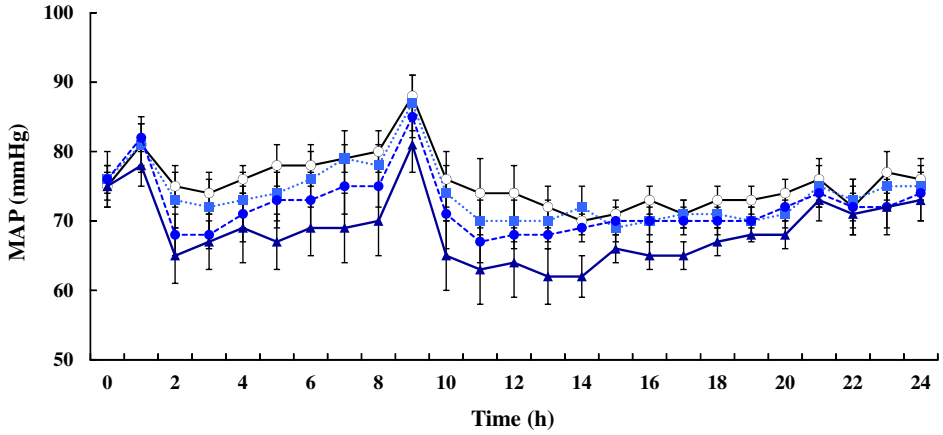


Figure 7. Changes in mean arterial pressure (MAP). 1% MC (○), 1 mg/kg (■), 3 mg/kg (●) and 10 mg/kg (▲) of **56** were orally administered to cynomolgus monkeys pretreated with furosemide. Data are expressed as mean ± SEM. See Ref. 13.

Table 7
Electrophysiological profile of compound **56**

	HR[a] (%)	PR(PQ)[a] (%)	QRS[a] (%)	QTc[a] (%)	Drug concn (μg/mL)
Ex vivo Langendorff rabbit heart (100 μM)	−5.8	1.7	0.1	−3.2	--
Anesthetized rabbit (1 mg/kg/min, iv infusion) at 10 min	3.9	−1.3	−1.3	2.7	NT[b]
Anesthetized dog (0.5 mg/kg/min, iv infusion) at 30 min	−5.4	−1.5	5.8	−1.4	38.1
Conscious monkey (1000 mg/kg, po) at 4 h	−4.2	0.3	−7.6	−0.4	5.4

[a] % Change from baseline.
[b] NT = not tested.

6.1.2. (3*R*,5*S*)-5-[(1*R*)-2-(Benzyloxy)-1-hydroxyethyl]-3-methyldihydrofuran-2(3*H*)-one (13c)

To a 2 L three-neck round-bottom flask, the buffer (0.05 M sodium tetraborate decahydrate in 0.4 mM aqueous ethylenediaminetetraacetic acid disodium salt, 400 mL), dimethoxymethane (333 mL), acetonitrile (167 mL), carboxylic acid **12c** (10.1 g, 43.2 mmol), tetra-*n*-butylammonium hydrogen sulfate (648 mg, 1.91 mmol), and 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiuro-2,6-pyranose (11.1 g, 43.0 mmol) were added. The reaction mixture was cooled with an ice bath. A solution of oxone (36.7 g, 59.6 mmol) in 0.4 mM aqueous ethylenediaminetetraacetic acid disodium salt (200 mL) and a solution of potassium carbonate (34.4 g, 247 mmol) in water (200 mL) were added dropwise through two separate additional funnels over a period of 8 h. The mixture was stirred at the same temperature for 1 h and then the reaction was quenched by the addition of AcOEt and water. The mixture was extracted with AcOEt, washed with brine, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (eluent, *n*-hexane/AcOEt = 6:1–1:1) to afford a diastereomeric mixture of **13c** (7.82 g, 72%, 88% de, two-steps) as a major product and a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): major isomer δ 7.42–7.29 (m, 5H), 4.59 (d, 1H, J = 11.7 Hz), 4.55 (d, 1H, J = 11.7 Hz), 4.48 (ddd, 1H, J = 8.2, 6.3, 3.9 Hz), 3.91–3.84 (m, 1H), 3.64 (dd, 1H, J = 9.8, 3.9 Hz), 3.56 (dd, 1H, J = 9.8, 6.3 Hz), 2.81–2.69 (m, 1H), 2.53 (ddd, 1H, J = 13.2, 9.4, 3.9 Hz), 2.47 (d, 1H, J = 5.1 Hz), 1.93 (dt, 1H, J = 13.2, 8.2 Hz), 1.28 (t, 3H, J = 7.0 Hz).

6.1.3. (3*R*,5*S*)-5-[(1*S*)-1-Azido-2-(benzyloxy)ethyl]-3-methyldihydrofuran-2(3*H*)-one (14c)

Methanesulfonyl chloride (5.36 g, 47.0 mmol) was added to a solution of **13c** (7.80 g, 31.2 mmol) and triethylamine (9.45 g, 93.6 mmol) in CH_2Cl_2 (200 mL) under ice-cooling, and the mixture was stirred at the same temperature for 3 h. 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 $MgSO_4$. After filtration, the solvent was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 4:1–1:1) to obtain a diastereomeric mixture of (1*R*)-2-(benzyloxy)-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl methanesulfonate (9.90 g, 97%, 88% de) as a major product and a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): major isomer δ 7.41–7.28 (m, 5H), 4.87–4.80 (m, 1H), 4.69–4.63 (m, 1H), 4.56 (s, 2H), 3.80–3.71 (m, 2H), 3.05 (s, 3H), 2.82–2.70 (m, 1H), 2.58 (ddd, 1H, J = 13.3, 9.4, 3.9 Hz), 1.99 (dt, 1H, J = 13.3, 8.2 Hz), 1.29 (d, 3H, J = 7.4 Hz). Sodium azide (2.93 g, 45.1 mmol) was added to a solution of (1*R*)-2-(benzyloxy)-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl methanesulfonate (9.90 g, 30.1 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) (100 mL) at room temperature, and the mixture was stirred at 60 °C for 3 days. The reaction mixture was cooled to room temperature and poured into ice water, followed by extraction with Et_2O. Then, the organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 5:1) to obtain a diastereomeric mixture of **14c** (7.56 g, 91%, 88% de) as a major product and a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.29 (m, 5H), 4.62–4.54 (m, 3H), 3.78–3.72 (m, 2H), 3.69–3.64 (m, 1H), 2.91–2.82 (m, 1H), 2.38 (ddd, 1H, J = 13.2, 9.8, 3.9 Hz), 2.00 (dt, 1H, J = 13.2, 8.3 Hz), 1.27 (d, 3H, J = 7.3 Hz).

6.1.4. *N*-{(1*S*)-1-[(2*S*,4*R*)-4-Methyl-5-oxotetrahydrofuran-2-yl]-2-hydroxyethyl}-2-nitrobenzenesulfonamide (10c)

To a solution of **14c** (7.56 g, 27.5 mmol) in EtOH (150 mL), a solution of 4 N HCl in dioxane (15.0 mL, 60.0 mmol) and 10% palladium–carbon (50% wet, 1.88 g) was added. The suspension was stirred under H_2 atmosphere at room temperature for 6 h. H_2 in

the reaction vessel was replaced by N_2, and then the reaction mixture was diluted with EtOH (100 mL). Palladium–carbon was separated by filtration and washed with EtOH. The solvent was evaporated from the filtrate under reduced pressure to obtain crude (3*R*,5*S*)-5-[(1*S*)-1-amino-2-hydroxyethyl]-3-methyldihydrofuran-2(3*H*)-one. Triethylamine (8.66 g, 85.7 mmol) and 2-nitrobenzenesulfonyl chloride (9.67 g, 41.3 mmol) were added to a solution of the crude (3*R*,5*S*)-5-[(1*S*)-1-amino-2-hydroxyethyl]-3-methyldihydrofuran-2(3*H*)-one in a mixed solvent of THF (120 mL) and water (12 mL) at 0 °C, and the mixture was stirred at the same temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and water (200 mL) was added, followed by extraction with AcOEt. Then, the organic layer was washed with water, saturated sodium bicarbonate aqueous solution and brine, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, AcOEt) to obtain **10c** (5.27 g, 56%, >99% ee, two-steps) as a single stereoisomer and a colorless solid after crystallization from diisopropyl ether (10 mL) and AcOEt (20 mL). The ee value of **10c** was determined by chiral HPLC analysis: column, Chiral Pack AD-H (4.6 × 250 mm); eluent, ethanol; flow rate = 0.8 mL/min; t_R of (1*S*,2*S*,4*R*)-isomer = 4.9 min; t_R of (1*R*,2*R*,4*S*)-isomer = 6.0 min. $[\alpha]_D^{23.8}$ +56.0 (*c* 1.00, MeOH). Mp = 131.0–131.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16–8.09 (m, 1H), 7.94–7.87 (m, 1H), 7.79–7.71 (m, 2H), 5.89 (br d, 1H, *J* = 6.8 Hz), 4.72–4.64 (m, 1H), 3.74–3.58 (m, 3H), 2.92–2.81 (m, 1H), 2.63 (ddd, 1H, *J* = 13.2, 9.8, 4.9 Hz), 2.03 (dt, 1H, *J* = 13.7, 8.3 Hz), 2.01–1.96 (m, 1H), 1.28 (d, 3H, *J* = 7.3 Hz). MS (FAB$^+$): *m*/*z* = 345 (M+H)$^+$.

6.1.5. (3*R*,5*S*)-3-Methyl-5-{(2*S*)-1-[(2-nitrophenyl)sulfonyl]aziridin-2-yl}dihydrofuran-2(3*H*)-one (15c)

A solution of diethyl azodicarboxylate in toluene (40%, 1.60 mL, 3.48 mmol) was added to a solution of **10c** (1.00 g, 2.90 mmol) and triphenylphosphine (912 mg, 3.48 mmol) in THF (30 mL) under ice-cooling over 5 min, and the mixture was stirred at the same temperature for 5 min. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, toluene/acetone = 5:1) to obtain **15c** (823 mg, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (dd, 1H, *J* = 7.4, 1.5 Hz), 7.83–7.73 (m, 3H), 4.76 (dt, 1H, *J* = 8.8, 2.0 Hz), 3.26–3.23 (m, 1H), 2.97–2.88 (m, 1H), 2.83 (d, 1H, *J* = 7.0 Hz), 2.65–2.60 (m, 2H), 2.18–2.10 (m, 1H), 1.26 (d, 3H, *J* = 6.8 Hz). MS (FAB$^+$): *m*/*z* = 327 (M+H)$^+$.

Experimental details for the synthesis and characterization of **15a** and **15b** are available in Refs.13,16.

6.1.6. *N*-{(1*S*)-2-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16a)

A solution of *N*-Ns-aziridine **15a** (192 mg, 0.541 mmol) and 1-(2-chlorophenyl)-5,5-dimethylpiperazin-2-one (**8a**) (181 mg, 0.758 mmol) in toluene (7 mL) was stirred at 110 °C for 90 min. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (eluent, CH_2Cl_2/AcOEt = 5:1) to obtain **16a** (299 mg, 93%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.18–8.16 (m, 1H), 7.93 (br s, 1H), 7.83–7.76 (m, 2H), 7.46 (dd, 1H, *J* = 7.8, 2.0 Hz), 7.36–7.27 (m, 2H), 7.22–7.11 (m, 1H), 5.93 (br s, 0.5H), 5.53 (br s, 0.5H), 4.88–4.84 (m, 1H), 3.62 (br s, 1H), 3.30–3.10 (m, 3H), 2.92–2.69 (m, 2.5H), 2.56 (br s, 1H), 2.44 (ddd, 1H, *J* = 13.7, 10.6, 5.9 Hz), 2.27–2.14 (m, 2.5H), 1.17 (br s, 3H), 1.08 (br s, 3H), 1.04 (d, 3H, *J* = 6.6 Hz), 0.98 (d, 3H, *J* = 6.6 Hz).

6.1.7. *N*-{(1*S*)-2-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-ethyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16b)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.17 (br d, 1H, *J* = 7.8 Hz), 7.93 (br s, 1H), 7.79 (br s, 2H), 7.45 (br d, 1H, *J* = 8.2 Hz), 7.33–7.26 (m, 2H), 7.19–7.10 (m, 1H), 5.90 (br s, 0.5H), 5.51 (br s, 0.5H), 4.89 (br s, 1H), 3.63 (br s, 1H), 3.30–3.10 (m, 3H), 2.91-2.45 (m, 4.5H), 2.15 (br s, 1.5H), 1.92–1.82 (m, 1H), 1.64–1.51 (m, 1H), 1.17 (br s, 3H), 1.07 (br s, 3H), 1.04 (br t, 3H, *J* = 7.4 Hz). IR: 1777, 1649, 1539, 1353, 1332, 1168, 1122 cm^{-1}.

6.1.8. *N*-{(1*S*)-2-[4-(2-Chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16c)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.17 (br d, 1H, *J* = 7.0 Hz), 7.93 (br s, 1H), 7.79 (br s, 2H), 7.44 (dd, 1H, *J* = 7.4, 2.0 Hz), 7.35–7.09 (m, 3H), 5.87 (br s, 0.5H), 5.53 (br s, 0.5H), 4.90 (br s, 1H), 3.63 (br s, 1H), 3.29–3.10 (m, 3H), 2.90–2.56 (m, 4.5H), 2.20–2.05 (m, 1.5H), 1.33 (br d, 3H, *J* = 7.0 Hz), 1.16 (br s, 3H), 1.07 (br s, 3H).

6.1.9. *N*-{(1*S*)-2-[4-(2-Chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16d)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 8.18–8.16 (m, 1H), 7.94 (br s, 1H), 7.83–7.77 (m, 2H), 7.42 (dd, 1H, *J* = 8.8, 5.4 Hz), 7.05–6.89 (m, 2H), 5.92 (br s, 0.6H), 5.57 (br s, 0.4H), 4.86–4.83 (m, 1H), 3.62 (br s, 1H), 3.30–3.10 (m, 3H), 2.88–2.40 (m, 4.4H), 2.27–2.15 (m, 2.6H), 1.17 (br s, 3H), 1.08 (br s, 3H), 1.03 (d, 3H, *J* = 6.8 Hz), 0.97 (d, 3H, *J* = 6.6 Hz).

6.1.10. *N*-{(1*S*)-2-[4-(2-Chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-ethyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16e)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 8.18–8.16 (m, 1H), 7.94 (br s, 1H), 7.83–7.78 (m, 2H), 7.42 (dd, 1H, *J* = 8.8, 5.4 Hz), 7.05–7.01 (m, 1H), 6.94–6.87 (m, 1H), 5.87 (br s, 0.6H), 5.54 (br s, 0.4H), 4.89 (br s, 1H), 3.62 (br s, 1H), 3.30–3.10 (m, 3H), 2.92–2.53 (m, 4.5H), 2.14 (br s, 1.5H), 1.91–1.82 (m, 1H), 1.63–1.56 (m, 1H), 1.17 (br s, 3H), 1.08 (br s, 3H), 1.03 (t, 3H, *J* = 7.6 Hz).

6.1.11. *N*-{(1*S*)-2-[4-(5-Fluoro-2-methylphenyl)-2,2-dimethyl-5-oxo-1-piperazinyl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydro-2-furanyl]ethyl}-2-nitrobenzenesulfonamide (16f)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.18–8.16 (m, 1H), 7.98–7.90 (m, 1H), 7.85–7.78 (m, 2H), 7.20 (dd, 1H, *J* = 8.2, 6.7 Hz), 6.96 (dt, 1H, *J* = 8.2, 2.7 Hz), 6.82–6.67 (m, 1H), 5.89 (br d, 0.6H, *J* = 6.7 Hz), 5.60 (br d, 0.4H, *J* = 7.0 Hz), 4.88–4.79 (m, 1H), 3.68–3.58 (m, 1H), 3.37–3.27 (m, 1H), 3.16–2.17 (m, 9H), 2.11 (s, 3H), 1.16–0.93 (m, 12H).

6.1.12. *N*-{(1*S*)-1-[(2*S*,4*R*)-4-Ethyl-5-oxotetrahydrofuran-2-yl]-2-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]ethyl}-2-nitrobenzenesulfonamide (16g)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.19–8.15 (m, 1H), 7.98–7.90 (m, 1H),

7.83–7.78 (m, 2H), 7.22–7.18 (m, 1H), 6.96 (dt, 1H, *J* = 8.2, 2.7 Hz), 6.82–6.66 (m, 1H), 5.85 (br d, 0.6H, *J* = 6.7 Hz), 5.57 (br d, 0.4H, *J* = 8.2 Hz), 4.93–4.83 (m, 1H), 3.69–3.59 (m, 1H), 3.37–2.47 (m, 7.6H), 2.21–2.11 (m, 4.4H), 1.90–1.82 (m, 1H), 1.62–1.54 (m, 1H), 1.17–1.00 (m, 9H).

6.1.13. *N*-{(1*S*)-2-[4-(5-Fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl}-2-nitrobenzenesulfonamide (16h)

In the same manner as the preparation of **16a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 8.18–8.16 (m, 1H), 7.99–7.78 (m, 3H), 7.22–7.18 (m, 1H), 6.98–6.93 (m, 1H), 6.81 (br d, 0.4H, *J* = 7.0 Hz), 6.66 (br d, 0.6H, *J* = 7.0 Hz), 5.83 (br d, 0.6H, *J* = 7.4 Hz), 5.57 (br d, 0.4H, *J* = 7.8 Hz), 4.94 (br s, 0.4H), 4.85 (br s, 0.6H), 3.70–3.26 (m, 2H), 3.12–2.50 (m, 7H), 2.18–2.05 (m, 4H), 1.34–1.32 (m, 3H), 1.17–1.05 (m, 6H).

6.1.14. *tert*-Butyl {(1*S*)-2-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17a)

Cesium carbonate (197 mg, 0.605 mmol) was added to a solution of **16a** (299 mg, 0.504 mmol) and thiophenol (110 μL, 1.00 mmol) in DMF (5 mL) under N_2 atmosphere at room temperature, and the mixture was stirred at the same temperature for 1 h. Brine was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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 = 20:1–10:1) to afford 4-{(2*S*)-2-amino-2-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}-1-(2-chlorophenyl)-5,5-dimethylpiperazin-2-one as a colorless solid. Triethylamine (210 μL, 1.51 mmol) and di-*t*-butyl dicarbonate (132 mg, 0.605 mmol) were added to a solution of 4-{(2*S*)-2-amino-2-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}-1-(2-chlorophenyl)-5,5-dimethylpiperazin-2-one obtained above in CH_2Cl_2 (5 mL), and the mixture was stirred at room temperature for 15 h. Brine was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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/AcOEt = 2:1) to obtain **17a** (205 mg, 80%, two-steps) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.47 (dd, 1H, *J* = 7.8, 2.0 Hz), 7.34-7.20 (m, 3H), 4.86–4.78 (m, 1H), 4.47–4.42 (m, 1H), 3.87–3.81 (m, 1H), 3.58–3.24 (m, 4H), 2.79–2.32 (m, 0.5H), 2.62–2.57 (m, 2H), 2.44–2.39 (m, 0.5H), 2.31–2.24 (m, 1H), 2.21–2.11 (m, 2H), 1.45 (s, 9H), 1.26–1.22 (m, 6H), 1.03 (d, 3H, *J* = 7.0 Hz), 0.97 (d, 3H, *J* = 6.6 Hz).

6.1.15. *tert*-Butyl {(1*S*)-2-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-ethyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17b)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.47 (br d, 1H, *J* = 7.4 Hz), 7.34–7.20 (m, 3H), 4.91–4.83 (m, 1H), 4.46–4.40 (m, 1H), 3.88–3.81 (m, 1H), 3.58–3.24 (m, 4H), 2.80–2.36 (m, 4H), 2.08–2.01 (m, 1H), 1.91–1.81 (m, 1H), 1.62–1.50 (m, 1H), 1.45 (s, 9H), 1.26 (br s, 3H), 1.22 (br s, 3H) 1.03 (br t, 3H, *J* = 7.4 Hz).

6.1.16. *tert*-Butyl {(1*S*)-2-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17c)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.49–7.46 (m, 1H), 7.35–7.21 (m, 3H), 4.90 (br s, 1H), 4.43 (br s, 1H), 3.88–3.82 (m, 1H), 3.58–3.24 (m, 4H), 2.80–2.40 (m, 4H), 2.04–1.96 (m, 1H), 1.45 (s, 9H), 1.31 (br d, 3H, *J* = 7.0 Hz), 1.26 (br s, 3H), 1.23 (br s, 3H).

6.1.17. *tert*-Butyl {(1*S*)-2-[4-(2-chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17d)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.43 (dd, 1H, *J* = 8.8, 5.3 Hz), 7.05–6.99 (m, 2H), 4.82 (br s, 1H), 4.44 (br s, 1H), 3.87–3.81 (m, 1H), 3.59–2.39 (m, 7H), 2.31–2.11 (m, 3H), 1.45 (br s, 9H), 1.25 (br s, 6H), 1.03 (br d, 3H, *J* = 6.7 Hz), 0.97 (br d, 3H, *J* = 7.0 Hz).

6.1.18. *tert*-Butyl {(1*S*)-2-[4-(2-chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-ethyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17e)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 7.45–7.42 (m, 1H), 7.05–6.96 (m, 2H), 4.87 (br s, 1H), 4.56 (br s, 1H), 3.85–3.84 (m, 1H), 3.57–2.36 (m, 8H), 2.08–2.02 (m, 1H), 1.90–1.82 (m, 1H), 1.61–1.52 (m, 1H), 1.46 (br s, 9H), 1.24 (br s, 6H), 1.03 (t, 3H, *J* = 7.3 Hz).

6.1.19. *tert*-Butyl {(1*S*)-2-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*S*)-4-isopropyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17f)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 7.21 (dd, 1H, *J* = 8.3, 6.4 Hz), 6.95 (dt, 1H, *J* = 8.3, 2.4 Hz), 6.86–6.80 (m, 1H), 4.83–4.76 (m, 1H), 4.47–4.44 (m, 1H), 3.87–3.82 (m, 1H), 3.55–3.16 (m, 4H), 2.75–2.41 (m, 3H), 2.30–2.11 (m, 6H), 1.45 (br s, 9H), 1.22 (br s, 6H), 1.03 (br d, 3H, *J* = 6.8 Hz), 0.98–0.96 (m, 3H).

6.1.20. *tert*-Butyl {(1*S*)-1-[(2*S*,4*R*)-4-ethyl-5-oxotetrahydrofuran-2-yl]-2-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]ethyl}carbamate (17g)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.22 (dd, 1H, *J* = 8.2, 6.3 Hz), 6.96 (dt, 1H, *J* = 8.2, 2.7 Hz), 6.87–6.80 (m, 1H), 4.89–4.82 (m, 1H), 4.46–4.42 (m, 1H), 3.88–3.82 (m, 1H), 3.56–3.15 (m, 4H), 2.77–2.36 (m, 4H), 2.19 (br s, 1.2H), 2.18 (br s, 1.8H), 2.08–2.01 (m, 1H), 1.92–1.81 (m, 1H), 1.62–1.51 (m, 1H), 1.45 (br s, 9H), 1.22 (br s, 6H), 1.03 (br t, 3H, *J* = 7.2 Hz).

6.1.21. *tert*-Butyl {(1*S*)-2-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-1-[(2*S*,4*R*)-4-methyl-5-oxotetrahydrofuran-2-yl]ethyl}carbamate (17h)

In the same manner as the preparation of **17a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.22 (dd, 1H, *J* = 8.6, 6.3 Hz), 6.98–6.93 (m, 1H), 6.87–6.79 (m, 1H), 4.92–4.85 (m, 1H), 4.45–4.41 (m, 1H), 3.88–3.82 (m, 1H), 3.56–3.16 (m, 4H), 2.77–2.41 (m, 4H), 2.19 (br s, 1.8H), 2.18 (br s, 1.2H), 2.04–1.96 (m, 1H), 1.45 (br s, 9H), 1.32 (br d, 3H, *J* = 7.4 Hz), 1.23 (br s, 6H).

6.1.22. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(2,2-dimethylpropyl)-4-hydroxy-2-isopropylhexanamide hemifumarate (5)

2-Hydroxypyridine (11.2 mg, 0.118 mmol) was added to a solution of **17a** (300 mg, 0.590 mmol) and neopentylamine (700 μL, 5.94 mmol), and the mixture was stirred at 80 °C for 3 h. After cooling, water was added to the reaction mixture, followed by

extraction with AcOEt. 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 = 40:1–20:1) to obtain *tert*-butyl {(2*S*,3*S*,5*S*)-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-[(2,2-dimethylpropyl)carbamoyl]-3-hydroxy-6-methylheptan-2-yl}carbamate (216 mg, 61%) as a colorless solid. Trifluoroacetic acid (837 μL, 10.9 mmol) was added to a solution of *tert*-butyl {(2*S*,3*S*,5*S*)-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-5-[(2,2-dimethylpropyl)carbamoyl]-3-hydroxy-6-methylheptan-2-yl}carbamate (216 mg, 0.363 mmol) in CH_2Cl_2 (1.7 mL) at room temperature, and the mixture was stirred at the same temperature for 30 min. After concentration under reduced pressure, saturated sodium bicarbonate aqueous solution was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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/Et_3N = 100:10:1) to obtain the free base of **5**. Fumaric acid (21.0 mg, 0.181 mmol) was added to a solution of the free base of **5** in MeOH (5 mL), and the mixture was stirred at room temperature for 5 min. The solvent was evaporated under reduced pressure to obtain **5** (163 mg, 81%, two-steps) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.92 (br s, 1H), 7.55–7.33 (m, 1H), 7.44–7.31 (m, 3H), 6.67 (s, 1H), 3.67–3.59 (m, 2H), 3.52–3.47 (m, 1H), 3.39–3.11 (m, 4H), 2.97–2.90 (m, 1.6H), 2.80–2.66 (m, 0.8H), 2.51 (dd, 0.6H, J = 13.7, 3.9 Hz), 2.43–2.38 (m, 1H), 1.91–1.80 (m, 2H), 1.74–1.68 (m, 1H), 1.31–1.26 (m, 6H), 1.01–0.99 (m, 6H), 0.94 (s, 9H). MS (FAB$^+$): m/z = 495 (M+H)$^+$. HPLC t_R = 4.14 min (>99.9% purity; column, Inertsil ODS-3, 4.6 × 250 mm; eluent, acetonitrile/0.1% ammonium acetate aqueous solution = 70:30; flow rate, 1 mL/min; wave length, 220 nm).

6.1.23. (2*S*,4*S*,5*S*)-5-Amino-*N*-butyl-6-[4-(2-chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropylhexanamide fumarate (4)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.04 (br s, 1H), 7.59–7.56 (m, 1H), 7.22–7.18 (m, 2H), 6.71 (s, 2H), 3.63–3.59 (m, 2H), 3.49–3.44 (m, 1H), 3.36–3.18 (m, 5H), 2.95 (br t, 0.6H, J = 13.3 Hz), 2.80–2.69 (m, 0.8H), 2.52–2.48 (m, 0.6 H), 2.32–2.28 (m, 1H), 1.85–1.78 (m, 2H), 1.74–1.68 (m, 1H), 1.55–1.48 (m, 2H), 1.41–1.33 (m, 2H), 1.31–1.26 (m, 6H), 1.00–0.93 (m, 9H). MS (FAB$^+$): m/z = 499 (M+H)$^+$.

6.1.24. (2*S*,4*S*,5*S*)-5-Amino-*N*-butyl-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropylhexanamide fumarate (20)

In a similar manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.03 (br s, 1H), 7.56–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.68 (s, 2H), 3.68–3.60 (m, 2H), 3.51–3.45 (m, 1H), 3.37–3.18 (m, 5H), 2.95 (br t, 0.6H, J = 11.0 Hz), 2.82–2.67 (m, 0.8H), 2.52 (dd, 0.6H, J = 13.7, 3.9 Hz), 2.33–2.28 (m, 1H), 1.87–1.78 (m, 2H), 1.75–1.68 (m, 1H), 1.55–1.48 (m, 2H), 1.42–1.33 (m, 2H), 1.31–1.26 (m, 6H), 1.00–0.93 (m, 9H). MS (FAB$^+$): m/z = 482 (M+H)$^+$.

6.1.25. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-isobutyl-2-isopropylhexanamide hemifumarate (21)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.56–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.67 (s, 1H), 3.67–3.60 (m, 2H), 3.52–3.46 (m, 1H), 3.37–3.11 (m, 4H), 2.97–2.89 (m, 1.6H), 2.80–2.67 (m, 0.8H), 2.53–2.49 (m, 0.6H), 2.37–2.32 (m, 1H), 1.88–1.68 (m, 4H), 1.31–1.26 (m, 6H), 1.00–0.93 (m, 12H). MS (FAB$^+$): m/z = 481 (M+H)$^+$.

6.1.26. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxo-piperazin-1-yl]-4-hydroxy-*N*,2-diisopropylhexanamide fumarate (22)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.90 (br d, 1H, J = 7.8 Hz), 7.55–7.54 (m, 1H), 7.43–7.32 (m, 3H), 6.68 (s, 2H), 4.06–3.98 (m, 1H), 3.66–3.60 (m, 2.4H), 3.53–3.46 (m, 1.4H), 3.37–3.35 (m, 0.8H), 3.23–3.19 (m, 0.8H), 3.17–3.13 (m, 0.6H), 2.97–2.92 (m, 0.6H), 2.80–2.69 (m, 0.8H), 2.54–2.51 (m, 0.6H), 2.29–2.25 (m, 1H), 1.85–1.78 (m, 2H), 1.74–1.68 (m, 1H), 1.31–1.26 (m, 6H), 1.19–1.15 (m, 6H), 1.00–0.96 (m, 6H). MS (FAB$^+$): m/z = 467 (M+H)$^+$.

6.1.27. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(2,2-dimethylpropyl)-2-ethyl-4-hydroxyhexanamide hemifumarate (23)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.98 (br t, 1H, J = 5.7 Hz), 7.56–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.67 (s, 1H), 3.66–3.50 (m, 3H), 3.37–3.09 (m, 4H), 2.95–2.89 (m, 1.6H), 2.78–2.66 (m, 0.8H), 2.61–2.48 (m, 1.6H), 1.90–1.83 (m, 1H), 1.72–1.48 (m, 3H), 1.30–1.26 (m, 6H), 0.97 (t, 3H, J = 7.4 Hz), 0.93 (s, 9H). MS (FAB$^+$): m/z = 481 (M+H)$^+$.

6.1.28. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(2,2-dimethylpropyl)-4-hydroxy-2-methylhexanamide hemifumarate (24)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.99 (br t, 1H, J = 5.9 Hz), 7.55–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.67 (s, 1H), 3.67–3.52 (m, 3H), 3.38–3.11 (m, 4H), 2.98–2.91 (m, 1.6H), 2.78–2.68 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.3, 3.9 Hz), 1.94–1.87 (m, 1H), 1.59–1.52 (m, 1H), 1.31–1.26 (m, 6H), 1.21 (d, 3H, J = 7.0 Hz), 0.92 (s, 9H). MS (FAB$^+$): m/z = 467 (M+H)$^+$.

6.1.29. (2*R*,4*S*,5*S*)-5-Amino-*N*-(2,2-dimethylpropyl)-6-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-methylhexanamide hemifumarate (25)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.33–7.29 (m, 1H), 7.06–6.93 (m, 2H), 6.66 (s, 1H), 3.68–3.48 (m, 3H), 3.39–3.11 (m, 4H), 2.95–2.89 (m, 1.6H), 2.81–2.73 (m, 1.4H), 2.66–2.62 (m, 0.4H), 2.50–2.46 (m, 0.6H), 2.19 (s, 3H), 1.93–1.87 (m, 1H), 1.58–1.51 (m, 1H), 1.27–1.25 (m, 6H), 1.21 (d, 3H, J = 7.0 Hz), 0.92 (s, 9H). MS (FAB$^+$): m/z = 465 (M+H)$^+$.

6.1.30. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-(2-hydroxy-2-methylpropyl)-2-isopropylhexanamide hemifumarate (27)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.91–7.87 (m, 1H), 7.56–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.70 (s, 1H), 3.67–3.60 (m, 2H), 3.55–3.48 (m, 1H), 3.38–3.13 (m, 5H), 2.98–2.92 (m, 0.6H), 2.80–2.68 (m, 0.8H), 2.54–2.50 (m, 0.6H), 2.42–2.36 (m, 1H), 1.91–1.69 (m, 3H), 1.31–1.27 (m, 6H), 1.21 (s, 6H), 1.00 (d, 3H, J = 4.3 Hz), 0.98 (d, 3H, J = 4.3 Hz). MS (FAB$^+$): m/z = 497 (M+H)$^+$.

6.1.31. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-(3-hydroxy-2,2-dimethylpropyl)-2-isopropylhexanamide fumarate (28)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 8.08–8.05 (m, 1H), 7.56–7.55 (m, 1H), 7.44–7.34 (m, 3H), 6.70 (s, 2H), 3.67–3.61 (m, 2H), 3.52–3.45 (m, 1H), 3.39–3.15 (m, 6H), 3.05–3.02 (m, 1H), 2.98–2.93 (m, 0.6H), 2.80–2.70 (m, 0.8H), 2.55–2.52 (m, 0.6H), 2.43–2.39 (m, 1H), 1.89–1.83 (m, 2H), 1.74–1.69 (m, 1H), 1.32–1.27 (m, 6H), 1.02–1.00 (m, 6H), 0.91–0.90 (m, 6H). MS (FAB$^+$): m/z = 511 (M+H)$^+$.

6.1.32. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(3-hydroxy-2,2-dimethylpropyl)hexanamide hemifumarate (29)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 8.10–8.06 (m, 1H), 7.57–7.52 (m, 1H), 7.44–7.33 (m, 3H), 6.68 (s, 1H), 3.73–3.68 (m, 2H), 3.67–3.60 (m, 2H), 3.53–3.45 (m, 1H), 3.39–3.13 (m, 6H), 3.04–3.01 (m, 1H), 2.97–2.92 (m, 0.6H), 2.80–2.72 (m, 0.8H), 2.55–2.50 (m, 0.6H), 1.89–1.83 (m, 1H), 1.72–1.60 (m, 2H), 1.32–1.26 (m, 6H), 0.99–0.96 (m, 3H), 0.90–0.89 (m, 6H). MS (FAB$^+$): m/z = 497 (M+H)$^+$.

6.1.33. (2*S*,4*S*,5*S*)-5-Amino-*N*-(3-amino-2,2-dimethyl-3-oxopropyl)-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropylhexanamide hemifumarate (30)

2-Hydroxypyridine (38.3 mg, 0.404 mmol) was added to a solution of **17a** (205 mg, 0.404 mmol) and 3-amino-2,2-dimethylpropanamide (140 mg, 1.21 mmol) in triethylamine (4 mL), and the mixture was stirred at 80 °C for 4 h. The reaction mixture was concentrated under reduced pressure, and then stirred at 80 °C for an additional 14 h. After cooling, water was added to the reaction mixture, followed by extraction with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, CH_2Cl_2/MeOH = 40:3) to obtain *tert*-butyl {(2*S*,3*S*,5*S*)-5-[(3-amino-2,2-dimethyl-3-oxopropyl)carbamoyl]-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-3-hydroxy-6-methylheptan-2-yl}carbamate (167 mg, 66%) as a yellow solid. Trifluoroacetic acid (412 μL, 5.35 mmol) was added to a solution of *tert*-butyl {(2*S*,3*S*,5*S*)-5-[(3-amino-2,2-dimethyl-3-oxopropyl)carbamoyl]-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-3-hydroxy-6-methylheptan-2-yl}carbamate (167 mg, 0.268 mmol) in CH_2Cl_2 (0.82 mL) at room temperature, and the mixture was stirred at the same temperature for 50 min. After concentration under reduced pressure, saturated sodium bicarbonate aqueous solution was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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/Et_3N = 100:10:1) to obtain the free base of **30**. Fumaric acid (10.0 mg, 0.0861 mmol) was added to a solution of the free base of **30** in MeOH (2 mL), and the mixture was stirred at room temperature for 5 min. The reaction mixture was concentrated under reduced pressure, and CH_2Cl_2 (0.8 mL) was added to the residue. Et_2O (8 mL) were further added and the solid was collected by filtration to obtain **30** (90.0 mg, 58%, two-steps) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.56–7.53 (m, 1H), 7.44–7.32 (m, 3H), 6.67 (s, 1H), 3.86–3.59 (m, 2H), 3.52–3.12 (m, 6H), 2.95 (br t, 0.6H, J = 12.5 Hz), 2.81–2.67 (m, 0.8H), 2.51 (dd, 0.6H, J = 13.5, 4.1 Hz), 2.38–2.33 (m, 1H), 1.88–1.67 (m, 3H), 1.31–1.27 (m, 6H), 1.22 (s, 3H), 1.20 (s, 3H), 0.99–0.96 (m, 6H). MS (FAB$^+$): m/z = 525 (M+H)$^+$.

6.1.34. Methyl 3-{[(2*S*,4*S*,5*S*)-5-amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropylhexanoyl]amino}-2,2-dimethylpropanoate hemifumarate (31)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.55–7.54 (m, 1H), 7.42–7.32 (m, 3H), 6.67 (s, 1H), 3.68 (s, 3H), 3.63–3.61 (m, 2H), 3.51–3.48 (m, 1H), 3.42–3.12 (m, 5H), 2.96 (br t, 0.6H, J = 12.2 Hz), 2.81–2.69 (m, 0.8H), 2.55–2.51 (m, 0.6H), 2.37–2.32 (m, 1H), 1.87–1.78 (m, 2H), 1.72–1.66 (m, 1H), 1.31–1.27 (m, 6H), 1.20–1.18 (m, 6H), 0.99–0.96 (m, 6H). MS (FAB$^+$): m/z = 539 (M+H)$^+$.

6.1.35. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-(*trans*-4-hydroxycyclohexyl)-2-isopropylhexanamide fumarate (33)

In the same manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.95–7.93 (m, 1H), 7.56–7.54 (m, 1H), 7.45–7.32 (m, 3H), 6.69 (s, 2H), 3.69–3.59 (m, 3H), 3.55–3.44 (m, 2H), 3.37–3.12 (m, 3H), 2.97–2.91 (m, 0.6H), 2.81–2.67 (m, 0.8H), 2.54–2.50 (m, 0.6H), 2.29–2.23 (m, 1H), 1.98–1.68 (m, 7H), 1.37–1.26 (m, 10H), 0.99–0.95 (m, 6H). MS (FAB$^+$): m/z = 522 (M+H)$^+$.

6.1.36. (2*S*,4*S*,5*S*)-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-(tetrahydro-2*H*-pyran-4-yl)hexanamide fumarate (34)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.56–7.54 (m, 1H), 7.44–7.38 (m, 2H), 7.35–7.32 (m, 1H), 6.70 (s, 2H), 3.95–3.92 (m, 3H), 3.67–3.59 (m, 2H), 3.51–3.45 (m, 4H), 3.36–3.14 (m, 2H), 2.97–2.93 (m, 0.6H), 2.81–2.68 (m, 0.8H), 2.52 (dd, 0.6H, J = 13.4, 3.7 Hz), 2.32–2.28 (m, 1H), 1.86–1.81 (m, 4H), 1.75–1.70 (m, 1H), 1.60–1.49 (m, 2H), 1.31–1.27 (m, 6H), 1.00–0.97 (m, 6H). MS (FAB$^+$): m/z = 509 (M+H)$^+$.

6.1.37. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-*N*-[3-ethyl-3-(hydroxymethyl)pentyl]-4-hydroxyhexanamide fumarate (52)

In the same manner as the preparation of **5**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 8.04–7.99 (m, 1H), 7.56–7.53 (m, 1H), 7.45–7.33 (m, 3H), 6.72 (s, 2H), 3.67–3.60 (m, 2H), 3.56–3.45 (m, 1H), 3.39–3.15 (m, 6H), 3.06–3.02 (m, 1H), 2.98–2.92 (m, 0.6H), 2.77–2.70 (m, 0.8H), 2.60–2.50 (m, 1.6H), 1.90–1.83 (m, 1H), 1.70–1.51 (m, 3H), 1.34–1.21 (m, 10H), 0.99–0.95 (m, 3H), 0.87–0.84 (m, 6H). MS (FAB$^+$): m/z = 525 (M+H)$^+$.

6.1.38. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-{2-[1-(hydroxymethyl)cyclopentyl]ethyl}hexanamide fumarate (53)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.54 (d, 1H, J = 7.3 Hz), 7.43–7.37 (m, 2H), 7.35–7.32 (m, 1H), 6.69 (s, 2H), 3.86–3.81 (m, 1H), 3.65–3.58 (m, 2H), 3.52–3.48 (m, 1H), 3.38–3.20 (m, 7H), 3.02–2.99 (m, 0.6H), 2.86–2.82 (m, 0.4H), 2.74–2.70 (m, 0.4H), 2.52 (dd, 0.6H, J = 13.4, 4.2 Hz), 1.97–1.85 (m, 5H), 1.76–1.71 (m, 1H), 1.34–1.24 (m, 12H), 1.05–1.02 (m, 1H), 0.86–0.79 (m, 2H). MS (ESI$^+$): m/z = 523 (M+H)$^+$.

6.1.39. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-{2-[1-(hydroxymethyl)cyclohexyl]ethyl}hexanamide fumarate (54)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a

mixture of rotamers δ 8.06 (t, 1H, *J* = 5.9 Hz), 7.54 (d, 1H, *J* = 7.3 Hz), 7.43–7.33 (m, 3H), 6.69 (s, 2H), 3.67–3.60 (m, 2H), 3.55–3.48 (m, 1H), 3.38–3.11 (m, 3H), 2.97–2.92 (m, 0.6H), 2.79–2.70 (m, 0.6H), 2.59–2.50 (m, 1.8H), 1.89–1.85 (m, 1H), 1.69–1.46 (m, 10H), 1.35–1.27 (m, 12H), 1.05–1.02 (m, 1H), 0.97 (t, 3H, *J* = 7.3 Hz). MS (ESI^+): *m*/*z* = 537 $(M+H)^+$.

6.1.40. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]-2-isopropylhexanamide fumarate (55)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.78 (br s, 1H), 7.54 (br d, 1H, *J* = 7.8 Hz), 7.43–7.32 (m, 3H), 6.69 (s, 2H), 3.97 (br s, 1H), 3.65–3.47 (m, 3H), 3.36–3.26 (m, 2H), 3.21–3.12 (m, 1H), 2.96–2.91 (m, 0.6H), 2.79–2.70 (m, 0.8H), 2.55–2.52 (m, 0.6H), 2.45 (br s, 1H), 2.11 (br s, 2H), 2.06 (br s, 1H), 1.98–1.70 (m, 11H), 1.50–1.48 (m, 2H), 1.31–1.26 (m, 6H), 1.00 (d, 3H, *J* = 6.8 Hz), 0.98 (d, 3H, *J* = 6.4 Hz). MS (FAB^+): *m*/*z* = 575 $(M+H)^+$.

6.1.41. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]hexanamide monofumarate dihydrate (56)

Experimental details for the synthesis and characterization of **56** are available in Ref. 13 $[\alpha]_D^{25.0}$ −31.6 (*c* 1.07, MeOH). ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.84 (d, 1H, *J* = 7.3 Hz), 7.55–7.54 (m, 1H), 7.43–7.32 (m, 3H), 6.68 (s, 2H), 3.97 (br s, 1H), 3.65–3.26 (m, 5H), 3.22–3.12 (m, 1H), 2.96–2.91 (m, 0.6H), 2.79–2.63 (m, 1.8H), 2.53 (dd, 0.6H, *J* = 13.7, 3.9 Hz), 2.12 (br s, 2H), 2.06 (br s, 1H), 1.98–1.82 (m, 5H), 1.77–1.74 (m, 4H), 1.69–1.47 (m, 5H), 1.31–1.26 (m, 6H), 0.95 (t, 3H, *J* = 7.3 Hz). ^{13}C NMR (125 MHz, CD_3OD): a mixture of rotamers δ 177.7, 171.5, 168.9, 168.7, 140.1, 139.9, 136.3, 133.33, 133.27, 131.6, 131.5, 130.9, 130.5, 130.3, 129.6, 129.5, 68.5, 68.3, 67.9, 61.9, 55.7, 54.9, 54.7, 54.45, 54.41, 54.0, 53.2, 50.7, 50.2, 45.9, 45.50, 45.47, 45.4, 45.2, 37.9, 35.2, 35.1, 31.4, 31.2, 31.1, 28.0, 24.8, 22.5, 20.2, 17.9, 12.2. IR: 3431, 2916, 1642, 1627, 1588, 1531, 1493, 1318, 1121, 750 cm^{-1}. HRMS (ESI^+): *m*/*z* calcd for $C_{30}H_{46}N_4O_4Cl$ $[M+H]^+$: 561.3208; found $[M+H]^+$: 561.3217. Anal. $C_{30}H_{45}N_4O_4Cl{\cdot}C_4H_4O_4{\cdot}2H_2O$ (713.26); calcd: C 57.25, H 7.49, N 7.86, Cl 4.97; found: C 57.21, H 7.55, N 7.89, Cl, 4.88. HPLC t_R = 2.88 min (>99.5% purity; column, Inertsil ODS-3, 4.6 × 250 mm; eluent, acetonitrile/0.1% ammonium acetate aqueous solution = 50:50; flow rate, 1 mL/min; wave length, 220 nm).

6.1.42. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]-2-methylhexanamide fumarate (57)

In a similar manner as the preparation of **30**, the title compound was obtained as a yellow solid. ^1H NMR (CD_3OD, 400 MHz): a mixture of rotamers δ 7.82 (br d, 1H, *J* = 7.4 Hz), 7.56–7.54 (m, 1H), 7.44–7.32 (m, 3H), 6.71 (s, 2H), 3.93 (br s, 1H), 3.66–3.13 (m, 6H), 2.98–2.72 (m, 2.4H), 2.53 (dd, 0.6H, *J* = 13.3, 4.3 Hz), 2.10 (br s, 3H), 1.97–1.73 (m, 9H), 1.60–1.48 (m, 3H), 1.31–1.26 (m, 6H), 1.20 (d, 3H, *J* = 7.0 Hz). MS (FAB^+): *m*/*z* = 547 $(M+H)^+$.

6.1.43. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(*cis*-5-hydroxyadamantan-2-yl)hexanamide fumarate (58)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.81 (br d, 1H, *J* = 6.3 Hz), 7.54 (br d, 1H, *J* = 7.8 Hz), 7.43–7.32 (m, 3H), 6.69 (s, 2H), 3.86 (br s, 1H), 3.65–3.52 (m, 3H), 3.37–3.27 (m, 2H), 3.23–3.13 (m, 1H), 2.97–2.92 (m, 0.6H), 2.80–2.59 (m, 1.8H), 2.53 (dd, 0.6H, *J* = 13.2, 3.4 Hz), 2.20 (br s, 1H), 2.16 (br s, 1H), 2.10 (br s, 1H), 1.95–1.84 (m, 3H), 1.74–1.48 (m, 12H), 1.31–1.26 (m, 6H), 0.97 (t, 3H, *J* = 7.6 Hz). MS (ESI^+): *m*/*z* = 561 $(M+H)^+$.

6.1.44. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]-2-isopropylhexanamide fumarate (61)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.78 (br d, 1H, *J* = 7.3 Hz), 7.58–7.55 (m, 1H), 7.21–7.18 (m, 2H), 6.69 (s, 2H), 3.97 (br s, 1H), 3.65–3.46 (m, 3H), 3.38–3.13 (m, 3H), 2.96–2.91 (m, 0.6H), 2.78–2.70 (m, 0.8H), 2.55-2.51 (m, 0.6H), 2.47–2.43 (m, 1H), 2.11 (br s, 2H), 2.06 (br s, 1H), 1.98–1.70 (m, 11H), 1.51–1.48 (m, 2H), 1.31–1.26 (m, 6H), 1.01–0.97 (m, 6H). MS (FAB^+): *m*/*z* = 593 $(M+H)^+$.

6.1.45. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chloro-5-fluorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]hexanamide fumarate (62)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.80 (m, 1H), 7.58–7.55 (m, 1H), 7.21–7.18 (m, 2H), 6.69 (s, 2H), 3.97 (br s, 1H), 3.64–3.12 (m, 6H), 2.95–2.91 (m, 0.6H), 2.75–2.61 (m, 1.8H), 2.53–2.51 (m, 0.6H), 2.11 (br s, 2H), 2.07 (br s, 1H), 1.97–1.82 (m, 5H), 1.77–1.75 (m, 4H), 1.68–1.48 (m, 5H), 1.30–1.26 (m, 6H), 0.96 (t, 3H, *J* = 7.3 Hz). MS (FAB^+): *m*/*z* = 579 $(M+H)^+$.

6.1.46. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]-2-isopropylhexanamide fumarate (63)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.79 (br d, 1H, *J* = 7.8 Hz), 7.33–7.30 (m, 1H), 7.06–6.93 (m, 2H), 6.69 (s, 2H), 3.97 (br s, 1H), 3.68–3.46 (m, 3H), 3.38–3.15 (m, 3H), 2.95–2.90 (m, 0.6H), 2.81–2.76 (m, 0.4H), 2.68–2.65 (m, 0.4H), 2.52–2.44 (m, 1.6H), 2.20 (br s, 3H), 2.12 (br s, 2H), 2.06 (br s, 1H), 1.99–1.71 (m, 11H), 1.51–1.48 (m, 2H), 1.27–1.25 (m, 6H), 1.01–0.97 (m, 6H). MS (FAB^+): *m*/*z* = 573 $(M+H)^+$.

6.1.47. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-[(2*s*,5*s*)-5-hydroxyadamantan-2-yl]hexanamide fumarate (64)

In a similar manner as the preparation of **30**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.80 (m, 1H), 7.33–7.30 (m, 1H), 7.06–7.02 (m, 1H), 6.98–6.93 (m, 1H), 6.69 (s, 2H), 3.97 (br s, 1H), 3.67–3.13 (m, 6H), 2.94–2.90 (m, 0.6H), 2.80–2.75 (m, 0.4H), 2.69–2.62 (m, 1.4H), 2.50 (dd, 0.6H, *J* = 13.4, 4.2 Hz), 2.20 (br s, 3H), 2.11 (br s, 2H), 2.07 (br s, 1H), 1.97–1.82 (m, 5H), 1.78–1.75 (m, 4H), 1.68–1.49 (m, 5H), 1.27–1.25 (m, 6H), 0.96 (t, 3H, *J* = 7.3 Hz). MS (FAB^+): *m*/*z* = 559 $(M+H)^+$.

6.1.48. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-phenylhexanamide hemifumarate (35)

A solution of Me_2AlCl in *n*-hexane (1.0 M, 2.70 mL, 2.70 mmol) was added to a solution of aniline (243 μL, 2.68 mmol) in CH_2Cl_2 (3 mL) under N_2 atmosphere at room temperature, and the mixture was stirred at room temperature for 1 h. A solution of **17a** (271 mg, 0.533 mmol) in CH_2Cl_2 (5 mL) was added to the solution obtained above, and the mixture was stirred at room temperature for

3.5 h. A 10% potassium sodium tartrate aqueous solution was added to the reaction mixture, followed by dilution with AcOEt. Then, the mixture was stirred at room temperature for 30 min. The reaction mixture was extracted with AcOEt, and then the organic layer was washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, CH_2Cl_2/MeOH = 40:1) to obtain *tert*-butyl [(2*S*,3*S*,5*S*)-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-3-hydroxy-6-methyl-5-(phenylcarbamoyl)heptan-2-yl]carbamate as a colorless solid. Trifluoroacetic acid (488 μL, 6.21 mmol) was added to a solution of *tert*-butyl [(2*S*,3*S*,5*S*)-1-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-3-hydroxy-6-methyl-5-(phenylcarbamoyl)heptan-2-yl]carbamate (125 mg, 0.207 mmol) in CH_2Cl_2 (1 mL) at room temperature, and the mixture was stirred at the same temperature for 1 h. After concentration under reduced pressure, saturated sodium bicarbonate aqueous solution was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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 = 20:1 - 8:1) to obtain the free base of **35**. Fumaric acid (12.0 mg, 0.103 mmol) was added to a solution of the free base of **35** in MeOH (5 mL), and the mixture was stirred at room temperature for 5 min. The solvent was evaporated under reduced pressure to obtain **35** (99.0 mg, 84%, two-steps) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 7.80 (d, 2H, J = 8.3 Hz), 7.55–7.53 (m, 1H), 7.43–7.37 (m, 2H), 7.33–7.28 (m, 3H), 7.11 (t, 1H, J = 7.3 Hz), 6.69 (s, 1H), 3.66–3.56 (m, 3H), 3.36–3.17 (m, 3H), 2.97–2.92 (m, 0.6H), 2.79–2.70 (m, 0.8H), 2.60–2.50 (m, 1.6H), 1.98–1.89 (m, 2H), 1.83–1.78 (m, 1H), 1.30–1.25 (m, 6H), 1.07–1.05 (m, 6H). MS (FAB$^+$): m/z = 501 (M+H)$^+$.

6.1.49. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-(pyridin-2-yl)hexanamide fumarate (36)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.32–8.30 (m, 1H), 8.12–8.10 (br d, 1H, J = 8.6 Hz), 7.81–7.76 (m, 1H), 7.55–7.52 (m, 1H), 7.44–7.27 (m, 3H), 7.14–7.11 (m, 1H), 6.69 (s, 2H), 3.66–3.54 (m, 3H), 3.35–3.18 (m, 3H), 2.97–2.91 (m, 0.6H), 2.76–2.60 (m, 1.8H), 2.55–2.50 (m, 0.6H), 2.00–1.90 (m, 2H), 1.84–1.78 (m, 1H), 1.29–1.24 (m, 6H), 1.05 (d, 6H, J = 7.0 Hz). MS (FAB$^+$): m/z = 502 (M+H)$^+$.

6.1.50. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-(pyridin-3-yl)hexanamide fumarate (37)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.81 (s, 1H), 8.28–8.27 (m, 1H), 8.15–8.13 (m, 1H), 7.55–7.53 (m, 1H), 7.43–7.29 (m, 4H), 6.68 (s, 2H), 3.66–3.53 (m, 3H), 3.36–3.18 (m, 3H), 2.98–2.91 (m, 0.6H), 2.81–2.62 (m, 1.8H), 2.55–2.51 (m, 0.6H), 2.02–1.91 (m, 2H), 1.84–1.77 (m, 1H), 1.30–1.25 (m, 6H), 1.05 (d, 6H, J = 6.6 Hz). MS (FAB$^+$): m/z = 502 (M+H)$^+$.

6.1.51. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(5-fluoro-2-methylphenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-(pyridin-3-yl)hexanamide fumarate (38)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 8.81 (s, 1H), 8.27 (d, 1H, J = 2.9 Hz), 8.14 (d, 1H, J = 7.3 Hz), 7.41 (dd, 1H, J = 8.3, 4.4 Hz), 7.31 (t, 1H, J = 7.3 Hz), 7.04 (td, 1H, J = 8.3, 2.4 Hz), 6.98–6.90 (m, 1H), 6.70 (s, 2H), 3.67–3.48 (m, 3H), 3.36–3.15 (m, 3H), 2.96–2.91 (m, 0.6H), 2.81–2.77 (m, 0.4H), 2.68–2.62 (m, 1.4H), 2.50 (dd, 0.6H, J = 13.2, 3.9 Hz), 2.18 (s, 3H), 2.00–1.91 (m, 2H), 1.81 (ddd, 1H, J = 13.7, 10.7, 2.9 Hz), 1.26–1.24 (m, 6H), 1.05 (d, 6H, J = 6.6 Hz). MS (FAB$^+$): m/z = 500 (M+H)$^+$.

6.1.52. ((2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-2-isopropyl-*N*-(pyridin-4-yl)hexanamide fumarate (39)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.39 (dd, 2H, J = 4.6, 1.5 Hz), 7.70 (dd, 2H, J = 5.0, 1.5 Hz), 7.56–7.52 (m, 1H), 7.44–7.36 (m, 2H), 7.34–7.28 (m, 1H), 6.68 (s, 2H), 3.67–3.51 (m, 3H), 3.37–3.18 (m, 3H), 2.97–2.91 (m, 0.6H), 2.81–2.64 (m, 1.8H), 2.54–2.50 (m, 0.6H), 2.02–1.92 (m, 2H), 1.83–1.76 (m, 1H), 1.30–1.25 (m, 6H), 1.04 (d, 3H, J = 2.3 Hz), 1.03 (d, 3H, J = 2.3 Hz). MS (FAB$^+$): m/z = 502 (M+H)$^+$.

6.1.53. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(4-fluorophenyl)-4-hydroxy-2-isopropylhexanamide hemifumarate (40)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.61–7.58 (m, 2H), 7.55–7.53 (m, 1H), 7.42–7.37 (m, 2H), 7.34–7.27 (m, 1H), 7.07–7.03 (m, 2H), 6.67 (s, 1H), 3.65–3.52 (m, 3H), 3.34–3.15 (m, 3H), 2.96–2.91 (m, 0.6H), 2.78–2.68 (m, 0.8H), 2.59–2.49 (m, 1.6H), 1.97–1.88 (m, 2H), 1.82–1.78 (m, 1H), 1.30–1.24 (m, 6H), 1.05–1.03 (m, 6H). MS (FAB$^+$): m/z = 519 (M+H)$^+$.

6.1.54. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-*N*-(4-fluorophenyl)-4-hydroxyhexanamide fumarate (41)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.62–7.53 (m, 2H), 7.55–7.53 (m, 1H), 7.43–7.37 (m, 2H), 7.33–7.28 (m, 1H), 7.08–7.03 (m, 2H), 6.69 (s, 2H), 3.66–3.56 (m, 3H), 3.34–3.16 (m, 3H), 2.97–2.92 (m, 0.6H), 2.79–2.69 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.7, 3.9 Hz), 1.97–1.92 (m, 1H), 1.79–1.58 (m, 3H), 1.30–1.24 (m, 6H), 1.03 (t, 3H, J = 7.3 Hz). MS (FAB$^+$): m/z = 505 (M+H)$^+$.

6.1.55. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(4-methylphenyl)hexanamide fumarate (42)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.56–7.52 (m, 1H), 7.46 (d, 2H, J = 8.2 Hz), 7.41–7.38 (m, 2H), 7.33–7.27 (m, 1H), 7.13 (d, 2H, J = 7.8 Hz), 6.69 (s, 2H), 3.66–3.55 (m, 3H), 3.35–3.12 (m, 3H), 2.94 (dd, 0.6H, J = 13.3, 11.3 Hz), 2.75–2.69 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.5, 4.1 Hz), 2.29 (s, 3H), 1.94 (ddd, 1H, J = 13.9, 11.2, 2.7 Hz), 1.78–1.58 (m, 3H), 1.29–1.24 (m, 6H), 1.01 (t, 3H, J = 7.4 Hz). MS (FAB$^+$): m/z = 501 (M+H)$^+$.

6.1.56. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(4-methoxyphenyl)hexanamide fumarate (43)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.55–7.27 (m, 6H), 6.90–6.86 (m, 2H), 6.69 (s, 2H), 3.76 (m, 3H), 3.65–3.55 (m, 3H), 3.35–3.15 (m, 3H), 2.98–2.92 (m, 0.6H), 2.75–2.68 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.5, 4.1 Hz), 1.93 (ddd, 1H, J = 13.8, 11.2, 2.3 Hz), 1.79–1.55

(m, 3H), 1.29–1.24 (m, 6H), 1.01 (t, 3H, J = 7.4 Hz). MS (ESI^+): m/z = 517 $(M+H)^+$.

6.1.57. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(3,4-difluorophenyl)-4-hydroxy-2-isopropylhexanamide hemifumarate (44)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 7.78–7.74 (m, 1H), 7.55–7.52 (m, 1H), 7.42–7.17 (m, 5H), 6.67 (s, 1H), 3.64–3.50 (m, 3H), 3.34–3.10 (m, 3H), 2.91 (t, 0.6H, J = 12.2 Hz), 2.73–2.68 (m, 0.8H), 2.58–2.48 (m, 1.6H), 1.95–1.88 (m, 2H), 1.82–1.76 (m, 1H), 1.29–1.24 (m, 6H), 1.04–1.02 (m, 6H). MS (FAB^+): m/z = 537 $(M+H)^+$.

6.1.58. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(2,4-difluorophenyl)-4-hydroxy-2-isopropylhexanamide hemifumarate (45)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.76–7.72 (m, 1H), 7.55–7.52 (m, 1H), 7.44–7.28 (m, 3H), 7.07–7.02 (m, 1H), 6.98–6.93 (m, 1H), 6.67 (s, 1H), 3.66–3.57 (m, 3H), 3.37–3.14 (m, 3H), 2.95 (dd, 0.6H, J = 13.2, 11.0 Hz), 2.79–2.62 (m, 1.8H), 2.55–2.50 (m, 0.6H), 1.98–1.88 (m, 2H), 1.84–1.77 (m, 1H), 1.30–1.25 (m, 6H), 1.06 (d, 6H, J = 6.9 Hz). MS (FAB^+): m/z = 537 $(M+H)^+$.

6.1.59. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(5-fluoropyridin-2-yl)-4-hydroxy-2-isopropylhexanamide fumarate (46)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (500 MHz, CD_3OD): a mixture of rotamers δ 8.21 (d, 1H, J = 2.9 Hz), 8.17 (dd, 1H, J = 9.0, 4.2 Hz), 7.61–7.57 (m, 1H), 7.54–7.53 (m, 1H), 7.42–7.37 (m, 2H), 7.33–7.29 (m, 1H), 6.68 (s, 2H), 3.65–3.54 (m, 3H), 3.34–3.19 (m, 3H), 2.96–2.91 (m, 0.6H), 2.76–2.61 (m, 1.8H), 2.52 (dd, 0.6H, J = 13.4, 4.2 Hz), 2.00–1.91 (m, 2H), 1.83–1.78 (m, 1H), 1.29–1.24 (m, 6H), 1.05–1.03 (m, 6H). MS (FAB^+): m/z = 521 $(M+H)^+$.

6.1.60. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-*N*-(5-fluoropyridin-2-yl)-4-hydroxyhexanamide fumarate (47)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.21–8.16 (m, 2H), 7.61–7.53 (m, 2H), 7.43–7.28 (m, 3H), 6.69 (s, 2H), 3.66–3.56 (m, 3H), 3.35–3.22 (m, 3H), 2.95 (t, 0.6H, J = 11.9 Hz), 2.80–2.72 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.3, 3.5 Hz), 1.97 (m, 1H), 1.80–1.59 (m, 3H), 1.29–1.24 (m, 6H), 1.00 (t, 3H, J = 7.4 Hz). MS (FAB^+): m/z = 507 $(M+H)^+$.

6.1.61. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-*N*-(3,5-difluoropyridin-2-yl)-4-hydroxy-2-isopropylhexanamide fumarate (48)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.25 (d, 1H, J = 2.7 Hz), 7.74–7.69 (m, 1H), 7.56–7.53 (m, 1H), 7.44–7.29 (m, 3H), 6.68 (s, 2H), 3.69–3.60 (m, 3H), 3.36–3.19 (m, 3H), 2.98 (dd, 0.6H, J = 13.5, 11.2 Hz), 2.84–2.62 (m, 1.8H), 2.53 (dd, 0.6H, J = 13.9, 4.1 Hz), 2.01–1.89 (m, 2H), 1.85–1.78 (m, 1H), 1.31–1.26 (m, 6H), 1.10–1.06 (m, 6H). MS (ESI^+): m/z = 538 $(M+H)^+$.

6.1.62. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(4-methoxypyridin-2-yl)hexanamide fumarate (49)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.11 (d, 1H, J = 5.9 Hz), 7.75 (br s, 1H), 7.55–7.52 (m, 1H), 7.43–7.28 (m, 3H), 6.73 (dd, 1H, J = 5.9, 2.3 Hz), 6.68 (s, 2H), 3.87 (s, 3H), 3.65–3.55 (m, 3H), 3.36–3.18 (m, 3H), 2.94 (dd, 0.6H, J = 13.5, 11.1 Hz), 2.80–2.71 (m, 1.8H), 2.53 (dd, 0.6H, J = 13.5, 4.1 Hz), 1.96 (ddd, 1H, J = 13.9, 11.1, 2.7 Hz), 1.81–1.59 (m, 3H), 1.29–1.24 (m, 6H), 1.01 (t, 3H, J = 7.4 Hz). MS (ESI^+): m/z = 518 $(M+H)^+$.

6.1.63. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-(6-methylpyridin-2-yl)-2-isopropylhexanamide hemifumarate (50)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.91 (d, 1H, J = 8.2 Hz), 7.66 (t, 1H, J = 7.8 Hz), 7.55–7.52 (m, 1H), 7.43–7.27 (m, 3H), 7.00 (d, 1H, J = 7.6 Hz), 6.70 (s, 1H), 3.65–3.55 (m, 3H), 3.35–3.18 (m, 3H), 2.96–2.90 (m, 0.6H), 2.75–2.73 (m, 0.6H), 2.63–2.58 (m, 1.2H), 2.52 (dd, 0.6H, J = 13.4, 4.1 Hz), 2.45 (s, 3H), 2.01–1.78 (m, 3H), 1.28–1.24 (m, 6H), 1.05–1.03 (m, 6H). MS (FAB^+): m/z = 516 $(M+H)^+$.

6.1.64. (2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxy-*N*-(3-methylpyridin-2-yl)hexanamide fumarate (51)

In the same manner as the preparation of **35**, the title compound was obtained as a colorless solid. ^1H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 8.14 (d, 1H, J = 2.3 Hz), 7.98 (d, 1H, J = 8.6 Hz), 7.62 (dd, 1H, J = 8.4, 2.2 Hz), 7.55–7.52 (m, 1H), 7.44–7.37 (m, 2H), 7.34–7.27 (m, 1H), 6.68 (s, 2H), 3.65–3.55 (m, 3H), 3.35–3.17 (m, 3H), 2.94 (dd, 0.6H, J = 13.3, 11.3 Hz), 2.79–2.72 (m, 1.8H), 2.51 (dd, 0.6H, J = 13.3, 3.9 Hz), 2.30 (s, 3H), 1.95 (ddd, 1H, J = 13.8, 11.2, 2.7 Hz), 1.82–1.57 (m, 3H), 1.29–1.24 (m, 6H), 1.01 (t, 3H, J = 7.4 Hz). MS (ESI^+): m/z = 502 $(M+H)^+$.

6.1.65. *tert*-Butyl (4*S*,5*S*)-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]methyl}-5-[(2*S*)-2-(methoxycarbonyl)-3-methylbutyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (18a)

2 N aqueous NaOH (2.95 mL, 5.83 mmol) was added to a solution of **17a** (1.48 g, 2.91 mmol) in a mixed solvent of MeOH (10 mL) and THF (10 mL) at room temperature and the mixture was stirred at the same temperature for 75 min. The mixture was neutralized with 2 N aqueous HCl (2.95 mL, 5.83 mmol), followed by extraction with AcOEt, and then the organic layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated, and the crude product was dissolved in a mixed solvent of toluene (30 mL) and MeOH (10 mL). $TMSCHN_2$ (2.0 M *n*-hexane solution, 2.20 mL, 4.40 mmol) was added to the mixture at 0 °C, and stirred at the same temperature for 30 min. Then, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 7:3–1:4) to obtain methyl 5-[(*tert*-butoxycarbonyl)amino]-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2,3,5,6-tetradeoxy-2-propan-2-yl-L-*lyxo*-hexonate (1.38 g, 88%, two-steps) as a colorless solid. To a solution of methyl 5-[(*tert*-butoxycarbonyl)amino]-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2,3,5,6-tetradeoxy-2-propan-2-yl-L-*lyxo*-hexonate (995 mg, 1.84 mmol) in DMF (20 mL) were added 2,2-dimethoxypropane (3.40 mL, 27.6 mmol) and PPTS (1.16 g, 4.60 mmol) at room temperature. After stirring at 65 °C for 90 min, water was added, and the water layer was extracted with AcOEt. The combined organics were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/AcOEt = 4:1-45:55) to obtain **18a** (727 mg, 68%) as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.48–7.46 (m, 1H), 7.33–7.25 (m, 3H), 4.02–3.96 (m, 1H), 3.69–1.46 (m, 29H), 1.26–1.20 (m, 6H), 0.97–0.94 (m, 6H).

6.1.66. *tert*-Butyl (4*S*,5*S*)-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]methyl}-5-[(2*R*)-2-(methoxycarbonyl) butyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (18b)

In the same manner as the preparation of **18a**, the title compound was obtained as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): a mixture of rotamers δ 7.47 (d, 1H, J = 7.8 Hz), 7.34–7.26 (m, 3H), 4.05–4.01 (m, 1H), 3.70–1.56 (m, 30H), 1.27–1.21 (m, 6H), 0.94–0.91 (m, 3H).

6.1.67. *tert*-Butyl (4*S*,5*S*)-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]methyl}-5-{(2*S*)-2-[(3-hydroxyadamantan-1-yl)carbamoyl]-3-methylbutyl}-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (19a)

2 N aqueous NaOH (590 μL, 1.18 mmol) was added to a solution of **18a** (172 mg, 0.296 mmol) in a mixed solvent of MeOH (1.5 mL) and THF (1.5 mL) at room temperature and the mixture was stirred at 65 °C for 11 h. After cooling to room temperature, the mixture was neutralized with 2 N aqueous HCl (590 μL, 1.18 mmol), followed by extraction with AcOEt, and then the organic layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent, AcOEt/MeOH = 99:1–9:1) to obtain (2*S*)-2-{[(4*S*,5*S*)-3-(*tert*-butoxycarbonyl)-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-methyl}-2,2-dimethyl-1,3-oxazolidin-5-yl]methyl}-3-methylbutanoic acid (150 mg, 89%) as a colorless solid. To a solution of (2*S*)-2-{[(4*S*,5*S*)-3-(*tert*-butoxycarbonyl)-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]methyl}-2,2-dimethyl-1,3-oxazolidin-5-yl]-methyl}-3-methylbutanoic acid (150 mg, 0.256 mmol) and 1-amino-3-hydroxyadamantane (128 mg, 0.768 mmol) in DMF (3 mL) were added Et_3N (214 μL, 1.54 mmol) and HATU (389 mg, 1.02 mmol) at room temperature, and the mixture was stirred at 40 °C for 2 h. Water was added, and the water layer was extracted with AcOEt. The combined organics were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (eluent, CH_2Cl_2/MeOH = 99:1–91:9) to obtain **19a** (160 mg, 87%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 8.02 (s, 1H), 7.47–7.24 (m, 4H), 5.44 (br s, 1H), 4.04–2.60 (m, 8H), 2.24–1.50 (m, 33H), 1.25–1.21 (m, 6H), 0.95–0.94 (m, 6H).

6.1.68. *tert*-Butyl (4*S*,5*S*)-5-{(2*R*)-2-[(*trans*-5-carbamoyladamantan-2-yl)carbamoyl]butyl}-4-{[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]methyl}-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (19b)

In a similar manner as the preparation of **19a**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): a mixture of rotamers δ 7.46 (d, 1H, J = 7.8 H), 7.36–7.26 (m, 3H), 6.11–6.01 (m, 1H), 5.70–5.61 (m, 1H), 5.28–5.17 (m, 1H), 4.11–4.01 (m, 2H), 3.69–3.18 (m, 6H), 2.68–1.44 (m, 34H), 1.23–1.15 (m, 6H), 0.92 (t, 3H, J = 7.2 Hz).

6.1.69. (2*S*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-4-hydroxy-*N*-(3-hydroxyadamantan-1-yl)-2-isopropylhexanamide fumarate (59)

Ten percent HCl in MeOH (3.0 mL) was added to **19a** (160 mg, 0.224 mmol) at room temperature and the mixture was stirred at the same temperature for 12 h. After concentration under reduced pressure, saturated sodium bicarbonate aqueous solution was added to the reaction mixture, followed by extraction with CH_2Cl_2. Then, the organic layer was 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 = 1:0–95:5) to obtain the free base of **59**. Fumaric acid (19.8 mg, 0.171 mmol) was added to a solution of the free base of **59** in MeOH (2 mL), and the mixture was stirred at room temperature for 5 min. The solvent was evaporated under reduced pressure to obtain **59** (93.0 mg, 60%, two-steps) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.56–7.51 (m, 1H), 7.44–7.32 (m, 3H), 6.69 (s, 2H), 3.66–2.73 (m, 9.6H), 2.52 (dd, 0.4H, J = 13.5, 4.5 Hz), 2.00–1.91 (m, 2H), 2.01–1.97 (m, 6H), 1.79–1.57 (m, 8H), 1.32–1.27 (m, 6H), 1.01–0.96 (m, 6H). MS (FAB$^+$): m/z = 575 (M+H)$^+$.

6.1.70. *trans*-4-({(2*R*,4*S*,5*S*)-5-Amino-6-[4-(2-chlorophenyl)-2,2-dimethyl-5-oxopiperazin-1-yl]-2-ethyl-4-hydroxyhexanoyl}amino) adamantane-1-carboxamide fumarate (60)

In the same manner as the preparation of **59**, the title compound was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): a mixture of rotamers δ 7.89 (d, 1H, J = 7.0 Hz), 7.56–7.54 (m, 1H), 7.44–7.31 (m, 3H), 6.69 (s, 2H), 4.00 (br s, 1H), 3.66–3.46 (m, 3H), 3.37–3.12 (m, 3H), 2.97–2.91 (m, 0.8H), 2.81–2.61 (m, 1.4H), 2.53 (dd, 0.8H, J = 13.3, 4.3 Hz), 2.06–1.85 (m, 12H), 1.70–1.51 (m, 5H), 1.31–1.26 (m, 6H), 0.98–0.94 (m, 3H). MS (FAB$^+$): m/z = 589 (M+H)$^+$.

6.2. Biological assays

6.2.1. 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 μL of the assay buffer (50 mM Tris–HCl (pH7.9), 100 mM NaCl) containing 5 μL of trypsin-activated recombinant human renin (final enzyme concentration of 50 μM), and the solution was pre-incubated at room temperature for 10 min. Next, 2 μM of the substrate (Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys (DABCYL)-Arg) in 100 μL of the assay buffer was added, and the resulting mixture was incubated at 37 °C for 90 min. After completion of incubation, the concentration of generated angiotensin I was measured by fluorescence at 492 nm (excitation at 340 nm) using a multilabel reader (Perkin–Elmer Inc.). The slope of the linear portion of the plot of fluorescence increase as a function of time was then determined, and the rate was used to calculate% inhibition in relation to uninhibited control. The % inhibition values were plotted as a function of inhibitor concentration, and the IC_{50} value was determined by probit analysis. The IC_{50} value is defined as the concentration of a particular inhibitor that reduces the formation of product by 50% relative to a control sample containing no inhibitor.

6.2.2. 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 cynomolgus monkey plasma stabilized with EDTA, 20 μL of pH adjusting solution, and 10 μL of Inhibitor A solution. The reaction mixture was incubated at 37 °C for 1 h. After incubation, angiotensin I in the reaction mixture was measured by competitive radioimmunoassay using commercial available RIA kit RENIN RIABEAD (Yamasa Co.). An uninhibited tube containing 1% DMSO and a control tube incubated at 4 °C were used to derive the % inhibition for each concentration of inhibitors. The % inhibition values were plotted as a 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.

6.3. Animal studies

6.3.1. Ex vivo PRA study in cynomolgus monkeys pre-treated with furosemide

A single oral dose administration of compound **56** was performed to furosemide-pretreated cynomolgus monkeys to determine the ex vivo plasma renin activity. Animals were dosed orally with compound **56** at doses of 1, 3, and 10 mg/kg, or vehicle (1% methylcellulose). Venous blood was collected into a Venoject tube (EDTA-2Na) and cooled immediately on ice. Subsequently, all the blood was centrifuged with a cooled centrifuge to obtain plasma. The plasma samples were stored in a deep freezer until the measurements. Plasma renin activity (PRA) was measured by a radioimmunoassay kit (Renin-Riabead, TFB, Japan). The plasma concentrations of the free form of **56** after administration of **56** at 1, 3, and 10 mg/kg were increased in a dose-dependent manner.

6.3.2. 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 h after dosing or later. The animals were allowed free access to water whole time. Furosemide at 5 mg/kg/day was intramuscularly administered for 3 days before drug administration. Cynomolgus monkeys received **56** at doses of 1, 3 and 10 mg/kg, or vehicle (1% methylcellulose), orally in a crossover design. 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 mean arterial blood pressure (MAP) was calculated.

6.3.3. Pharmacokinetics

The pharmacokinetics of compounds was determined in cynomolgus monkeys. Compounds 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 the HPLC-MS/MS method. PK parameters were calculated from non-compartmental analysis of the plasma concentration-time curves.

6.4. Cardiac studies

6.4.1. hERG currents assay

Potassium currents in CHO-K1 cells stably expressing the hERG potassium channel were measured using the patch clamp technique on an IonWorks Quattro system (Molecular Devices, USA). hERG channels were activated from a holding potential of −80 mV by a step to +50 mV for 2 s, followed by a step to −40 mV for 2 s, and then tail peak currents were measured. Test compounds were incubated with the cells for 5 min.

6.4.2. hCa$_v$1.2 currents assay

Calcium currents in HEK293 cells stably expressing the hCa$_v$1.2 calcium channel were measured using the patch clamp technique on a PatchXpress 7000A system (Molecular Devices). hCa$_v$1.2 channels were activated by 125 m pulses to +25 mV from a holding potential of −80 mV. Peak calcium currents were recorded upon repolarization to −40 mV. This voltage-clamp pulse protocol was performed continuously during the experiment. An interpulse interval of 20 s allowed the recovery of hCa$_v$1.2 at −80 mV. Test compounds were incubated with the cells between 2 and 5 min until the current reached a steady state level.

6.4.3. hNa$_v$1.5 currents assay

Sodium currents in HEK293 cells stably expressing the hNa$_v$1.5 sodium channel were measured using the patch clamp technique on PatchXpress 7000A system. hNa$_v$1.5 channels were activated by 20 ms pulses to −10 mV from a holding potential of −120 mV, and peak sodium currents were measured. This voltage-clamp pulse protocol was performed continuously during the experiment at a frequency of 1 Hz. Test compounds were incubated with the cells for 2 min.

6.4.4. Sodium channel (Site 2) binding assay

Wister rat brain membranes in modified Tris–HCl buffer pH 7.4 were incubated with 5 nM [^{3}H]batrachotoxin for 1 h at 37 °C. Non-specific binding was estimated in the presence of 100 μM veratridine. Binding affinity of test compounds (10 μM) was expressed as a percent inhibition referred to the vehicle control.

6.4.5. Guinea pig right ventricular papillary muscle action potential assay

Action potentials were measured using the glass microelectrode method. Male Hartley guinea pigs were sacrificed by cervical dislocation and exsanguination. The heart was immediately excised. A strand of free-running papillary muscle from the right ventricle was dissected and immediately mounted into a perfusion chamber. The perfusing Tyrode solution was oxygenated with a gas mixture of 5% CO_2 and 95% O_2, and kept at 36.5 °C with a temperature-controlled circulator. Transmembrane action potentials were recorded using a 3 M KCl glass microelectrode with a tip resistance of 5–30 megohms, which was coupled to an Ag-AgCl bath electrode and connected to a microelectrode amplifier (MEZ-8300 or MEZ-8301, Nihon Kohden, Japan). The tissue preparation was electrically driven at 1 Hz through bipolar electrodes using a stimulator (SEN-3201 or SEN-3301, Nihon Kohden). The stimulation pulse was square in shape, 1 ms duration, with an intensity of about 1.3 times the diastolic threshold. The action potentials were displayed on an oscilloscope (CS-4025 or DSC-7040, Kenwood, Japan), and recorded and analyzed using a computer system (WinCA-PA1.6.6.Aui, Physio-Tech, Japan). Resting membrane potential, action potential amplitude, maximal upstroke velocity, and action potential duration at 30%, 60% and 90% repolarization levels were quantified. After confirming that these parameters were stabilized, test compounds were incubated with the tissue preparation for 30 min.

6.4.6. Isolated Langendorff-perfused rabbit heart model

Female NZW rabbits were anesthetized with thiopental (30 mg/kg, iv). The heart was immediately excised and transferred to chilled Krebs Henseleit solution. The isolated hearts were attached to a Langendorff apparatus via an aortic cannula, perfused with Krebs Henseleit solution at 37 °C, gassed with 95% O_2 and 5% CO_2, and pressurised to approximately 70 mmHg. Electrocardiogram (ECG) and monophasic action potential (MAP) electrodes were placed on the surface of the heart. The ECG and MAP were continuously monitored using a polygraph system (RMP-6008M, Nihon Kohden), and recorded on a thermal array recorder (RTA-1300M, Nihon Kohden). Heart rate (HR), PR interval, QRS width, QT interval, QTc (corrected with Bazett's formula), and monophasic action potential duration at 90% repolarization levels were quantified. After confirming that these parameters were stabilized, test compounds (10, 30, and 100 μM) were perfused through the heart for 20 min.

6.4.7. Electrophysiological rat model (anesthetized condition)

Male Sprague-Dawley rats were anesthetized with inactin (100 mg/kg, ip). The surface lead II ECG was continuously recorded on a recticorder (RJG-4124, Nihon Kohden). A 30-gauge needle connected with PE-10 polyethylene tube was inserted into the right jugular vein to infuse the test compounds. After the stabilization, test compounds (3 and 10 mg/kg) were injected for the assessment of QRS complex abnormality.

6.4.8. Electrophysiological rabbit model (anesthetized condition)

Female NZW rabbits were anesthetized with ketamine (35 mg/kg, ip) and xylazine (5 mg/kg, ip). A tracheotomy was performed to control the respiration using a volume-limited ventilator (SN-480-6, Shinano, Japan) with room air. Tidal volume and respiratory rate were set at 5–10 mL/kg and 35 strokes/min, respectively. The surface lead AB ECG was obtained from the electrodes. Catheters were inserted into the right femoral artery and vein to monitor the systemic blood pressure and to infuse the test compounds, respectively. The ECG and systemic blood pressure were continuously monitored using a polygraph system (RMP-6008M, Nihon Kohden), and recorded on a thermal array recorder (RTA-1300M, Nihon Kohden). HR, PR interval, QRS width, QT interval, QTc, and systemic blood pressure were quantified. After confirming that these parameters were stabilized, test compounds (1 mg/kg/min) were continuously infused for 1 h.

6.4.9. Electrophysiological dog model (anesthetized condition)

Female beagle dogs were initially anesthetized with thiopental (30 mg/kg, iv). After incubation with a cuffed endotracheal tube, 1% halothane vaporized with 100% O_2 was inhaled with a volume-limited ventilator (SN-480-3, Shinano). The tidal volume and respiratory rate were set at 20 mL/kg and 15 strokes/min, respectively. The surface lead II ECG was obtained from the limb electrodes. The systemic blood pressure was measured at the right femoral artery. A pig tail catheter was positioned at the left ventricle through the right femoral artery to measure the left ventricular pressure. The ECG, systemic blood pressure, and left ventricular pressure were continuously monitored using a polygraph system (RMP-6008M, Nihon Kohden), and recorded on a thermal array recorder (RTA-1300M, Nihon Kohden). A 7.5 French Swan-Ganz catheter (Baxter Healthcare, USA) connected with a CEDV monitor (Model: VGSVSYS, Baxter Healthcare) was inserted into the femoral vein, and positioned in the pulmonary artery for measuring cardiac output and mixed venous blood oxygen saturation. HR, PR interval, QRS width, QT interval, QTc, systemic blood pressure, left ventricular pressure, and cardiac output were quantified. After confirming that these parameters were stabilized, test compounds (0.5 mg/kg/min) were continuously infused for 1 h. A volume of 2 mL of blood was drawn from the left femoral artery to measure the plasma drug concentration.

6.4.10. Monkey telemetry model (conscious condition)

Male cynomolgus monkeys were anesthetized with cefazolin (15 mg/kg, im), ketamine (im), and xylazine (im). A telemetry transmitter (TL11M2-D70-PCT, Data Sciences International) was implanted in the abdominal cavity for measuring ECG and systemic blood pressure. ECG electrodes were implanted subcutaneously on the right side of the chest and the left side of the abdomen. A catheter containing a built-in pressure sensor connected to the transmitter was inserted into the left femoral artery. The animals were allowed to recover for at least 3 weeks before any experiment. The animals were fasted from the morning of the dosing day. Feeding on the dosing day was conducted 7 h after dosing. The animals were allowed free access to water whole time. HR, PR interval, QRS width, QT interval, QTc and systemic blood pressure were monitored by the telemetry method, and analyzed before administration and at 1, 2, 4, 7, and 24 h after administration. Data were analyzed using data acquisition and a real-time analysis system (Dataquest™ OpenART™, Data Sciences International). Test compounds (30, 100, and 1000 mg/kg) were orally administered with a dose escalation design at 6 day intervals.

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Supplementary data

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