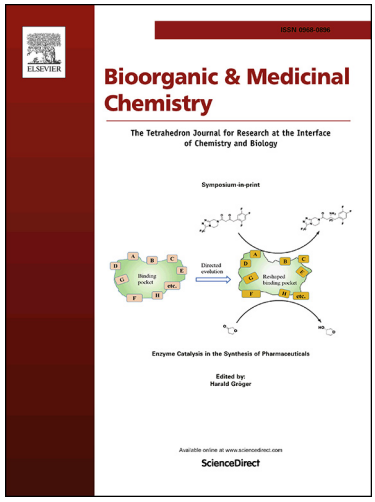
Accepted Manuscript



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Discovery of Benzimidazole Derivatives as Orally Active Renin Inhibitors: Optimization of 3,5-Disubstituted Piperidine to Improve Pharmacokinetic Profile

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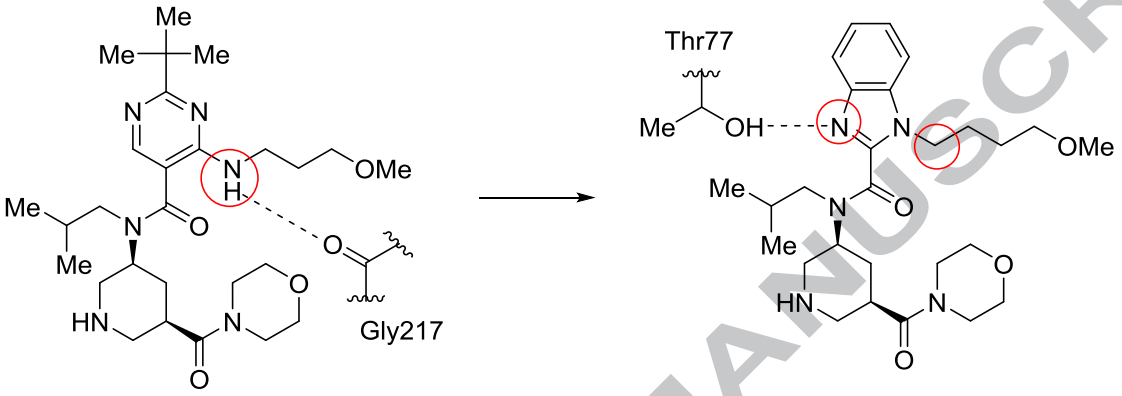
ABSTRACT We previously identified 2-*tert*-butyl-4-[(3-methoxypropyl)amino]-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]pyrimidine-5-carboxamide **3** as a potent renin inhibitor. Since **3** showed unacceptably low bioavailability (BA) in rats, structural modification, using SBDD and focused on physicochemical properties was conducted to improve its PK profile while maintaining renin inhibitory activity. Conversion of the amino group attached at the 4-position of pyrimidine to methylene group improved PK profile and decreased renin inhibitory activity. New central cores with carbon side chains were explored to improve potency. We had designed a series of 5-membered azoles and fused heterocycles that interacted with the

lipophilic S3 pocket. In the course of modification, renin inhibitory activity was enhanced by the formation of an additional hydrogen bonding with the hydroxyl group of Thr77. Consequently, a series of novel benzimidazole derivatives were discovered as potent and orally bioavailable renin inhibitors. Among those, compound **13** exhibited more than five-fold of plasma renin inhibition than aliskiren in cynomolgus monkeys at dose ratio.



3: hPRA: IC_{50} = 0.79 nM
TPSA: 109 $Å^2$
LogD: 2.99
PAMPA: 390 nm/sec
BA: <1%

13: hPRA: IC_{50} = 1.4 nM
TPSA: 88.9 $Å^2$
LogD: 2.11
PAMPA: 345 nm/sec
BA: 14%

KEYWORDS: renin inhibitor, crystal structure, bioavailability, piperidine, topological polar surface area

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INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) is a well-studied pathway, which regulates mainly blood pressure (BP) and renal function.[1] The control of BP is essential for the prevention of cardiovascular outcomes such as heart failure, stroke, atherosclerosis, and kidney disease.[2] Aspartic protease renin exclusively cleaves the protein angiotensinogen to release the physiologically inert angiotensin I (Ang I).[3] Angiotensin-converting enzyme (ACE) then converts Ang I to angiotensin II (Ang II), which is a potent vasoconstrictor agent that acts through binding to the Ang II type I receptor (AT1) and a stimulant of mineralocorticoid (aldosterone) secretion from the adrenal cortex resulting in sodium and water retention.[4] The effectiveness of RAAS cascade blockade is proven by extensive clinical studies using ACE inhibitors and AT1 receptor blockers.[5] On the other

hand, these agents stimulate elevation in plasma renin concentration and plasma renin activity due to a feedback mechanism.[6] The inhibition of renin, which controls the first and rate-limiting step of the RAAS, should result in complete blockade of the angiotensin signal even in situations of elevated circulating or tissue active renin.[7] Thus, renin has long been recognized as a desirable target for antihypertensive drugs.

The development of clinically effective renin inhibitors was a major challenge for medicinal chemistry because of the difficulties in identifying suitable agents with the required profiles such as high affinity for renin's active site and sufficient bioavailability (BA) to permit chronic oral administration.[8] Effort to identify clinically efficacious direct renin inhibitors (DRIs) were made by many pharmaceutical companies. First generation DRIs were peptide mimetics like **1** (zankiren),[9] which suffered from too low a BA to justify clinical testing. Second generation DRI **2** (aliskiren) was approved in the US in 2007,[10] despite modest oral BA in humans (BA = 2.6% in humans). This was an important milestone in the history of RAAS blockade development and the first new class of antihypertensive drugs in more than a decade.[11] Based on the positive results reported for **2**,[12] the interest in DRIs with a desirable PK profile for the control of hypertension has intensified and encouraged us to identify a "best-in-class" third-generation DRI.

Compound **2** characterized by its four exposed hydrogen bond donors (HBD) and high number of rotatable bonds**,** suffered from poor intrinsic cell permeability, which could be caused in part by high topological polar surface area (TPSA) value (146 $Å^2$).[13,14] In the preceding paper,[15] the optimization of a highly attractive small molecule lead series has been described, yielding compound **3** (Figure 1). Despite compound **3** has only two HBD

and good membrane permeability, PK study revealed that **3** exhibited a low BA value in animals (BA = <1% in rats). We hypothesized that by adjusting physicochemical properties, such as TPSA, with keeping rein inhibitory activity by SBDD approach, we would be able to refine PK profile and identify a "best-in-class" renin inhibitor. Previous study revealed that the amino group of the piperidine ring on compound **3** forms a tight hydrogen bonding with the catalytic site (Asp32 and Asp215) of the enzyme (Figure 2). In addition, the isobutyl group occupies the S1 site. This hydrophobic interaction is essential for strong potency. In contrast, the S1', S3, and $S3^{sp}$ sites (which are occupied by a morpholine group, *tert*-butylpyrimidine group, and methoxybutyl group, respectively) are targetable for modification.

SAR investigation of compound **3** showed that the removal of the hydrogen bonding donor, replacement of the amino group on the pyrimidine in the 4-position improved PK profile.[14] In this report, we describe the investigation of the S3site binder with considering physicochemical properties, which lead to the discovery of a series of novel benzimidazole derivatives.

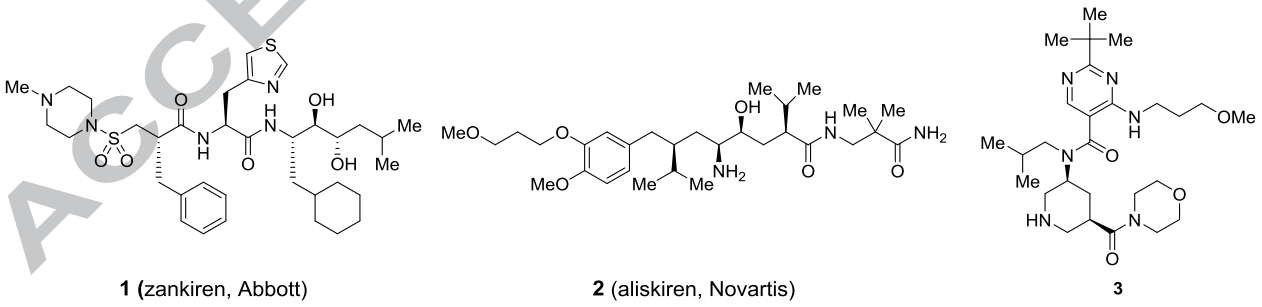


Figure 1. Structure of previously reported renin inhibitors and **3**.

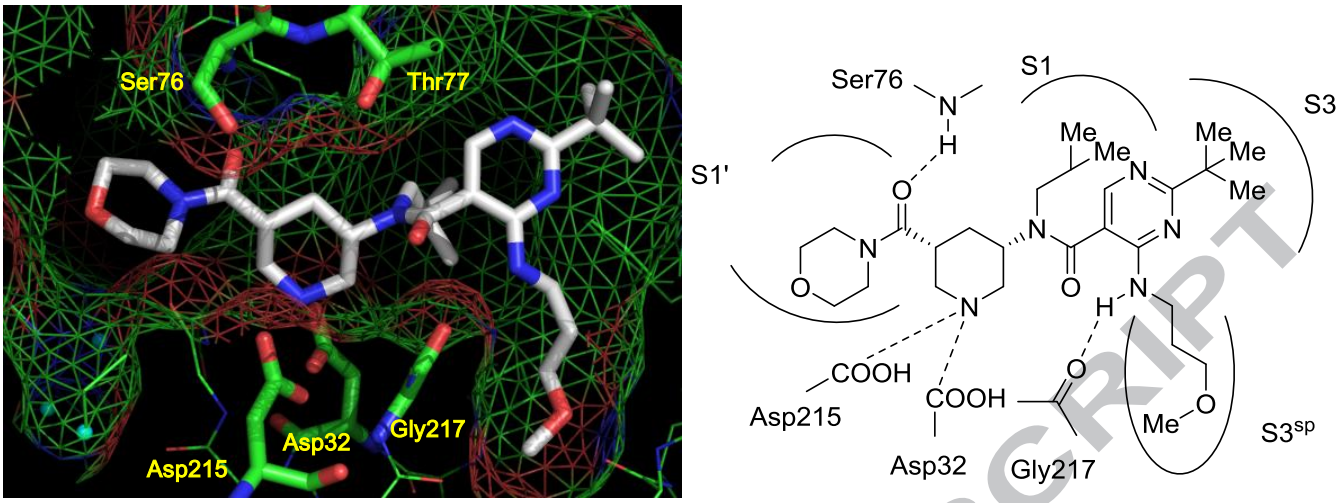
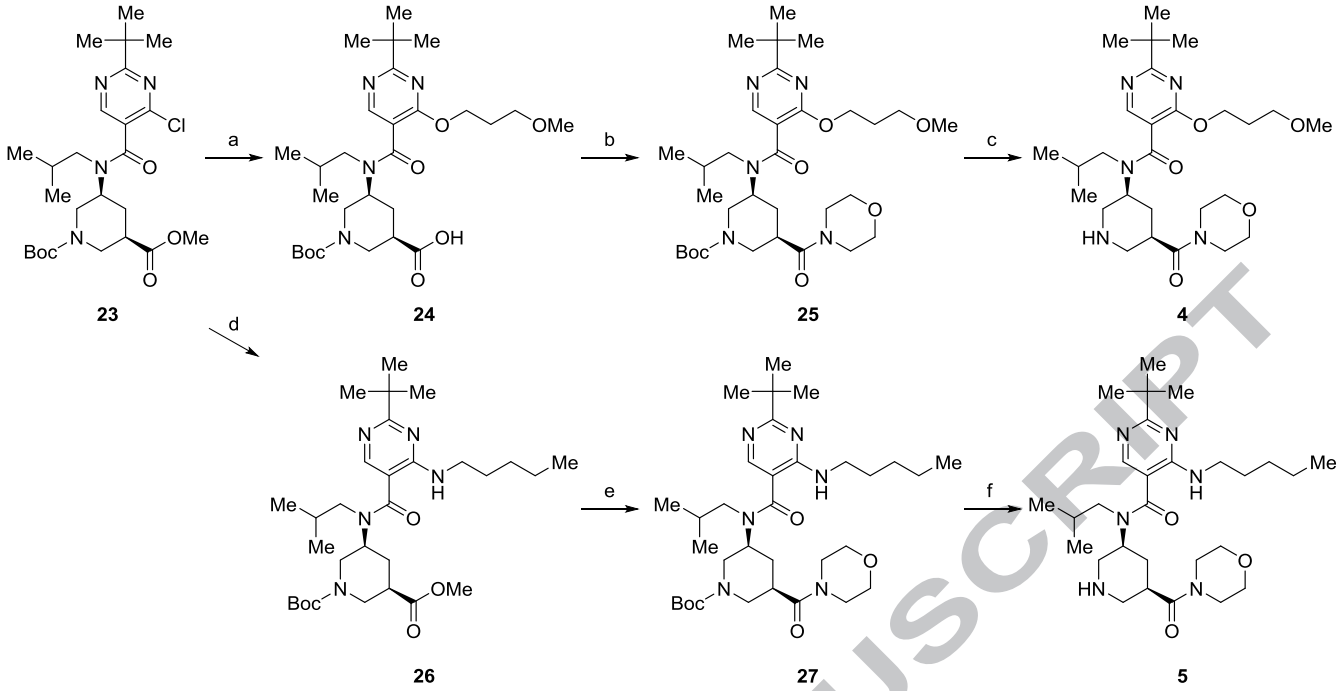


Figure 2. The X-ray crystal structure (left) and binding mode (right) of **3** with renin.

CHEMISTRY

The synthesis of pyrimidine derivatives is outlined in Scheme 1. Methoxypropanol was deprotonated by sodium hydride and reacted with the common intermediate chloropyrimidine **23**[13]. Upon quenching with water, carboxylic acid **24** was obtained. It was then converted to **4** in two steps. The pentylamine side chain was introduced to afford **26**. The obtained ester **26** was then hydrolyzed, reacted with morpholine, and deprotected to give **5** in three steps with a good yield.

Scheme 1.[a] Synthesis of **4** and **5**.

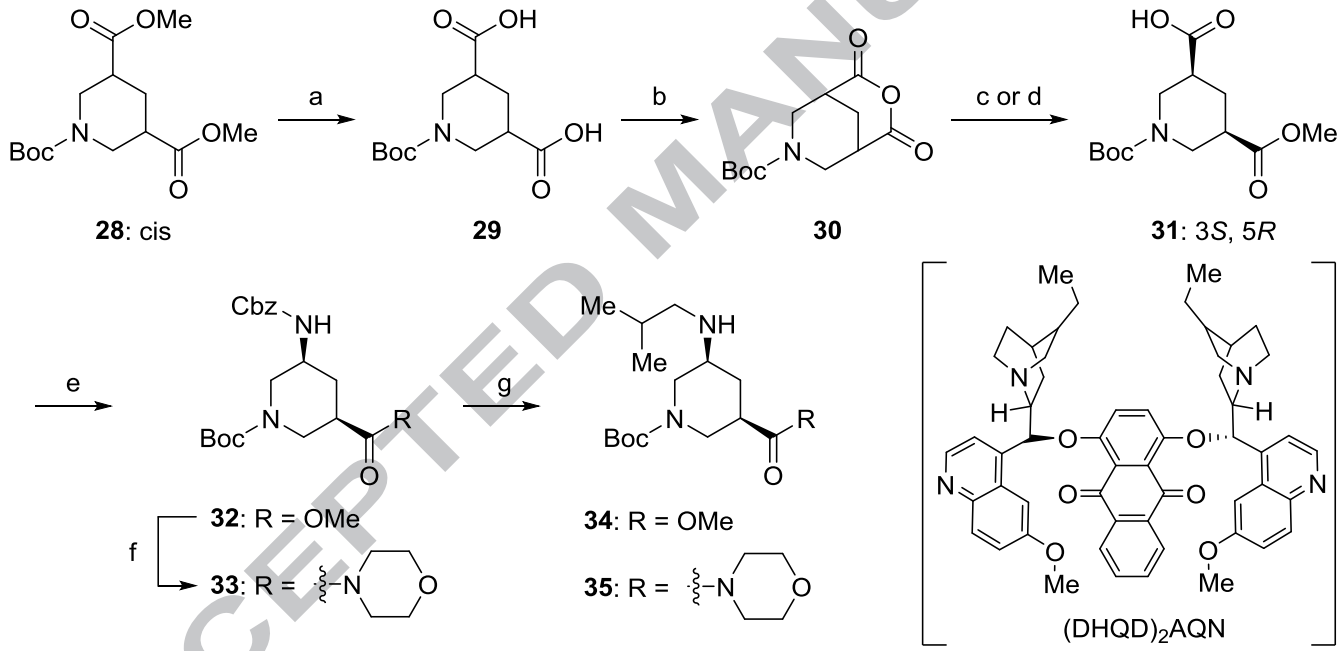


[a]Reagents and conditions: (a) 3-methoxypropan-1-ol, NaH, DMF, rt, then water, rt, 36%; (b) morpholine, WSC, HOBt, Et_3N, DCE, rt, 81%; (c) 1 M HCl in EtOAc, rt, 38%; (d) pentan-1-amine, DIEA, DMF, 80 °C, 95%; (e) (1) 0.5 M NaOH, MeOH, rt, quant.; (2) morpholine, WSC, HOBt, DCE, rt, 82%; (f) 1 M HCl in EtOAc, rt, 98%.

The asymmetric synthesis of key intermediate **35** was investigated as shown in Scheme 2. Hydrolysis of the cis-dimethyl ester **28**[15] and subsequent treatment with Ac_2O provided acid anhydride **30**. A catalytic enantioselective methanolysis reaction was performed in a manner reported by Deng[16] to afford 3*S*,5*R*-isomer in excellent enantiomeric excess (95%ee) when 0.2 equivalent of $(DHQD)_2AQN$ was used. Despite the excellent specificity of the asymmetric methanolysis reaction, use of the precious reagent $(DHQD)_2AQN$ is problematic for large-scale experiments. Hence alternative methods for this methanolysis reaction were investigated. In Deng's report, quinidine was also demonstrated to be a useful catalyst in the same asymmetric reaction. Quinidine is derived from a natural source (the bark of the cinchona tree) in large quantity; therefore, it is easily available for large

scale use. One equivalent of quinidine was applied to the large scale synthesis of mono-ester **31**. Further optical resolution by recrystallization of its (*R*)-(+)-1-phenylethylamine salt provided the desired mono-ester **31** in 97%ee. The carboxyl group of **31** was converted to a protected amine group by Curtius rearrangement. After the modification of ester moiety of **32** to morpholine amide **33**, the desired intermediates **34** and **35** were obtained in two steps with good yield.

Scheme 2.[a] Asymmetric synthesis of the key intermediate **35**.

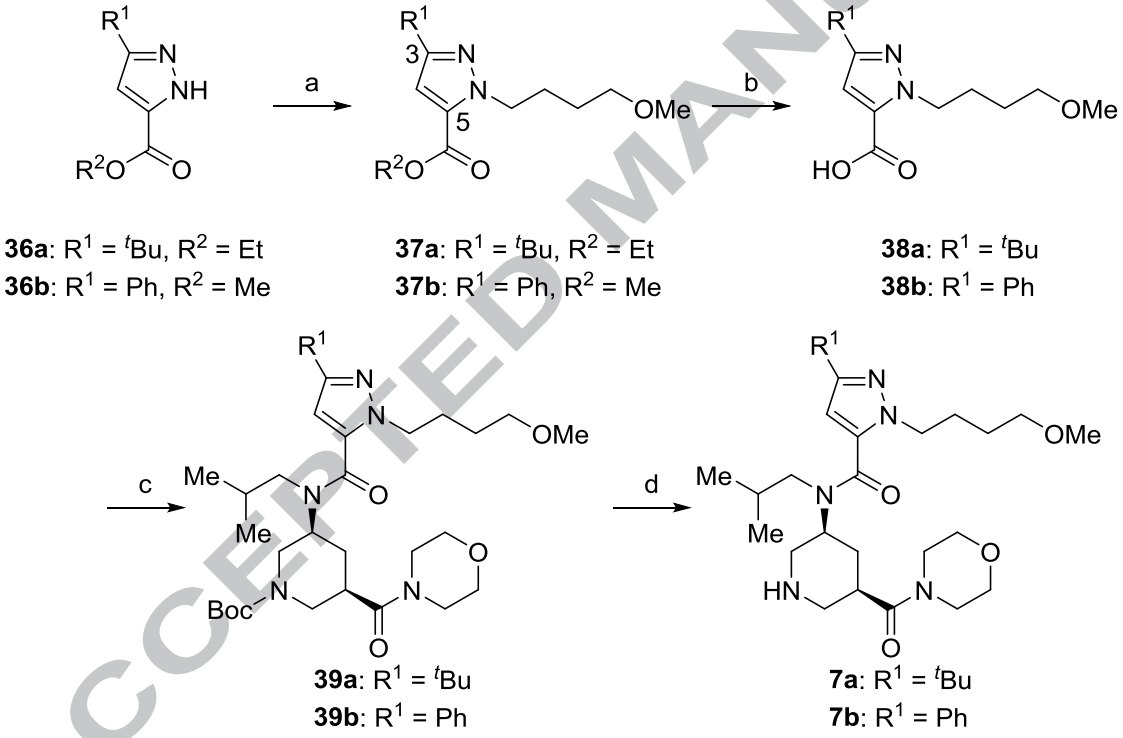


[a]Reagents and conditions: (a) K_2CO_3, MeOH, water, reflux, 97%; (b) Ac_2O, reflux, 99%; (c) $(DHQD)_2AQN$, MeOH, Et_2O, THF, –40 °C, 57%; (d) (1) quinidine, MeOH, THF, –40°C, 93%; (2) (*R*)-1-phenylethylamine, EtOH then $KHSO_4$aq, 69%; (e) Et_3N, DPPA, toluene, 100 °C, BnOH, Et_3N, 80 °C, 73%; (f) (1) 1 M NaOHaq, MeOH, rt, 90%; (2) morpholine, WSC, HOBt, Et_3N, DMF, rt, quant.; (g) (1) $Pd(OH)_2/C$, H_2, MeOH, rt; (2) 2-methylpropanal, $NaH(OAc)_3$, MeOH, rt, 65–71%.

Scheme 3 illustrates the synthesis of pyrazole derivatives **7a**,**b**. Alkylation of **36a** and **36b** with 4-methoxybutyl methanesulfonate in the presence of cesium carbonate occurred

at the 2-position of the pyrazole ring to give **37a** and **37b**. The ester moieties of **37a** and **37b** were hydrolyzed to afford carboxylic acids **38a** and **38b**. Coupling of these acids with the amine **35** obtained above was performed using *N*,*N*,*N*',*N*'-tetramethylchloroformamidinium hexafluorophosphate (TCFH) in the presence of *N*,*N*-diisopropylethylamine (DIEA).[17] Finally, **39a** and **39b** were deprotected to afford the desired 7**a** and 7**b**.

Scheme 3.[a] Synthesis of **7a,b**.



36a: R^1 = ^{t}Bu, R^2 = Et
36b: R^1 = Ph, R^2 = Me

37a: R^1 = ^{t}Bu, R^2 = Et
37b: R^1 = Ph, R^2 = Me

38a: R^1 = ^{t}Bu
38b: R^1 = Ph

39a: R^1 = ^{t}Bu
39b: R^1 = Ph

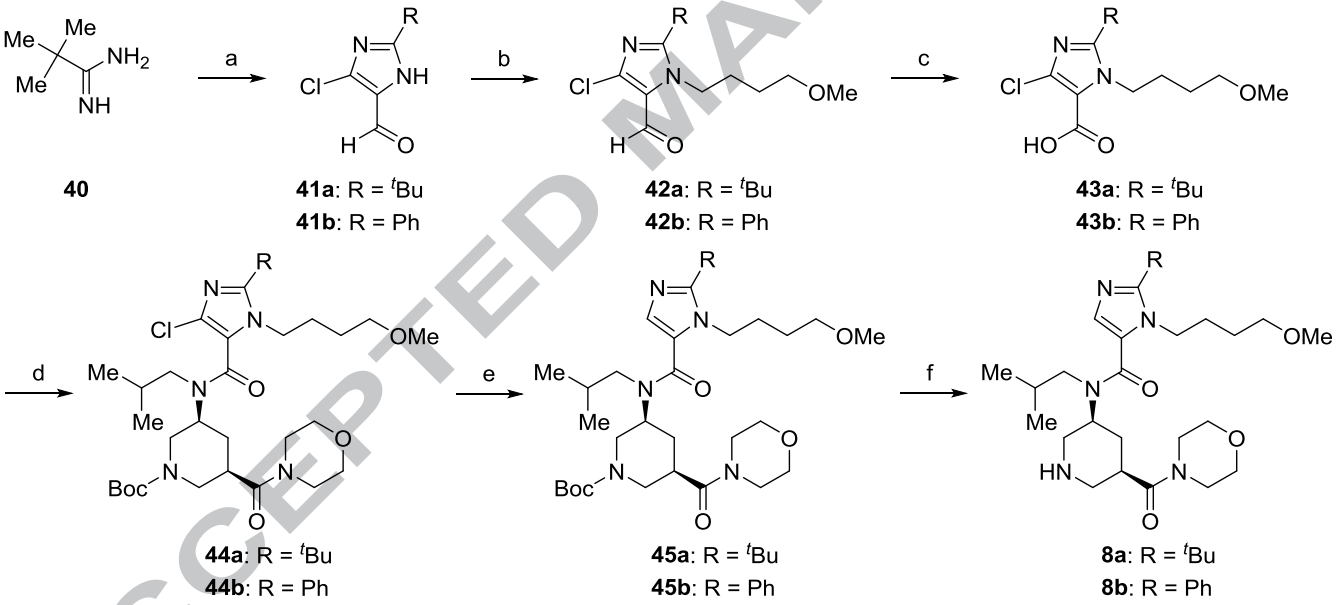
7a: R^1 = ^{t}Bu
7b: R^1 = Ph

[a]Reagents and conditions: (a) 4-methoxybutyl methanesulfonate, Cs_2CO_3, DMA, 65 °C, 75–82%; (b) $LiOH{\cdot}H_2O$, EtOH, water, 65 °C, 91–97%; (c) **35**, TCFH, DIEA, DCE, rt, 66–98%; (d) 4 M HCl in EtOAc, EtOAc, rt, 95–97%.

The imidazole ring was constructed by the synthetic route described in Scheme 4. Amidine **40** was reacted with glycine *tert*-butyl ester and a subsequent ring closing

reaction was accomplished with phosphoryl chloride to yield **41a**.[18] A methoxybutyl side chain was introduced onto the imidazole ring of the obtained **41a** and commercially available **41b** to give **42a** and **42b**. The aldehyde groups of those compounds were oxidized to afford **43a** and **43b**. After amidation with **35**, the chloro group was removed by catalytic hydrogenation in the presence of potassium acetate. Removal of a Boc group provided the imidazole derivatives **8a** and **8b**, respectively.

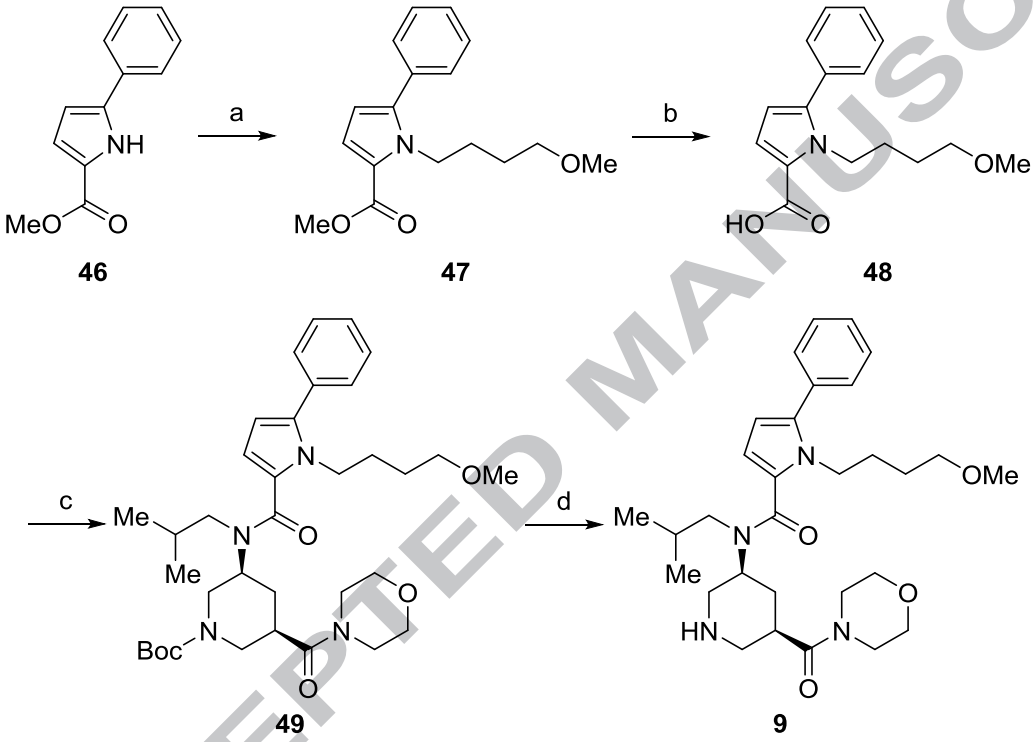
Scheme 4.[a] Synthesis of **8a,b**.



[a]Reagents and conditions: (a) (1) *tert*-butyl glycinate hydrochloride, Et_3N, DMF, 80 °C, 55%; (2) TFA, DCE, rt, then $POCl_3$, toluene, DMF, 100 °C, 22%; (b) 4-methoxybutyl methanesulfonate, Cs_2CO_3, DMA, 90 °C, 52–99%; (c) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl-2-butene, rt, 99–100%; (d) **35**, TCFH, DIEA, DCE, rt, 64–84%; (e) $Pd(OH)_2/C$, H_2, KOAc, MeOH, rt, 38–48%; (f) 1 M HCl in EtOAc, rt, 76–99%.

The pyrrole derivative **9** was synthesized according to the method in Scheme 5. Commercially available **46** was alkylated to give **47**. After hydrolysis of ester **47,** the desired **9** was obtained by amidation with **35**, followed by treatment with hydrochloric acid for deprotection.

Scheme 5.[a] Synthesis of **9**.

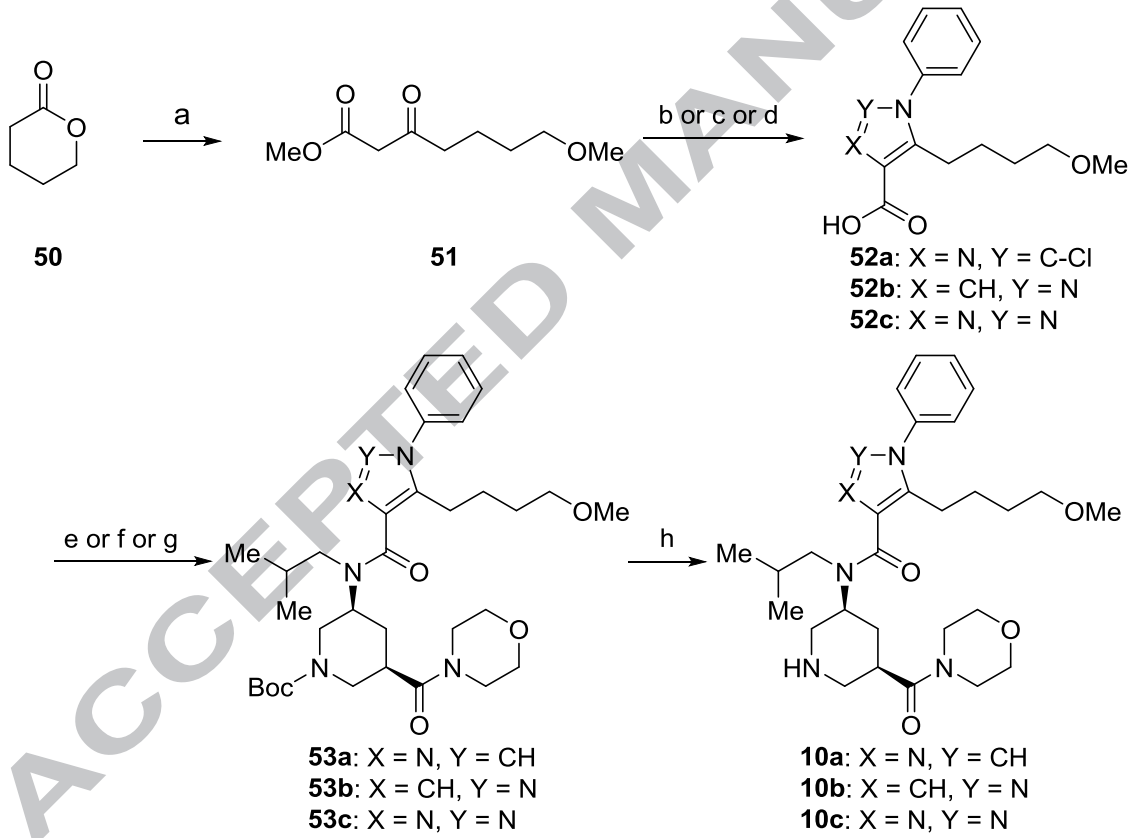


[a]Reagents and conditions: (a) 4-methoxybutyl methanesulfonate, Cs_2CO_3, DMF, 60 °C, 75%; (b) 2 M NaOH, MeOH, quant.; (c) **35**, TCFH, DIEA, DCE, rt, 48%; (d) 4 M HCl in EtOAc, EtOAc, rt, 2 h, 82%.

Scheme 6 summarizes the synthesis of imidazole **10a**, pyrazole **10b**, and 1,2,3-triazole **10c**. □-Keto ester **51**,[19] a common intermediate, was synthesized from **50**. Rhodium-catalyzed ring closure and then chlorination afforded imidazole **52a**, which was converted to **10a** in the same manner giving rise to imidazole derivatives **8a** and **8b**. The pyrazole

intermediate **52b** was obtained by treatment of **51** with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA), ring closure using phenylhydrazine, and hydrolysis of the ester moiety. A 1,2,3-triazole ring was constructed by treating **51** with phenylazide and sodium hydride. Then, the ester moiety was hydrolyzed without purification to afford **52c**. Condensation of these acids with amine **35** was performed by using TCFH. Deprotection of the Boc group was carried out under acidic conditions to afford **10b** and **10c**.

Scheme 6.[a] Synthesis of **10a-c**.

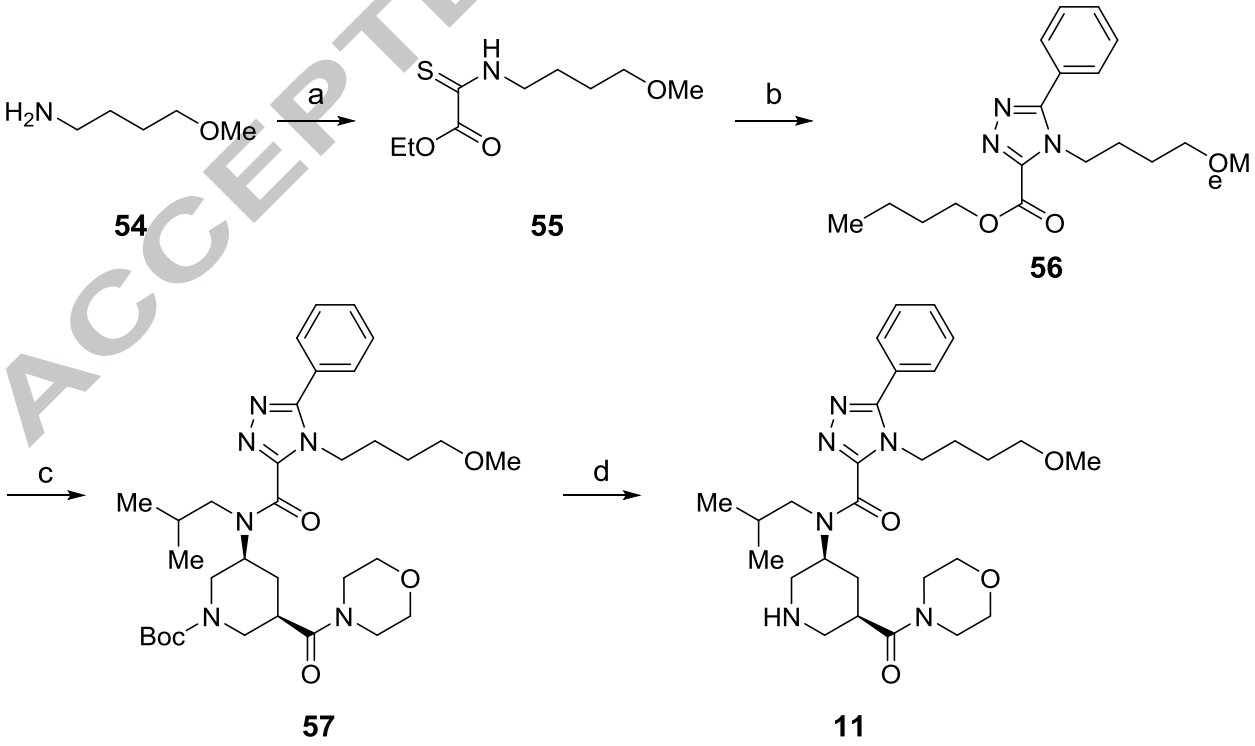


[a]Reagents and conditions: (a) (1) trimethyl orthoformate, H_2SO_4, MeOH, 65 °C, then 8 M NaOH, 95 °C, 81%; (2) oxalyl chloride, THF, 0 °C to rt, then Meldrum's acid, pyridine, DCM, 0 °C, (3) MeOH, reflux, 89% for 2 steps; (b) (1) 4-(acetylamino)benzenesulfonyl azide, Et_3N, MeCN, rt, 2 days, quant.; (2) 1-phenylurea, $Rh_2(OAc)_4$, toluene, DCE, 80 °C, then TFA, rt, 92%; (3) $POCl_3$, 100 °C, 29%; (4) 1 M NaOH, MeOH, 80 °C, 87%; (c) (1) DMF-DMA, toluene, 80 °C, then $PhNHNH_2$, EtOH, 80 °C, 77%; (2) NaOH, MeOH, rt,

quant.; (d) NaH, phenyl azide, DMF, rt, then 4 M NaOH, MeOH, 60 °C, 52%; (e) (1) **35**, TCFH, DIEA, DCE, rt, 2 h, 92%; (2) $Pd(OH)_2/C$, H_2, KOAc, MeOH, rt, 54%; (f) $SOCl_2$, DMF, toluene then **35**, THF, DIEA, 28%; (g) **35**, TCFH, DIEA, DCE, rt, 15 h, 75%; (h) 1–4 M HCl in EtOAc, rt, 91–99%.

In order to construct a 1,2,4-triazole ring, precursor **55** was synthesized in two steps (Scheme 7). **54** was acylated with ethyl chloroglyoxylate in the presence of triethylamine, followed by treatment with Lawesson's reagent gave the desired thioamide intermediate **55**. Ring closure was achieved by heating with benzohydrazide in 1-butanol to afford **56** in poor yield. The ester moiety was exchanged for a butyl group at the same time. Conversion of **56** to **11** was accomplished in good yield.

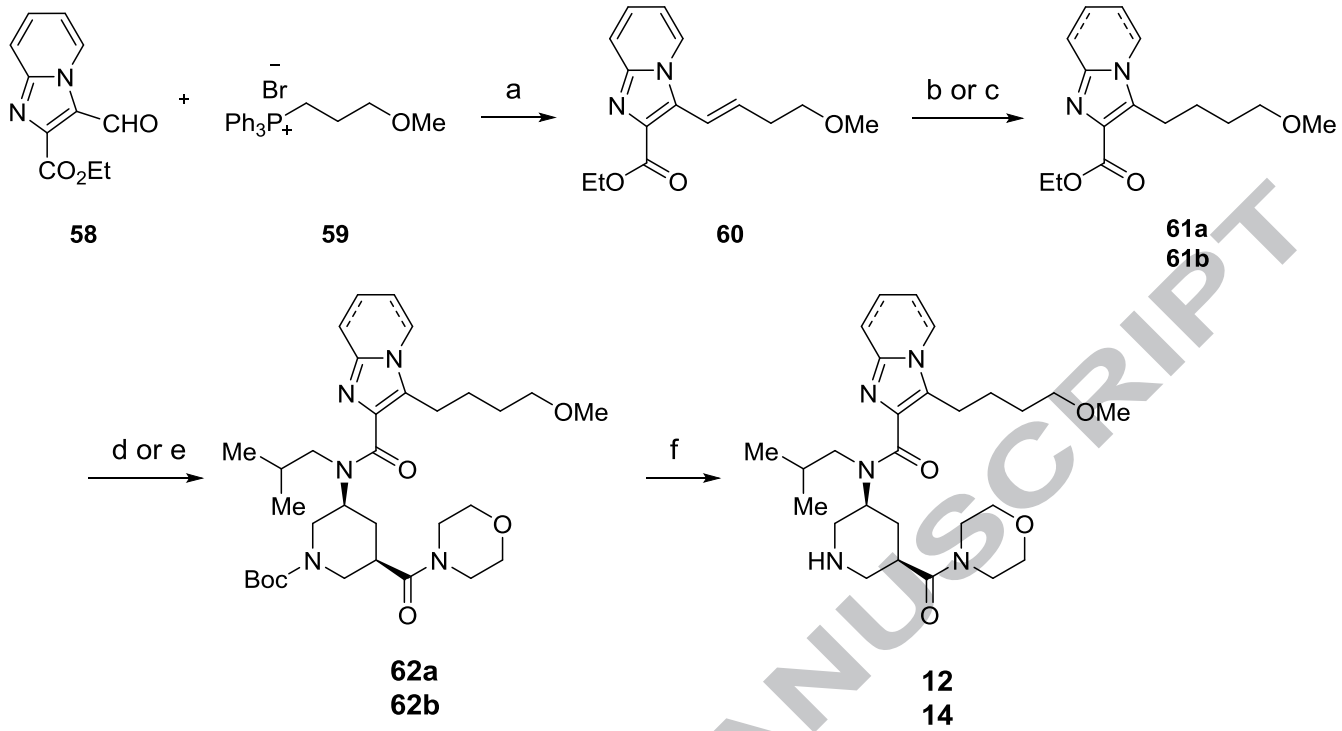
Scheme 7.[a] Synthesis of **11**.



[a]Reagents and conditions: (a) (1) Et_3N, ethyl chloroglyoxylate, THF, 0 °C to rt; (2) Lawesson's reagent, toluene, 90 °C, 55% for 2 steps; (b) $PhC(O)NHNH_2$,1-BuOH, 140 °C, 4.4%; (c) (1) $LiOH \cdot H_2O$, EtOH, water, rt; (2) **35**, TCFH, DIEA, MeCN, rt, 74% for 2 steps; (d) 4 M HCl in EtOAc, rt, 75%.

Syntheses of imidazopyridine **12** and tetrahydroimidazopyridie **14** were depicted in Scheme 8. Introduction of methoxybutyl group to imidazopyridine **58** was accomplished by Wittig reaction. Palladium catalyzed hydrogenation of olefin **60** reduced both the olefin moiety and the imidazopyridine core to give **61b**. Selective olefin reduction was achieved with palladium catalyst poisoned by diphenylsulfide to yield **61a**. Esters **61a** and **61b** were converted to the desired **12** and **14**.

Scheme 8.[a] Synthesis of **12** and **14**.

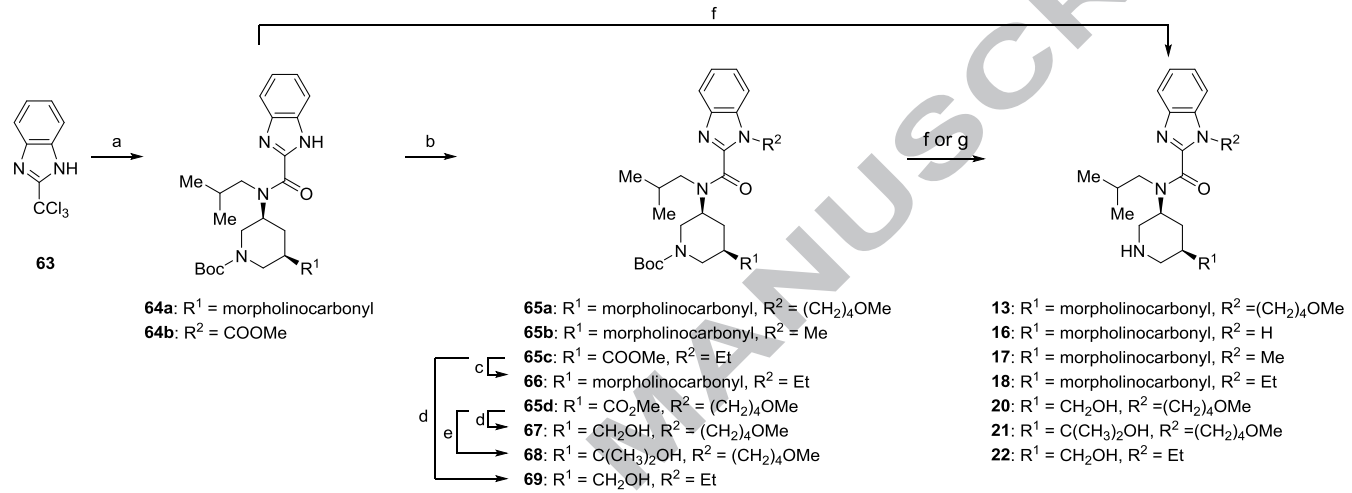


[a]Reagents and conditions: (a) KO^{t}Bu, THF, –78 °C to rt, 68%; (b) Pd/C, Ph_2S, H_2, EtOAc, rt, 49%; (c) Pd/C, H_2, EtOAc, rt, 80%; (d) (1) LiOH·H_2O, EtOH, 60 °C, then **34**, TCFH, DIEA, MeCN, rt, 62%; (2) 2 M NaOH, MeOH, 60 °C, quant.; (3) morpholine, WSC, HOBt, DIEA, DMF, rt, 85%; (e) LiOH·H_2O, 8 M NaOH, EtOH, 60 °C, then **35**, TCFH, DIEA, MeCN, rt, 22%; (f) 4 M HCl in EtOAc, EtOAc rt, 89–97%.

Scheme 9 showed the syntheses of benzimidazole derivatives **13**, **16**–**18** and **20**–**22**. Coupling reactions of **63** and **35** or **34** under basic conditions afforded **64a** and **64b**, respectively. Alkyl groups were introduced into the NH group of the benzimidazole ring using cesium carbonate to afford **65a–d**. The ester moiety of **65c** was converted to morpholine amide group in two steps affording **66**. Reduction of ester groups was accomplished by calcium borohydride to give **67** and **69**. The reaction of ester **65d** with methyl magnesium bromide yielded **68**. Boc deprotection was carried out by using

hydrogen chloride in ethyl acetate. As for **67** and **69**, methanol solution of hydrogen chloride was used to prevent the esterification of the hydroxymethyl moiety.

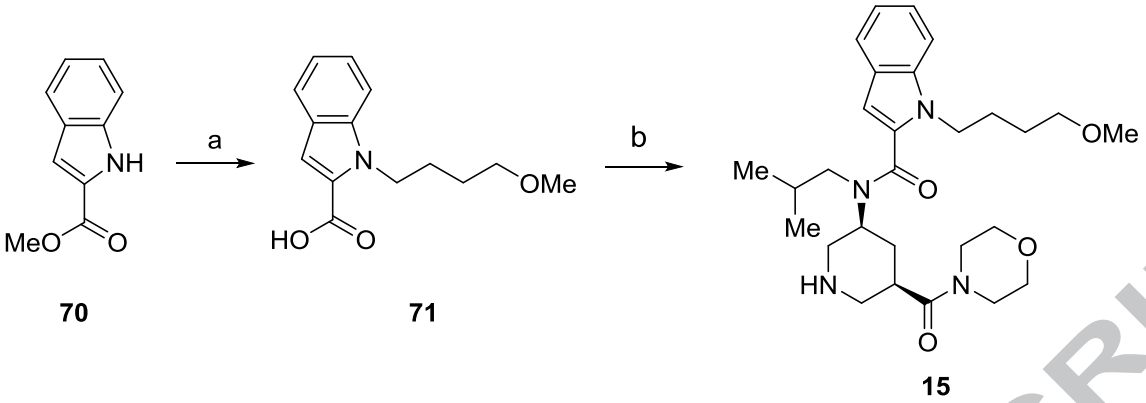
Scheme 9.[a] Synthesis of **13, 16–18** and **20–22**.



[a]Reagents and conditions: (a) **34** or **35**, THF, water $NaHCO_3$, rt, 77–84%; (b) 4-methoxybutyl methanesulfonate or MeI or EtI, $CsCO_3$, DMF or DMA, 50–70 °C, 80–87%; (c) (1) 1 M NaOH, MeOH, THF, rt, quant.; (2) morpholine, WSC, HOBt, Et_3N, MeCN, rt, 63%; (d) $NaBH_4$, $CaCl_2$, EtOH, THF, 0 °C to rt, 47–63%; (e) MeMgBr, THF, –40 °C to rt, 54%, (f) 4 M HCl in EtOAc, rt, 76–99%; (g) 10–20% HCl in MeOH, rt, 91–99%.

Scheme 10 described the synthesis of indole **15**. Displacement of indole **70** with 4-methoxybutyl methanesulfonate and cesium carbonate, followed by hydrolysis afforded **71**. Subsequent amide coupling and deprotection of **71** provided **15**.

Scheme 10.[a] Synthesis of **15**.

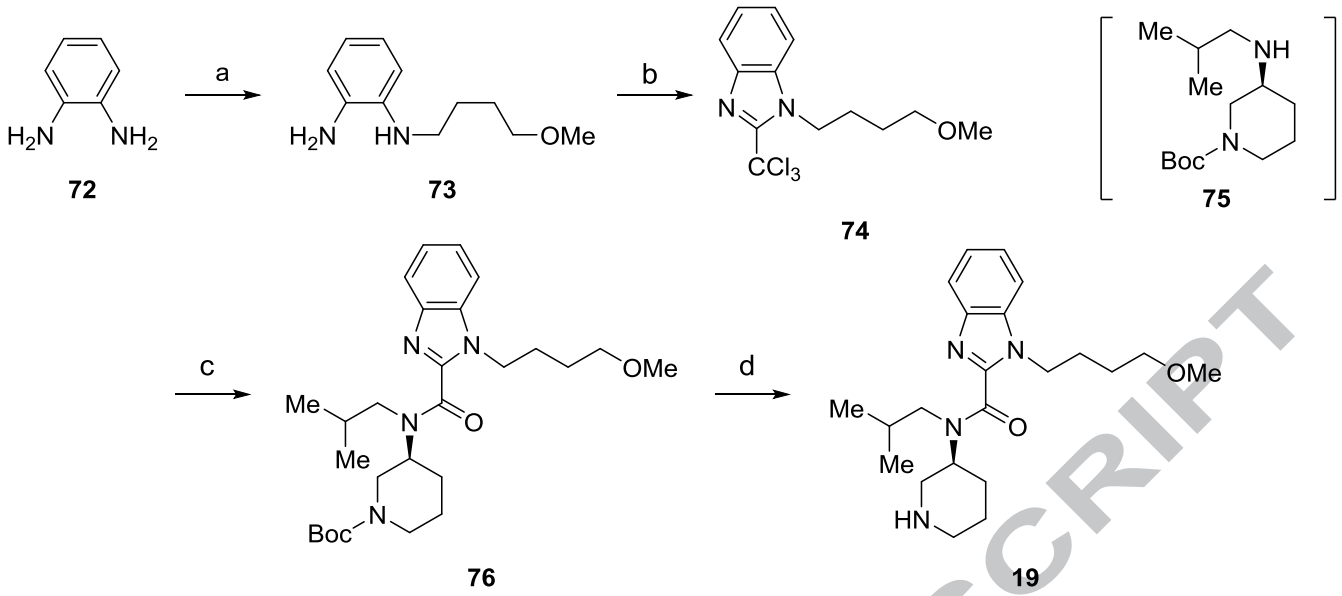


70 **71** **15**

[a]Reagents and conditions: (a) (1) 4-methoxybutyl methanesulfonate, Cs_2CO_3, DMF, 60 °C; (2) 4M NaOHaq 80 °C, 82% for 2 steps; (b) (1) **35**, TCFH, DIEA, DCE, rt, 19%; (2) 4 M HCl in EtOAc, EtOAc, rt, 89%.

Synthesis of **19** was shown in Scheme 11. Mono-alkylation of phenylenediamine **72** gave **73**. The benzimidazole ring was constructed by the reaction of **73** with methyl 2,2,2-trichloroethanimidate to provide **74**. The desired **19** was obtained by the coupling reaction of **74** and **75**[20], followed by the deprotection.

Scheme 11.[a] Synthesis of **19**.



[a]Reagents and conditions: (a) 4-methoxybutyl methanesulfonate, K_2CO_3, MeCN, reflux, 56%; (b) CCl3 C(NH)Ome, AcOH, rt, 96%; (c) **75**, MeCN, water K_2CO_3, 80 °C, 63%; (d) 4 M HCl in EtOAc, EtOAc, rt, quant..

RESULTS AND DISCUSSION

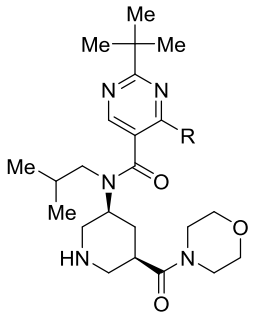
The compounds listed in Tables 1–5 inhibited recombinant human renin activity in the absence or presence of human serum albumin (HSA– or HSA+, respectively) and endogenous renin activity in human plasma (human plasma renin activity, hPRA). These inhibitory activities were evaluated and reported as IC_{50} values.

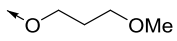
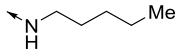
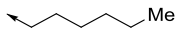
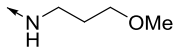
As the first step toward our goal, the effect for the potency and PK profiles by replacing the amino group on the pyrimidine ring at the 4-position was investigated to know the contribution of HBD (Table 1). Although the renin inhibitory activity of oxa-analogue **4** was diminished, the carba-analogue **6**[13] exhibited moderate renin inhibitory activity. These decreases in renin inhibitory activity could be rationalized by the loss of hydrogen bonding to the Gly217 backbone oxygen of the renin protein observed in X-ray co-crystal studies. In particular, repulsion between the lone pair of the ether oxygen atom of **4** and the

carbonyl group of Gly217 caused the disappearance of renin inhibitory activity. The importance of the oxygen atom in the methoxy group was also investigated. Although, under HSA+ conditions, the renin inhibitory activities of **3** and **5** were similar, **5** showed weaker hPRA inhibition than **3** (5.4 nM and 0.79 nM, respectively). The lipophilicity of compounds (LogD: 4.45 for **5** and 2.99 for **3**) dramatically affected their activity in the presence of proteins such as HSA and in plasma, which has relevance to in vivo potency. Reducing the LogD value would thus be important for optimizing compounds to improve their efficacy in vivo.

In the rat cassette dosing study, the high clearance value (7261 mL/h/kg) and undetected AUC_{po} value of **3** indicated that the poor PK profile of **3** was due to rapid excretion and/or poor absorption rather than to metabolic stability, which was shown to be good (data not shown). On the other hand, **6** showed improved clearance value (2407 mL/h/kg) and AUC_{po} value (20.9 ngh/mL), giving the improved BA (5.0%). Both **3** and **6** possessed good parallel artificial membrane permeability assay (PAMPA) permeability. The improved PK profile of **6** seemed to be due to a difference in the rate of drug excretion from the bloodstream. The reduced TPSA value of **6** would explain the difference of clearance of compound (TPSA: 87.7 $Å^2$ for **6** and 109 $Å^2$ for **3**). Lowering the TPSA of compound was seemed the key factor for the orally bioavailable renin inhibitors. These results inspired us to prepare a new S3 site binder with methoxybutyl side chain for the recovery of potency.

Table 1. SAR and ADME profiles of pyrimidine derivatives **3–6**.



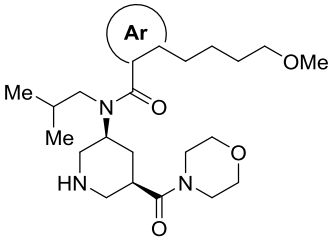
cmpd	R	IC_{50} (nM)[a]			LogD	rat cassette dosing[b]				PAMPA	TPSA
		HSA–	HSA+	hPRA		AUC_{iv}	AUC_{po}	CL	F[c]	pH7.4	($Å^2$)
						(ngh/mL)		(mL/h/kg)	(%)	(nm/sec)	
4		> 100	> 100	NT[d]	2.81	NT[d]	NT[d]	NT[d]	NT[d]	272	106
5		0.20 (0.15–0.26)	0.64 (0.36–1.1)	5.4 (3.5–8.3)	4.45	27.3	ND[e]	3745	< 1	252	99.7
6		29 (22–38)	24 (19–31)	> 10	4.77	42.0	20.9	2407	5.0	435	87.7
3		1.5 (0.60–3.6)	0.38 (0.24–0.62)	0.79 (0.61–1.0)	2.99	14.1	ND[e]	7261	< 1	390	109

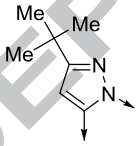
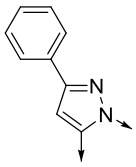
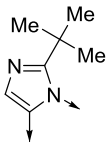
[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Dose: 1 mg/kg, *po*, 0.1 mg/kg, *iv*. Average of 3 rats. [c] F is the measure of bioavailability. [d] Not tested. [e] Not detected.

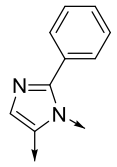
The vast lipophilic S3 site would accept various substituents and permitted us to introduce different scaffolds. To explore more preferable S3 site binders, we initially examined replacement of the 6-membered pyrimidine ring by a 5-membered azole (Table 2). Pyrazole derivatives **7a,b** and imidazole derivatives **8a,b** with *tert*-butyl or phenyl group were initially synthesized as new S3 site binders. Compound **7a** showed potent

renin inhibitory activity under HSA– and HSA+ conditions, while potency of hPRA inhibition was somewhat decreased, maybe due to the lipophilicity (LogD = 2.73). Interestingly, changing *tert*-butyl to a phenyl group resulted in the disappearance of potency (**7a** vs **7b**). On the other hand, the imidazole derivative **8a** showed moderate potency under each of the conditions and **8b** possessed comparable potency to **7a**, indicating that the substitution manner of **8b** can be used for further substituent modification. The scaffold of **8b**, on which a phenyl group is located vicinal to the methoxybutyl side chain, was employed to further explore 5-membered azole cores.

Table 2. SAR of 5-membered azoles **7a,b** and **8a,b**.



cmpd	Ar	IC_{50} (nM)[a]			LogD
		HSA–	HSA+	hPRA	
7a		2.0 (1.6–2.4)	3.6 (2.5–5.2)	52.8% inhibition at 10 nM	2.73
7b		>100	>100	NT[b]	2.68
8a		48 (34–69)	35 (25–50)	NT[b]	2.12

8b		4.4 (3.0–6.3)	3.3 (2.6–4.3)	2.8 (1.2–7.0)	2.03

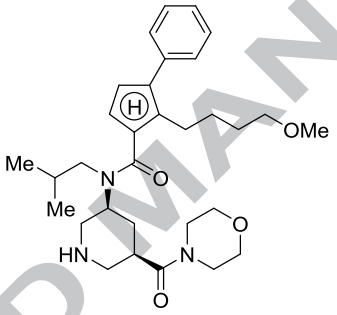
[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Not tested.

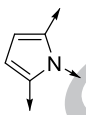
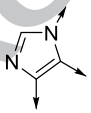
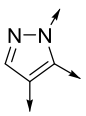
All 5-membered azole derivatives (**9, 10a–c, and 11**) listed in Table 3 showed potent renin inhibitory activity in the absence of HSA. The level of hPRA inhibition of pyrrole **9** was decreased despite strong activity under HSA– conditions as reflected by higher LogD value (3.08). The potency of imidazole **10a** was increased as compared to that of **8b**, while the difference between these compounds is in the position of the nitrogen atom and not in LogD value. Notably, the presence of the nitrogen atom next to the carbonyl group resulted in relatively stronger hPRA inhibition (**10b** vs **10c** and **8b** vs **11**, respectively). The X-ray co-crystal structures of **9** and **10c** revealed that the distance between the hydroxyl group of Thr77 and **9** were separated by 3.7 Å (Figure 3). On the other hand, a hydrogen bond interaction was observed between Thr77 and the nitrogen atom of **10c** with a distance of 2.6 Å. The rotation of Thr77 residue was observed and indicated that formation of a hydrogen bonding between the nitrogen atom of **10c** and Thr77 could account for the increase in potency.

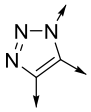
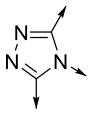
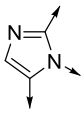
Rat cassette dosing study of synthesized compounds revealed that the oral absorption of compounds **9** and **10c** (AUC_{po}: 18.5 and 3.5 ngh/mL) was better than that of other compounds, which is probably due to their better PAMPA membrane permeabilities (217 and 369 nm/sec). The compounds with low logD value tend to exhibit low membrane

permeability (**10a, 10b**, and **11**). Compound **9**, with the lowest TPSA (76.0 Å^2), exhibited lowest clearance value among the compounds in Table 3. Compound **10c** had good in vitro profiles, but its high clearance value resulted in an unacceptable PK profile for oral administration. The high TPSA value (102 Å^2) could be the reason for that unfavorable drug excretion as in the case of compound **3**. These results encouraged us to explore other S3 site binders in more detail.

Table 3. SAR and ADME profiles of phenylazoles **9**, **10a–c**, **11**.



Cmpd	Ar	IC$_{50}$ (nM)[a]			LogD	rat cassette dosing[b]				PAMPA	T
		HSA–	HSA+	hPRA		AUC$_{iv}$ (ngh/mL)	AUC$_{po}$ (ngh/mL)	CL (mL/h/kg)	F[c] (%)	pH7.4 (nm/sec)	(
9		0.16 (0.082–0.3)	1.4 (0.97–2.1)	3.4 (1.6–6.8)	3.08	34.6	18.5	2968	5.4	217	7
10a		0.83 (0.59–1.2)	1.1 (0.66–1.8)	1.2 (0.93–1.5)	1.84	13.3	ND[d]	7604	< 1	78	8
10b		1.1 (0.93–1.4)	1.2 (0.93–1.6)	1.3 (0.84–2.2)	1.78	20.8	0.7	4826	0.3	78	8

10c		1.0 (0.61–1.7)	0.65 (0.50–0.86)	0.40 (0.0044–1.2)	1.94	18.4	3.5	5544	1.9	369	1
11		1.4 (0.88–2.1)	1.8 (1.2–2.6)	1.2 (0.74–2.0)	1.79	15.3	ND[d]	6740	< 1	116	1
8b		4.4 (3.0–6.3)	3.3 (2.6–4.3)	2.8 (1.2–7.0)	2.03	17.6	ND[d]	5804	< 1	72	8

[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Dose: 1 mg/kg, *po*, 0.1 mg/kg, *iv*. Average of 3 rats. [c] F is the measure of bioavailability. [d] Not detected.

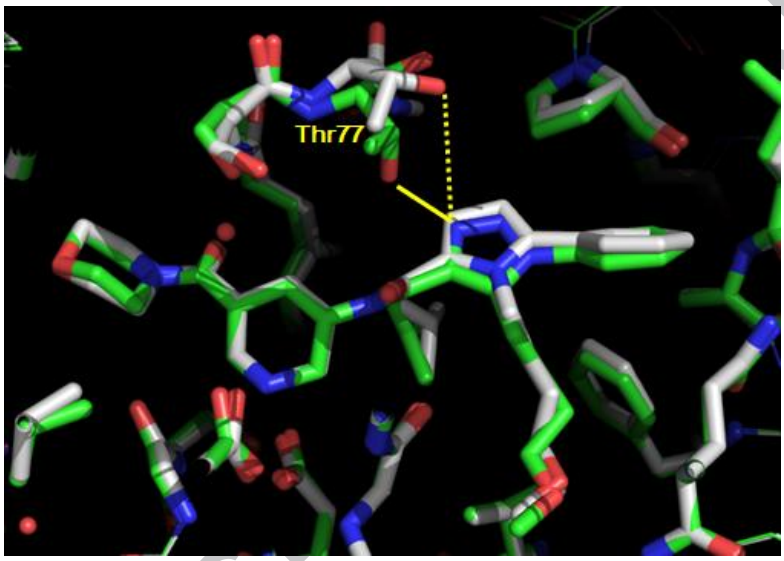
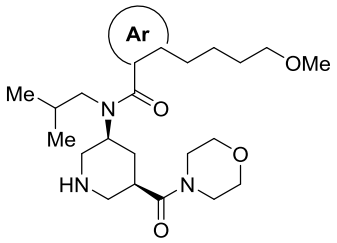


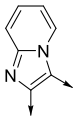
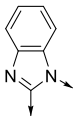
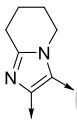
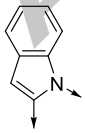
Figure 3. Overlay of crystal structures of **9** (white) and **10c** (green) bound to renin. The solid yellow line indicates the hydrogen bonding in the structure of **10c**. The dashed-yellow line indicates the distance between hydroxyl group of Thr77 and carbon atom measured within the structure of **9**.

The strategy to further improve PK profile is adjusting physicochemical properties, such as reducing the molecular weight (all the compound listed in Table 1–3 were greater than 500), TPSA and the number of rotatable bonds without losing the interaction as described above.[13,14] Imidazopyridine **12** and benzimidazole **13** were designed on the basis of concepts as follows: 1) the nitrogen atom of the new central core would interact with the Thr77, 2) fused heterocycles would reside in the same lipophilic S3 site as **3** occupies, 3) the molecular weight and number of rotatable bonds were reduced by forming a fused ring as a new scaffold (Table 4). Both **12** and **13** exhibited potent hPRA inhibition and lowered TPSA value. Although the imidazopyridine **12** showed moderate membrane permeability (63 nm/sec), which is probably due to low LogD value (1.54), its oral absorption was confirmed (AUC_{po}: 9.5 ngh/mL) and clearance from the bloodstream was mitigated (CL: 2304 mL/h/kg), reflecting its TPSA value (88.4 $Å^2$). Furthermore, **13** exhibited a significant improvement in rat AUC_{po} (92.2 ngh/mL) and BA (14%) with good membrane permeability (345 nm/sec). These results indicate our optimization strategy worked well. Adjusting the lipophilicity and TPSA of compounds is \essential for good BA in this chemotype. To improve poor absorption, imidazopyridine **12** was reduced to provide compound **14**. However, **14** showed three-fold weaker hPRA inhibition than **12**. To confirm the importance of the hydrogen bonding with Thr77, **15** was synthesized. Interestingly, **15** exhibited strong potency under HSA– condition, but considerable decrease of hPRA inhibition was observed. We concluded that this lost in potency could be derived from the loss of the hydrogen bonding. Therefore, forming a suitable interaction with renin protein is essential to overcome this gap in this scaffold.

The X-ray crystal structure of **13** bound to renin protein is shown in Figure 4. As expected, the binding mode of **13** was similar to that of **3**. Hydrogen bonding between the nitrogen atom of the benzimidazole and Thr77 was observed.

Table 4. SAR and ADME profiles of fused heterocycles **12**–**15**.



cmpd	Ar	IC_{50} (nM)[a]			LogD	rat cassette dosing[b]				PAMPA	TPSA
		HSA–	HSA+	hPRA		AUC_{iv} (ngh/mL)	AUC_{po} (ngh/mL)	CL (mL/h/kg)	F[c] (%)	pH7.4 (nm/sec)	($Å^2$)
12		1.6 (0.84–3.1)	2.1 (1.2–3.5)	1.1 (0.97–1.3)	1.54	43.5	9.5	2340	2.2	63	88.4
13		0.43 (0.29–0.66)	0.84 (0.68–1.0)	1.4 (1.1–2.7)	2.11	68.0	92.2	1554	14	345	88.9
14		2.2 (1.0–4.4)	2.7 (1.4–5.1)	3.3 (1.5–8.0)	NT[d]	NT[d]	NT[d]	NT[d]	NT[d]	NT[d]	88.9
15		1.5 (1.1–2.2)	0.81 (0.64–1.0)	> 10	2.66	127.9	NT[d]	799	NT[d]	282	76

[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Dose: 1 mg/kg, *po*, 0.1 mg/kg, *iv*. Average of 3 rats. [c] F is the measure of bioavailability. [d] Not tested.

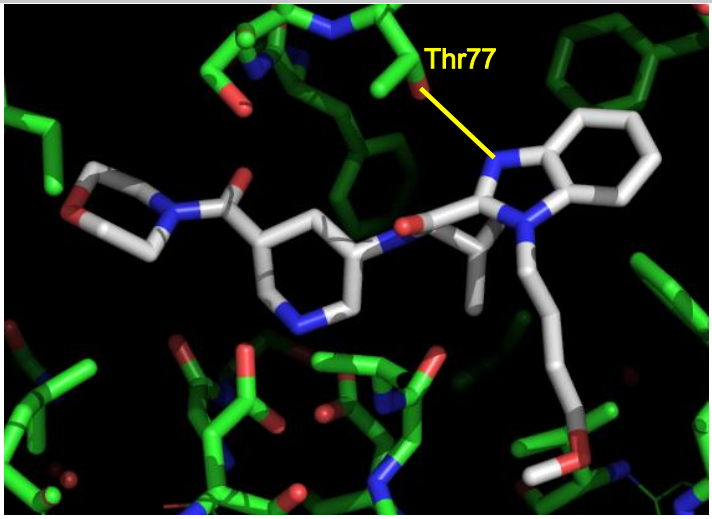
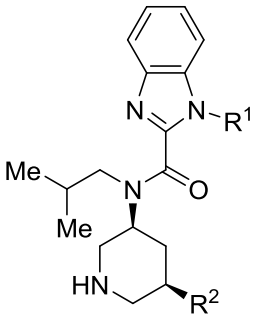


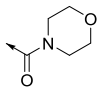
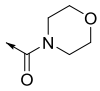
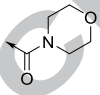
Figure 4. Crystal structure of **13** bound to renin. The solid yellow line indicates the hydrogen bonding in the structure of **13**.

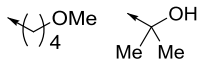
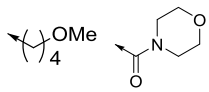
Finally, reinvestigation of the S1' and $S3^{sp}$ site binders was conducted with the benzimidazole core (Table 5). **16** maintained potency under both HSA– and HSA+ conditions in spite of the absence of the $S3^{sp}$ site binder, but hPRA inhibition of **16** was decreased (IC_{50} = 4.0 nM). Introduction of methyl group increased hPRA inhibition (**17**, IC_{50} = 2.9 nM), and ethyl analog showed comparable hPRA inhibition to **13** (**18**, IC_{50} = 1.3 nM). This finding showed that occupation of the $S3^{sp}$ site was not necessary for the potent hPRA inhibition in this benzimidazole scaffold. Rat cassette dosing study revealed that compounds with small substituents, such as **16**–**18** did not show superior PK profiles over **13**, nonetheless, some acceptable PK profiles were observed. Next, we explored S1' site binders. As in the case of the $S3^{sp}$ site binder, compound **19**, which did not have S1'site binder, showed comparable potency under HSA– and HSA+ conditions and decreased hPRA inhibition. X-ray crystal structure study of **3** revealed that the carbonyl

group of the morpholine amide moiety forms hydrogen bonding interaction with Ser76. Also, the morpholine moiety was occupying the S1' site (Figure 2). Instead of morpholinocarbonyl group, a hydroxyl group was introduced as a small substituent for the better PK profiles. **20** exhibited comparable potency to **13**, suggesting that a hydrogen bonding interaction between the hydroxyl group of **20** and Ser76 was formed successfully. However, **20** showed lower Log D and PAMPA value than **13**; rat PK profile of **20** was comparable to **13**. To improve permeability by masking the polarity of hydroxyl group, the conversion to the tertiary alcohol was attempted (**21**). The PAMPA value was improved as expected, but hPRA inhibition was decreased. This suggested that the hydrogen bonding interaction with Ser76 might be disrupted by the dimethyl group. Finally, combination study of the $S3^{sp}$ and S1' site binders was investigated by employing the hydroxymethyl group of **20** as the S1' site binder, with the ethyl group as the $S3^{sp}$ site binders. Contrary to our expectation, **22** showed decreased renin inhibitory activity. This result indicated that both of the hydrogen bonding interaction with Ser76 and occupation of the morpholine and methoxybutyl groups at the S1' site and $S3^{sp}$ sites had contributed to the strong potency (**18** and **20** vs **22**). Utilizing both binding sites would be better to show potent renin inhibition in human. All the benzimidazole compounds showed improved clearance value. This fact supports that the strategy of lowering TPSA is effective to enhance BA by improving the drug clearance from bloodstream. Compound **13** exhibited strong potency, good membrane permeability and reliable PK profiles, and was selected for further in vivo evaluation.

Table 5. SAR and ADME profiles of benzimidazole derivatives **16**–**22**.



cmpd	R^1	R^2	IC_{50} (nM)[a]			Log D	rat cassette dosing[b]				PAMPA	TPSA
			HSA–	HSA+	hPRA		AUC_{iv} (ngh/mL)	AUC_{po} (ngh/mL)	CL (mL/h/kg)	F[c] (%)	pH7.4 (nm/sec)	($Å^2$)
16	H		0.76 (0.46–1.2)	0.59 (0.42–0.85)	4.0 (2.7–6.0)	1.74	50.7	41.7	2053	8.2	60	90.6
17	Me		0.63 (0.43–0.93)	0.66 (0.46–0.95)	2.9 (2.0–4.4)	1.71	43.9	74.2	2332	17	274	79.7
18	Et		0.66 (0.41–1.0)	0.89 (0.64–1.2)	1.3 (0.90–2.3)	1.79	43.4	58.9	2312	14	242	79.7
19		H	0.9 (0.64–1.3)	1.6 (1.3–1.9)	9.1 (4.8–29)	2.00	52.1	59.7	1978	12	324	59.4
20			0.28 (0.22–0.35)	0.32 (0.22–0.47)	1.5 (0.82–2.9)	1.77	107.7	103.4	931	9.6	82	79.6

21		0.72 (0.55 – 0.95)	1.5 (1.0–2.1)	2.6 (1.0–8.4)	1.98	NT[d]	NT[d]	NT[d]	NT[d]	202	79.6
22		16 (10–25)	21 (13–36)	> 10	1.73	NT[d]	NT[d]	NT[d]	NT[d]	55	70.4
13		0.43 (0.29 – 0.66)	0.84 (0.68 –1.0)	1.4 (1.1–2.7)	2.11	68.0	92.2	1554	14	345	88.9

[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Dose: 1 mg/kg, *po*, 0.1 mg/kg, *iv*. Average of 3 rats. [c] F is the measure of bioavailability. [d] Not tested.

In a species difference study between **13** and **2**, reflecting the homology difference (human vs rat: 67.0% and human vs monkey 98.8%), the IC_{50} value of **13** for inhibition of rat plasma renin activity was 200 times lower than that for inhibition of monkey plasma renin activity (310 nM and 1.5 nM, respectively, Table 6).[21] In vivo study using primate animal would give us good clinical extrapolation and measuring PRA inhibition is enough meaningful as a PD marker of antihypertensive effect.[21] Therefore, compound **13** was evaluated in a cynomolgus monkey in vivo study (Figure 5). Oral administration of **13** (1 and 3 mg/kg) exhibited potent and long-lasting PRA inhibition in a dose-related manner. At four hours after oral administration, PRA inhibition by compound **13** at a dosage of 1 mg/kg was similar to that of **2** at a dosage of 15 mg/kg. PRA inhibition by compound **13** at a dosage of 1 and 3 mg/kg was comparable to that of **2** at a dosage of 5 and 15 mg/kg at 24 hours after oral administration, respectively. Rats and monkey PK profiles and in vivo

study of compound **13** raised our expectations that the plasma renin inhibitory activity of compound **13** could be more potent than that of **2** in humans.

Table 6. PRA inhibition by **2** and **13** and PK profile of **2** and **13** in cynomolgus monkeys.

cmpd	PRA IC_{50} (nM)[a]			monkey cassette dosing[b]					
	human	rat	monkey	AUC_{iv} (ngh/mL)	CL (mL/h/kg)	AUC_{po} (ngh/mL)	C_{max} (ng/mL)	T_{max} (h)	F[c] (%)
13	1.4 (1.1-2.7)	310 (240-390)	1.5 (1.1-3.5)	128	855	323	29.1	1.67	25
2	0.84 (0.39-1.8)	88 (73-110)	1.2 (1.1-1.3)	501	202	25.9	3.5	3.33	0.6

[a] IC_{50} values and 95% confidence limits are calculated from the concentration-response curves generated by GraphPad Prism. [b] Dose: 1 mg/kg, *po*, 0.1 mg/kg, *iv*. Average of 3 monkeys. [c] F is the measure of bioavailability.

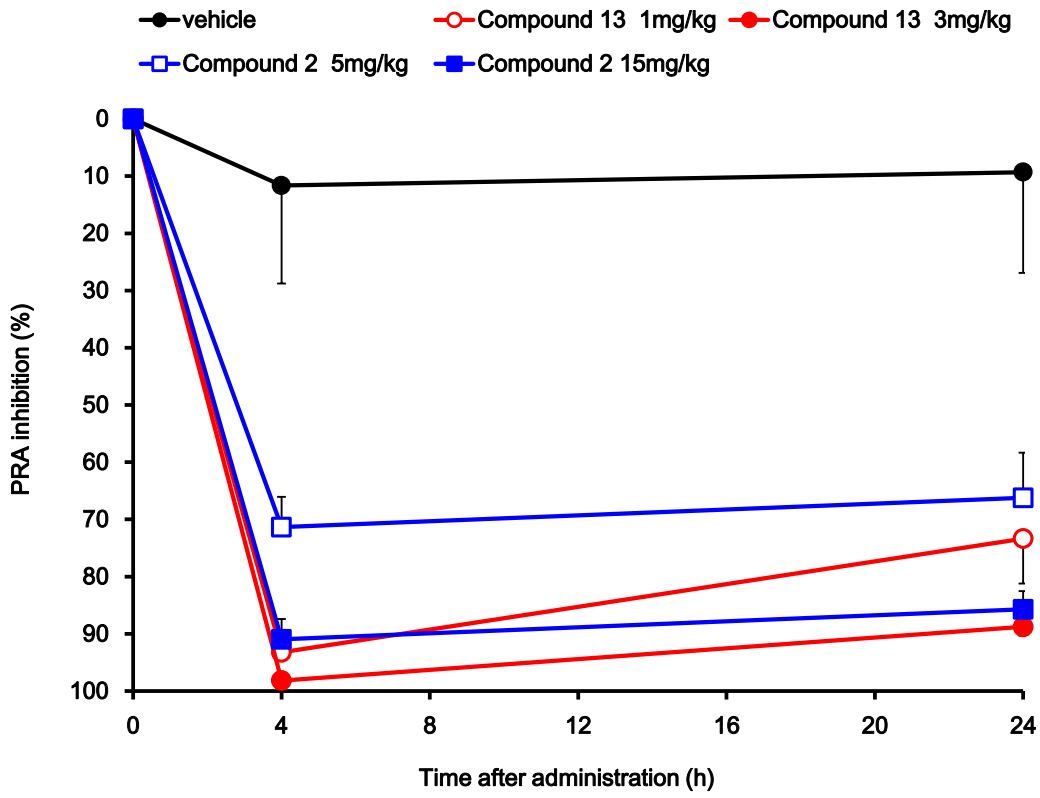


Figure 5. PRA inhibition by compound **13** (1 and 3 mg/kg, *po*) and **2** (5 and 15 mg/kg, *po*) in plasma samples collected from cynomolgus monkeys at the times indicated. Data represent mean ± standard error of the mean (n=4).

Conclusion

Exploration and optimization of new central cores that act as S3 site binders of the lead compound **3** have been successfully carried out to yield potent renin inhibitors. We demonstrated that adjusting the physicochemical properties, such as TPSA, HBD, number of rotatable bonds and molecular weight resulted in the improvement of PK profiles. In the course of modification, we discovered phenylazole derivatives with carbon side chains, which allowed an additional hydrogen bonding interaction with Thr77, increase potency. Furthermore, fused heterocycles were designed and benzimidazole core was found to be

an attractive scaffold with good PK profiles. The TPSA value would be the good index to avoid high clearance and discover orally bioavailable renin inhibitor. In this study, clearance value in rat cassette dosing was improved from 7261 mL/h/kg of **3** to 1554 mL/h/kg of **13** by lowering TPSA value (109 $Å^2$ and 88.9 $Å^2$, respectively). Compound **13** was identified as a potent renin inhibitor with favorable PK profiles in rats and monkeys. It exhibited potent and long-lasting PRA inhibition in cynomolgus monkeys.

Experimental Section

General. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-300 (300 MHz) or Bruker AV-600 (600 MHz) or Varian VNMRS-400 (400 MHz) spectrometer, and are reported in parts per million (δ) relative to tetramethylsilane (TMS: δ 0.0 ppm). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublet, brs = broad singlet), and coupling constants (*J*, Hz). Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. Column chromatography was performed using Merck silica gel 60 (70–230 mesh). Basic silica gel column chromatography was performed using Chromatorex NH-DM 1020 (100–200 mesh, Fuji Silysia Chemical, Ltd.). Thin-layer chromatography (TLC) was performed on Merck silica gel plates 60F254. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mmφ x 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes

were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmφ x 50 mm), eluting at 25 mL/min with a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). UV detection was at 220 nm. Purity data were collected by a HPLC with Corona CAD(Charged Aerosol Detector) detector. The column was L-column 2 ODS (30 mm x 2.0 mm I.D., CERI, Japan) or Acquity UPLC BEH C18 (50 mm x 2.1 mm I.D., Waters, MA, USA) with a temperature of 50 ºC and a flow rate of 0.5 mL/min. Mobile phase A and B under a neutral condition were a mixture of 5 mmol/L Ammonium acetate and 5 mmol/L ammonium acetate in 98% acetonitrile, respectively. Mobile phase A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min or 5% to 99% over 3.2 min, 99% over the next 0.4 min.. Or purity measurements were carried out using a Shimadzu UFLC system employing the following conditions: column; L-column2 ODS (3.0 mmIDx30 mmL, 2 μm); mobile phase; MeCN/H_2O/TFA = 5:95:0.1 (0 min) → 90:10:0.1 (2.00 min) → 90:10:0.1 (3.3 min); flow rate; 1.2 ml/min; temperature; 40 ºC; detection; UV 220 mm. Unless otherwise noted, the purity of all compounds was ≥95%. Elemental analyses, HRMS and optical rotations were performed by Takeda Analytical Research Laboratories, Ltd. Melting points were determined on Yanagimoto micro melting-point apparatus and are uncorrected.

2-*tert*-Butyl-4-(3-methoxypropoxy)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]pyrimidine-5-carboxamide dihydrochloride (**4**). A solution of **25** (128 mg, 0.206 mmol) in 1 M hydrogen chloride in ethyl acetate (3 mL) was stirred at room temperature for 2 h and then concentrated in vacuo. The residue was subjected to preparative HPLC, and a fraction was concentrated in vacuo. The residue was dissolved in ethyl acetate, and to the solution 1 M hydrogen chloride in ethyl acetate was added. The precipitations was filtered to give the object product (46.9 mg, 38%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.71 (3H, brs), 0.80–1.06 (3H, m), 1.13–1.39 (10H, m), 1.39–1.58 (1H, m), 1.72–2.11 (5H, m), 2.74–3.08 (4H, m), 3.08–3.32 (6H, m), 3.32–3.62 (10H, m), 4.48 (2H, brs), 8.34–8.63 (1H, m), 9.18 (1H, brs), 9.41 (1H, brs). MS (ESI+) *m/z* 520.0 $(M+1)^+$. Anal. Calcd for $C_{27}H_{45}N_5O_5 \cdot 2HCl \cdot 0.5H_2O$: C, 53.90; H, 8.04; N, 11.64. Found: C, 54.08; H, 8.22; N, 11.59. $[\alpha]^{25}_D$ +2.1º (c 0.2705, $CHCl_3$).

2-*tert*-Butyl-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-4-(pentylamino)pyrimidine-5-carboxamide dihydrochloride (**5**). A solution of **27** (58.1 mg, 0.09 mmol) in 1 M hydrogen chloride in ethyl acetate (3 mL) was stirred at room temperature for 2 h and then concentrated in vacuo to give the object product (54.2 mg, 98%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.63–0.99 (9H, m), 1.15–1.45 (13H, m), 1.47–1.71 (2H, m), 1.71–1.98 (1H, m), 1.98–2.16 (3H, m), 2.94 (1H, brs), 3.06–3.29 (5H, m), 3.59–3.68 (6H, m), 3.41–3.59 (6H, m), 4.32 (1H, brs), 8.21 (1H, brs), 9.38 (1H, brs). MS (ESI+) *m/z* 517.1 $(M+1)^+$. Anal. Calcd for $C_{28}H_{48}N_6O_3 \cdot 2HCl \cdot 1.4H_2O$: C, 54.70; H, 8.66; N, 13.67. Found: C, 55.00; H, 8.75; N, 13.38. $[\alpha]^{25}_D$ +5.7º (c 0.4250, $CHCl_3$).

3-*tert*-Butyl-1-(4-methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-pyrazole-5-carboxamide hydrochloride (**7a**). A solution of **39b** (400 mg, 0.66 mmol) in ethyl acetate (1 mL), 4 M hydrogen chloride-ethyl acetate solution (2 mL) was added, and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was dried under reduced pressure to give the object product as white solid (340 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.47–1.05 (6H, m), 1.23 (9H, s), 1.36–1.51 (2H, m), 1.61–2.44 (6H, m), 2.77–2.99 (1H, m), 3.01–3.69 (18H, m), 3.91–4.10 (2H, m), 4.11–4.37 (1H, m), 5.63–7.29 (2H, m), 9.23–10.10 (1H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.7 (2C), 26.1, 26.8, 28.4, 30.3 (3C), 31.6, 34.7, 41.6, 43.5, 44.0, 45.4, 49.4, 51.5, 52.0, 55.5, 57.7, 65.9 (2C), 71.2, 101.8, 136.2, 159.2, 163.4, 171.9. MS (ESI+) *m/z* 506.0 $(M+1)^+$. Anal. Calcd for $C_{27}H_{47}N_5O_4 \cdot HCl \cdot H_2O$: C, 57.89; H, 9.00; N, 12.50. Found: C, 57.61; H, 8.98; N, 12.25. $[\alpha]^{25}_{D}$ –3.1° (c 0.2665, $CHCl_3$).

3-*tert*-Butyl-1-(4-methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-pyrazole-5-carboxamide hydrochloride (**7b**). A solution of **39b** (400 mg, 0.64 mmol) in ethyl acetate (1 mL), 4 M hydrogen chloride-ethyl acetate solution (2 mL) was added, and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was dried under reduced pressure to give the object product as a white solid (350 mg, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.61–1.04 (6H, m), 1.39–1.62 (2H, m), 1.66–2.45 (5H, m), 2.82–3.83 (20H, m), 4.01–4.47 (3H, m), 6.76–7.19 (1H, m), 7.24–7.53 (3H, m), 7.72–7.98 (2H, m), 8.88–10.23 (2H, m). ^{13}C NMR (151 MHz, DMSO-d_6) δ 19.6, 20.0, 21.0, 26.1, 26.8, 28.3, 30.4,

34.7, 41.6, 43.5, 44.1, 45.3, 48.2, 49.9, 51.7, 52.2, 55.7, 57.8, 65.9, 66.1, 71.2, 102.9, 125.2, 127.6, 127.7, 128.5, 128.6, 132.6, 136.9, 137.7, 148.8, 162.9, 168.6, 171.9. (observed complexity is due to rotameric effects at the experimental temperature). MS (ESI+) *m/z* 526.0 $(M+1)^+$. Anal. Calcd for $C_{29}H_{43}N_5O_4 \cdot HCl \cdot 0.5H_2O$: C, 60.98; H, 7.94; N, 12.26. Found: C, 61.08; H, 8.06; N, 12.26. $[\alpha]^{25}_D$ +0.5º (c 0.2750, $CHCl_3$).

2-*tert*-Butyl-1-(4-methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-imidazole-5-carboxamide dihydrochloride (**8a**). A solution of **45a** (79 mg, 0.13 mmol) in 1 M hydrogen chloride in ethyl acetate (3 mL) was stirred at room temperature for 2 h and then concentrated in vacuo to give the object product (75 mg, 99%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (5H, dd, *J* = 6.4, 2.7 Hz), 1.34–.63 (9H, m), 1.69 (2H, brs), 1.93–2.09 (2H, m), 2.09–2.38 (2H, m), 2.73–2.99 (4H, m), 3.05 (1H, brs), 3.13–3.35 (12H, m), 3.51–3.90 (9H, m), 7.75 (1H, brs), 9.52 (2H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.0, 20.1, 21.0, 25.8, 26.8, 28.4 (3C), 33.5, 34.4, 41.6, 42.5, 43.6, 45.4, 45.7, 50.4, 50.9, 52.0, 57.8, 65.9, 66.0, 70.9, 127.0, 153.6, 160.9, 168.7, 171.9 (the minor rotamer's signals are omitted). MS (ESI+) *m/z* 506.0 $(M+1)^+$. Anal. Calcd for $C_{27}H_{47}N_5O_4 \cdot 2HCl \cdot 3.5H_2O$: C, 50.54; H, 8.80; N, 10.91. Found: C, 50.70; H, 8.44; N, 10.47. ESI-HRMS calcd for $C_{27}H_{47}N_5O_4$ m/z 506.3701 (M+H), Found 506.3636 (M+H). $[\alpha]^{25}_D$ +11.5º (c 0.2250, $CHCl_3$).

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-2-phenyl-1*H*-imidazole-5-carboxamide dihydrochloride (**8b**). A solution of **45b** (90.0 mg, 0.14 mmol) in 1 M hydrogen chloride in ethyl acetate (3 mL) was stirred at room temperature for 2 h and then concentrated in vacuo. The residue

was triturated in ethyl acetate-diethyl ether (1:1), and insoluble materials were filtered to give the object product (65.4 mg, 76%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (6H, dd, J = 6.3, 2.0 Hz), 1.21–1.46 (2H, m), 1.50–1.75 (2H, m), 1.90–2.29 (3H, m), 2.77–2.98 (2H, m), 3.06–3.26 (5H, m), 3.26–3.66 (14H, m), 4.05–4.31 (2H, m), 4.76 (1H, brs), 7.55–7.76 (3H, m), 7.76–7.88 (2H, m), 8.14 (1H, brs), 9.54–9.75 (2H, m). ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.0 (2C), 25.6, 26.6, 26.8, 34.4, 41.6 (2C), 43.6, 44.6, 45.4, 45.7, 48.4, 52.1, 57.8, 65.9, 66.0, 70.7, 126.8, 128.9, 129.0 (2C), 129.8 (2C), 130.0, 131.7, 146.1, 160.6, 168.7 (the minor rotamer's signals are omitted). MS (ESI+) *m/z* 526.0 $(M+1)^+$. Anal. Calcd for $C_{29}H_{43}N_5O_4 \cdot 2HCl \cdot 2H_2O$: C, 54.88; H, 7.73; N, 11.11. Found: C, 55.24; H, 7.73; N, 11.11. $[\alpha]^{25}_D$ –3.5° (c 0.3790, $CHCl_3$). HPLC purity: 90%.

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-5-phenyl-1*H*-pyrrole-2-carboxamide hydrochloride (**9**). **49** (150 mg, 0.24 mmol) was dissolved in ethyl acetate (5 mL), 4 M hydrogen chloride in ethyl acetate (5 mL) was added and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure to give the object product (110 mg, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.74–0.95 (6H, m), 1.09–1.27 (2H, m), 1.36–1.53 (2H, m), 1.75–2.30 (3H, m), 2.82–3.69 (20H, m), 4.01–4.20 (2H, m), 4.28–4.59 (1H, m), 6.15 (1H, d, J = 3.8 Hz), 6.50 (1H, d, J = 3.8 Hz), 7.31–7.56 (5H, m), 8.98 (1H, brs), 9.58 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.9, 20.1, 25.9, 26.6, 27.8, 29.6, 34.8, 38.2, 41.6, 43.8, 44.1, 44.2, 45.4, 52.3, 57.7, 65.9, 66.0, 71.0, 108.1, 112.0, 126.5, 127.6, 128.5 (2C), 128.9 (2C), 132.3, 137.4, 165.0,

168.8. MS (ESI+) *m/z* 525.4 $(M+1)^+$. Anal. Calcd for $C_{29}H_{45}N_5O_4 \cdot 1HCl \cdot 2H_2O$: C, 60.34; H, 8.27; N, 9.38. Found: C, 60.65; H, 8.03; N, 9.65. $[\alpha]^{25}_D$ –4.5º (c 0.1255, $CHCl_3$).

5-(4-Methoxybutyl)-*N*-(2-methylpropyl)-N-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1-phenyl-1*H*-imidazole-4-carboxamide dihydrochloride (**10a**). A solution of **53a** (180 mg, 0.288 mmol) in 1 M hydrogen chloride in ethyl acetate (4 mL) was stirred at room temperature for 2 h and then concentrated in vacuo to give the object product (170 mg, 99%) as white solid.. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.65–0.99 (6H, m), 1.29 (4H, brs), 1.95–2.09 (2H, m), 2.09–2.38 (2H, m), 2.57–2.87 (2H, m), 2.90–3.05 (2H, m), 3.09 (3H, s), 3.17–3.44 (4H, m), 3.44–3.66 (6H, m), 3.66–4.04 (6H, m), 7.38–7.76 (5H, m), 8.00–8.38 (1H, m), 9.22–9.49 (2H, m). ^{13}C NMR (151 MHz, DMSO-d_6) δ 19.4, 19.5, 20.1, 20.3, 21.0, 22.4, 24.4, 27.2, 28.1, 28.5, 30.6, 34.4, 34.9, 38.2, 41.6, 42.5, 43.5, 44.1, 44.9, 45.4, 48.2, 51.7, 52.2, 55.6, 57.7, 63.1, 65.9, 66.1, 71.0, 126.4, 126.7, 129.6, 130.1, 133.7, 134.1, 134.4, 135.8, 136.2, 162.4, 168.9, 171.9 (observed complexity is due to rotameric effects at the experimental temperature). MS (ESI+) *m/z* 525.9 $(M+1)^+$ Anal. Calcd for $C_{29}H_{43}N_5O_4 \cdot 2HCl \cdot H_2O$: C, 56.49; H, 7.68; N, 11.36. Found: C, 56.64; H, 7.74; N, 11.16. $[\alpha]^{25}_D$ –8.5º (c 0.3815, $CHCl_3$).

5-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1-phenyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**10b**). **53b** (550 mg, 0.88 mmol) was dissolved in 2 M hydrogen chloride in ethyl acetate (2 mL), and the mixture was stirred at room temperature for 15 h and concentrated under reduced pressure. The residue was triturated with ethyl acetate-hexane. The precipitate was collected by filtration to give the object product as white powder (526 mg, 91%). ^{1}H

NMR (300 MHz, $CDCl_3$) δ 0.85 (6H, brs), 1.34 (4H, brs), 1.89 (2H, brs), 2.74 (2H, d, *J* = 7.5 Hz), 2.96 (1H, brs), 3.10 (3H, s), 3.11–3.17 (2H, m), 3.17–3.39 (5H, m), 5.47 (5H, d, *J* = 4.0 Hz), 3.57 (5H, d, *J* = 16.2Hz), 7.42–7.64 (5H, m), 7.79 (1H, s), 9.00 (1H, brs), 9.36 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 19.9 (2C), 24.0, 24.7, 27.0, 28.4, 34.7, 41.6, 42.5, 43.8, 44.1, 45.4, 51.8, 57.7, 63.1, 65.9, 66.0, 71.0, 116.0, 125.6 (2C), 128.4, 129.2, 129.2, 138.1, 139.0, 143.2, 166.2, 172.2 (the minor rotamer's signals are omitted). Anal. Calcd for $C_{29}H_{43}N_5O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 55.67; H, 7.73; N, 11.19. Found: C, 55.32; H, 7.73; N, 11.19. $[\alpha]^{25}_D$ –13.5º (c 0.2775, $CHCl_3$). MS (ESI+) *m/z* 526.3 $(M+1)^+$.

5-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide hydrochloride (**10c**). **53c** (235 mg, 0.38 mmol) was dissolved in ethyl acetate (0.5 mL), 4 M hydrogen chloride-ethyl acetate solution (0.5 mL) was added, and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was dried under reduced pressure to give the object product as a white solid (200 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.71–0.99 (6H, m), 1.22–1.54 (4H, m), 1.86–2.47 (4H, m), 2.68–3.83 (21H, m), 4.07–4.74 (1H, m), 7.51–7.78 (5H, m), 9.69 (2H, brs). ^{13}C NMR (151 MHz, DMSO-d_6) δ 19.4, 19.5, 20.0, 20.1, 21.0, 22.2, 24.3, 27.1, 27.3, 28.2, 28.5, 31.3, 34.9, 35.0, 41.6, 41.7, 43.7, 44.2, 44.9, 45.3, 48.2, 51.9, 52.3, 54.8, 57.7, 65.9, 66.0, 66.1, 70.9, 71.0, 125.7, 125.8, 129.7, 130.1, 135.4, 135.5, 139.5, 139.7, 139.8, 140.4, 163.1, 163.5, 168.8, 168.9, 171.9. (observed complexity is due to rotameric effects at the experimental temperature). Anal. Calcd for $C_{28}H_{42}N_6O_4 \cdot HCl \cdot H_2O$: C, 57.87; H,

7.80; N, 14.46. Found: C, 58.20; H, 7.90; N, 14.14. ESI-HRMS calcd for $C_{28}H_{42}N_6O_4$ m/z 527.3340 (M+H), Found 527.3323 (M+H). $[\alpha]^{25}_D$ +14.6º (c 0.1535, $CHCl_3$).

4-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-5-phenyl-4*H*-1,2,4-triazole-3-carboxamide dihydrochloride (**11**). **57** (112 mg, 0.17 mmol) was dissolved in 4 M hydrogen chloride-ethyl acetate (3 mL), and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated, subjected to reversed-phase preparative HPLC and the eluted fraction was concentrated under reduced pressure. The residual aqueous layer was neutralized with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate, 4 M hydrogen chloride-ethyl acetate (1 mL) was added, and the solvent was evaporated under reduced pressure to give the object product as a white solid (71 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.74–0.99 (6H, m), 1.19–1.41 (2H, m), 1.49–1.67 (2H, m), 1.82–2.21 (2H, m), 2.25–2.47 (1H, m), 2.86–3.71 (20H, m), 4.01–4.64 (3H, m), 7.46–7.86 (5H, m), 8.95–9.90 (2H, m). MS (ESI+) *m/z* 527.0 $(M+1)^+$. ESI-HRMS calcd for $C_{28}H_{42}N_6O_4$ m/z 527.3340 (M+H), Found 527.3316 (M+H). $[\alpha]^{25}_D$ +19.6º (c 0.6200, $CHCl_3$). HPLC purity: 99.4%.

3-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]imidazo[1,2-*a*]pyridine-2-carboxamide dihydrochloride (**12**). **62a** (102 mg, 0.17 mmol) was dissolved in 4 M hydrogen chloride in ethyl acetate (3 mL), and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure to give the object product as a white solid (85 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61–1.03 (6H, m), 1.49–1.72 (4H, m), 1.80–2.18

(3H, m), 2.23–2.46 (1H, m), 2.69–4.60 (23H, m), 7.31–7.42 (1H, m), 7.67–7.88 (2H, m), 8.63–8.80 (1H, m), 9.32–9.73 (2H, m). MS (ESI+) *m/z* 500.7 $(M+1)^+$. Anal. Calcd for $C_{27}H_{41}N_5O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 54.09; H, 7.73; N, 11.68. Found: C, 53.98; H, 7.84; N, 11.53. $[\alpha]^{25}_D$ +22.4º (c 0.2650, $CHCl_3$).

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide dihydrochloride (**13**). **65a** (5.85 g, 9.75 mmol) was dissolved in methanol (20 mL), 4 M hydrogen chloride in ethyl acetate (20 mL) was added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated, and the residue was diluted with aqueous sodium bicarbonate, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate-methanol (9:1) was concentrated under reduced pressure to give 1-(4-methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide (4.40 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.66–1.04 (6H, m), 1.55–1.73 (2H, m), 1.86–2.40 (5H, m), 2.54–3.00 (4H, m), 3.03–3.44 (8H, m), 3.46–3.76 (9H, m), 4.13–4.46 (3H, m), 7.27–7.40 (2H, m), 7.40–7.47 (1H, m), 7.62–7.82 (1H, m). MS (ESI+) *m/z* 499.9 $(M+1)^+$. 1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide (2.20 g, 4.4 mmol) was dissolved in ethyl acetate (20 mL), 4 M hydrogen chloride in ethyl acetate (5 mL) and methanol (20 mL) were added, and the mixture was stirred at room temperature for 5 min. The reaction mixture was

concentrated under reduced pressure to give the object product as white solid (2.52 g, quant.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.63–0.76 (2H, m), 0.85–1.00 (4H, m), 1.40–1.60 (2H, m), 1.68–1.89 (2H, m), 1.93–2.17 (2H, m), 2.20–2.44 (2H, m), 2.81–3.81 (20H, m), 4.19–4.39 (3H, m), 7.23–7.46 (2H, m), 7.57–7.81 (2H, m), 8.38–9.77 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6, 333K) δ 19.5, 19.2, 25.9, 26.1, 26.8, 26.9, 34.6, 37.7, 42.3, 44.0, 43.8, 51.9, 52.0, 57.3, 62.7, 65.4, 65.7, 71.0, 110.6, 119.4, 122.3, 123.4, 134.0, 140.5, 145.1, 161.9, 168.6. MS (ESI+) *m/z* 499.9 $(M+1)^+$. Anal. Calcd for $C_{27}H_{41}N_5O_4 \cdot 2HCl \cdot H_2O$: C, 54.91; H, 7.68; N, 11.86. Found: C, 55.16; H, 7.75; N, 11.69. mp 137–140 °C. $[\alpha]^{25}_D$ +14.7º (c 0.2465, $CHCl_3$). ESI-HRMS calcd for $C_{27}H_{41}N_5O_4$ m/z 500.3231 (M+H), Found 500.3211 (M+H).

Compound **14** was prepared following similar procedures to the synthesis of compound **12**.

3-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carboxamide dihydrochloride (**14**). Yield: 97%, a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.64–0.99 (6H, m), 1.44–1.61 (4H, m), 1.79–2.10 (7H, m), 2.59–2.68 (2H, m), 2.82–3.80 (22H, m), 3.95–4.53 (3H, m), 9.15–9.90 (2H, m), 10.38 (1H, brs). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 13.9, 17.5, 19.4, 20.1, 20.8, 21.9, 24.4, 27.1, 28.4, 30.3, 41.6, 42.6, 43.1, 43.5, 44.8, 45.4, 48.1, 51.5, 57.8, 65.9, 66.1, 71.2, 121.6, 128.4, 130.4, 139.3, 168.8.MS (ESI+) *m/z* 504.4 $(M+1)^+$. Anal. Calcd for $C_{27}H_{47}N_5O_4 \cdot 2HCl \cdot 2.5H_2O$: C, 52.17; H, 8.43; N, 11.27. Found: C, 52.33; H, 8.45; N, 11.09.

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-indole-2-carboxamide hydrochloride (**15**). **71** (210 mg, 0.85 mmol), **35** (270 mg, 0.73 mmol) and DIEA (560 μL) were dissolved in 1,2-dichloroethane (10 mL), TCFH (360 mg, 0.85 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated, and the residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate–hexane (0:10 – 10:0) was concentrated under reduced pressure to give *tert*-butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-1*H*-indol-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate (83 mg, 19%). *tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-1*H*-indol-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate (83 mg, 0.14 mmol) was dissolved in 4 M hydrogen chloride in ethyl acetate (3 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated to give the object product (67 mg, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.71–1.03 (6H, m), 1.34–1.54 (2H, m), 1.58–1.77 (2H, m), 1.85–2.35 (5H, m), 2.65–4.57 (12H, m), 6.72 (1H, brs), 7.02–7.13 (1H, m), 7.18–7.29 (1H, m), 7.58 (2H, dd, *J* = 19.8, 8.1 Hz), 8.75–10.06 (2H, m). MS (ESI+) *m/z* 499.0 $(M+1)^+$. HPLC purity: 87%.

Compounds **16**–**19** were prepared following similar procedures to the synthesis of compound **12**.

***N*-(2-Methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide dihydrochloride** (**16**). Yield: 99%, a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.69–0.98 (6H, m), 1.82–2.16 (2H, m), 2.21–2.48 (1H, m),

2.82–5.34 (17H, m), 7.24–7.44 (2H, m), 7.55–7.33 (2H, m), 8.73–9.97 (3H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 20.0, 20.2, 27.2, 35.1, 41.5, 42.5, 43.6, 44.6, 45.3, 51.7, 52.8, 65.9, 66.0, 115.8, 123.6, 123.8, 136.8, 144.9, 145.5, 160.5, 160.6, 168.9. MS (ESI+) *m/z* 414.0 $(M+1)^+$. Anal. Calcd for $C_{22}H_{31}N_5O_3 \cdot 2HCl \cdot 0.6H_2O$: C, 53.14; H, 6.93; N, 14.08. Found: C, 52.88; H, 7.14; N, 14.34. $[\alpha]^{25}_D$ +17.4º (c 1.0000, MeOH).

1-Methyl-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide dihydrochloride (**17**). Yield: 98%, a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61–1.00 (6H, m), 1.69–2.45 (3H, m), 2.82–3.14 (2H, m), 3.19–3.75 (14H, m), 3.75–3.93 (3H, m), 4.21–4.43 (1H, m), 7.26–7.47 (2H, m), 7.61–7.76 (2H, m), 8.54–9.50 (1H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.4, 20.2, 27.3, 30.8, 31.0, 34.9, 41.6, 43.4, 44.5, 45.3, 48.4, 52.3, 63.1, 65.9, 111.3, 119.1, 123.3, 124.1, 134.3, 139.1, 145.3, 161.1, 168.7. MS (ESI+) *m/z* 428.0 $(M+1)^+$. Anal. Calcd for $C_{23}H_{33}N_5O_3 \cdot 2HCl \cdot 0.9H_2O$: C, 53.47; H, 7.18; N, 13.55. Found: C, 53.29; H, 7.01; N, 13.34. $[\alpha]^{25}_D$ +5.9º (c 1.0000, MeOH).

1-Ethyl-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide dihydrochloride (**18**). Yield: 99%, a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.62–0.98 (6H, m), 1.38 (3H, t, *J* = 7.1 Hz), 1.66–2.42 (4H, m), 2.78–4.44 (16H, m), 7.20–7.44 (2H, m), 7.59–7.79 (2H, m), 8.35–9.35 (1H, m), 9.45–9.72 (1H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 15.3, 19.5, 20.0, 26.9, 27.3, 34.9, 41.6, 42.5, 43.5, 44.5, 45.3, 48.3, 52.3, 63.1, 65.9, 111.1, 119.6, 122.8, 123.9, 133.9, 140.4, 145.1, 161.8, 168.7. MS

(ESI+) *m/z* 442.4 $(M+1)^+$. Anal. Calcd for $C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot H_2O$: C, 54.13; H, 7.38; N, 13.15. Found: C, 54.34; H, 7.47; N, 12.97.

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*)-piperidin-3-yl]-1*H*-benzimidazole-2-carboxamide dihydrochloride (**19**). Yield: 63%, a white powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.68–0.98 (6H, m), 1.42–1.59 (3H, m), 1.72–2.02 (5H, m), 2.08–2.31 (1H, m), 2.69–2.89 (1H, m), 3.02–3.55 (10H, m), 4.05–4.24 (1H, m), 4.27–4.42 (2H, m), 7.30–7.45 (2H, m), 7.73 (1H, t, *J* = 7.0 Hz), 7.78 (1H, t, *J* = 8.3 Hz), 8.61–9.43 (1H, m), 9.56–9.90 (1H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.5, 20.2, 21.2, 24.9, 26.2, 26.5, 27.1, 41.8, 44.1, 45.0, 48.5, 53.0, 57.8, 71.2, 111.4, 119.5, 123.1, 124.1, 134.0, 139.7, 145.0, 161.4. MS (ESI+) *m/z* 487.0 $(M+1)^+$. Anal. Calcd for $C_{22}H_{34}N_4O_2 \cdot 2HCl \cdot 0.1H_2O$: C, 57.29; H, 7.91; N, 12.15. Found: C, 57.03; H, 8.16; N, 12.17. $[\alpha]^{25}_D$ –6.1° (c 1.0000, MeOH).

***N*-[(3*S*,5*R*)-5-(Hydroxymethyl)piperidin-3-yl]-1-(4-methoxybutyl)-*N*-(2-methylpropyl)-1*H*-benzimidazole-2-carboxamide dihydrochloride** (**20**). To a solution of **67** (1.4 g, 2.71 mmol) was dissolved in 10–20% hydrogen chloride in methanol (30 mL) and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure to give the object product as a white solid (1.31 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.60–7.02 (6H, m), 1.42–2.21 (8H, m), 2.42–2.76 (2H, m), 3.04–3.53 (12H, m), 4.09–4.45 (3H, m), 7.25–7.46 (2H, m), 7.62–7.83 (2H, m), 8.55–9.41 (1H, m), 9.48–9.76 (1H, m). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.5, 20.2, 26.2, 26.4, 27.1, 30.1, 35.5, 44.2, 44.2, 44.8, 48.4, 52.7, 57.8, 62.5, 71.2, 111.5, 119.4, 123.2, 124.1, 133.9, 139.6, 145.0, 161.3. MS

(ESI+) *m/z* 417.0 $(M+1)^+$. Anal. Calcd for $C_{23}H_{36}N_4O_3 \cdot 2HCl \cdot 0.4H_2O$: C, 55.62; H, 7.87; N, 11.28. Found: C, 55.84; H, 7.79; N, 11.08. $[\alpha]^{25}_D$ +7.5º (c 1.0000, MeOH).

Compound **21** was prepared following similar procedures to the synthesis of compound **12**.

***N*-[(3*S*,5*R*)-5-(1-Hydroxy-1-methylethyl)piperidin-3-yl]-1-(4-methoxybutyl)-*N*-(2-methylpropyl)-1*H*-benzimidazole-2-carboxamide dihydrochloride** (**21**). Yield: 76%, a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63–1.0 ^{1}H NMR (DMSO-d_6) δ 0.64–0.75 (2H, m), 0.86–0.98 (4H, m), 1.40–1.58 (2H, m), 1.65–1.88 (2H, m), 1.88–2.36 (4H, m), 2.69–3.63 (9H, m), 3.79–3.95 (3H, m), 4.07–4.40 (5H, m), 4.99 (2H, brs), 7.22–7.44 (2H, m), 7.62–7.79 (2H, m), 8.41 (1H, brs), 8.67–8.87 (1H, m), 9.14 (1H, br s). ^{13}C NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 19.6, 20.2, 22.7, 26.1, 26.5, 27.1, 27.6, 29.0, 43.1, 43.4, 44.1, 52.7, 53.3, 57.8, 67.2, 69.1, 71.2, 111.4, 119.6, 123.0, 124.0, 133.8, 139.9, 145.1, 161.5. MS (ESI+) *m/z* 444.9 $(M+1)^+$. Anal. Calcd for $C_{25}H_{40}N_4O_3 \cdot 2HCl \cdot 0.2H_2O$: C, 57.62; H, 8.20; N, 10.75. Found: C, 57.45; H, 8.45; N, 10.61. $[\alpha]^{25}_D$ +4.6º (c 1.0000, MeOH).

Compound **22** was prepared following similar procedures to the synthesis of compound **20**.

1-Ethyl-*N*-[(3*S*,5*R*)-5-(hydroxymethyl)piperidin-3-yl-]-*N*-(2-methylpropyl)-1*H*-benzimidazole-2-carboxamide dihydrochloride (**22**). Yield: 63%, a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.60–0.99 (6H, m), 1.38 (3H, t, *J* = 6.9 Hz), 1.57–2.21 (4H, m), 2.53–2.76 (1H, m), 3.01–3.18 (1H, m), 3.22–3.58 (6H, m), 4.00–4.42 (5H, m), 7.23–7.44 (2H, m), 7.62–7.84 (2H, m), 8.48–9.26 (1H, m), 9.38–9.62 (1H, m). ^{13}C NMR

(151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 15.1, 19.4, 20.2, 27.1, 30.1, 35.6, 44.5, 44.9, 48.3, 52.1, 52.6, 62.5, 111.1, 119.8, 122.8, 123.8, 133.5, 140.5, 145.2, 161.7. MS (ESI+) *m/z* 359.5 $(M+1)^+$. Anal. Calcd for $C_{20}H_{30}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 54.54; H, 7.55; N, 12.72. Found: C, 54.49; H, 7.66; N, 12.68. $[\alpha]^{25}_D$ +8.6º (c 1.0000, MeOH).

(3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-[{[2-*tert*-butyl-4-(3-methoxypropoxy)pyrimidin-5-yl]carbonyl}(2-methylpropyl)amino]piperidine-3-carboxylic acid (**24**). To an ice-cooled solution of 1-*tert*-butyl 3-methyl (3*R*,5*S*)-5-{[(2-*tert*-butyl-4-chloropyrimidin-5-yl)carbonyl](2-methylpropyl)amino}piperidine-1,3-dicarboxylate (**23**) (365 mg, 0.714 mmol) and 3-methoxypropan-1-ol (0.11 mL, 1.123 mmol) in DMF (6 mL) was added NaH (55 mg, 50% in oil, 1.15 mmol). After being stirred for 2 h, the reaction mixture was quenched with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (141 mg, 36%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, brs), 1.33 (9H, s), 1.38 (9H, s), 1.78–1.94 (2H, m), 1.92–2.18 (2H, m), 2.19–3.24 (8H, m), 3.34 (3H, s), 3.47 (2H, t, *J* = 5.2 Hz), 3.55–4.15 (3H, m), 7.95–8.46 (1H, m). MS (ESI+) *m/z* 551.2 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[2-*tert*-butyl-4-(3-methoxypropoxy)pyrimidin-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**25**). A solution of **24** (140 mg, 0.254 mmol), morpholine (0.027 mL, 0.31 mmol), WSC (75 mg, 0.39 mmol), HOBt (20 mg, 0.13 mmol), and triethylamine (0.090

mL, 0.65 mmol) in 1,2-dichloroethane (DCE) (4.0 mL) was stirred at room temperature for 16 h. The mixture was diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (5:95–80:20) was concentrated under reduced pressure to give the object product (128 mg, 81%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6H, brs), 1.30–1.40 (18H, m), 1.78–1.94 (2H, m), 1.91–2.23 (3H, m), 2.19–3.24 (8H, m), 3.35–3.75 (8H, m), 7.99–8.32 (1H, m). MS (ESI+) *m/z* 620.3 $(M+1)^+$.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[{[2-*tert*-butyl-4-(pentylamino)pyrimidin-5-yl]carbonyl}(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (**26**). A mixture of **23** (200 mg, 0.391 mmol), pentan-1-amine (0.091 mL, 0.79 mmol), and DIEA (0.21 mL, 1.18 mmol) in DMF (4.0 mL) was stirred at 80 °C for 7 h. After being cooled to room temperature, the reaction mixture was diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (208 mg, 95%) as pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80–0.87 (7H, m), 1.01 (2H, dd, *J* = 6.2, 3.6 Hz), 1.34–1.50 (18H, m), 1.83–1.99 (1H, m), 2.15–2.48 (6H, m), 2.60 (2H, brs), 2.77–2.96 (4H, m), 3.02 (3H, s), 3.47 (2H, d, *J* = 6.1 Hz), 3.59–3.75 (3H, m), 4.30–4.44 (4H, m), 8.55 (1H, s). MS (ESI+) *m/z* 562.3 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[2-*tert*-butyl-4-(pentylamino)pyrimidin-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**27**). **26**

(130 mg, 0.231 mmol) was dissolved in methanol (4.0 mL), 0.5 M aqueous sodium hydroxide solution (4.0 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the aqueous layer of the mixture was adjusted to pH 5–6 with saturated aqueous ammonium chloride solution, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give (3*R*,5*S*)-1-(*tert*-butoxycarbonyl)-5-[{[2-*tert*-butyl-4-(pentylamino)pyrimidin-5-yl]carbonyl}(2-methylpropyl)amino]piperidine-3-carboxylic acid (128 mg, quant.) as white solid. The obtained carboxylic acid (63 mg, 0.12 mmol), morpholine (0.015 mL, 0.17 mmol), HOBt (9.0 mg, 0.06 mmol) and triethylamine (0.026 mL, 0.19 mmol) were dissolved in DCE (2.0 mL), WSC (35 mg, 0.18 mmol) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was poured into water, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (58.1 mg, 82%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87–0.92 (6H, m), 1.30–1.40 (18H, m), 1.83–1.99 (3H, m), 2.0–2.35 (6H, m), 2.40–2.60 (2H, m), 2.67–2.96 (6H, m), 3.00–3.35 (3H, m), 3.47–3.52 (3H, m), 3.59–3.70 (4H, m), 4.20–4.32 (4H, m), 7.99–8.30 (1H, s). MS (ESI+) *m/z* 617.3 $(M+1)^+$.

3,5-(*Z*)-1-(*tert*-Butoxycarbonyl)piperidine-3,5-dicarboxylic acid (**29**). A mixture of the mono-ester **28** (5.0 g, 16.6 mmol) and K_2CO_3 (6.9 g, 49.9 mmol) in MeOH (80 mL)

and water (20 mL) was stirred under reflux for 10 h. After being cooled to room temperature, the reaction mixture was concentrated in vacuo. The resulting aqueous solution was acidified with 1M HCl aq. to pH = 2 and extracted twice with EtOAc. The combined organic layer was dried over $MgSO_4$, and concentrated in vacuo to afford object product as white powder (4.6 g, 97%). mp 255–256 °C. NMR (300 MHz, DMSO-d_6) δ ppm 1.32–1.48 (1H, m), 1.40 (8H, s), 2.23 (1H, d, J = 13.3 Hz), 2.36 (2H, dd, J = 11.9, 3.6 Hz), 2.62 (3H, bs), 4.11 (2H, bs), 12.49 (2H, bs). Anal. Calcd for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01; N, 5.13. Found: C, 52.94; H, 7.04; N, 5.01.

***tert*-Butyl 2,4-dioxo-3-oxa-7-azabicyclo[3.3.1]nonane-7-carboxylate** (**30**). A mixture of the dicarboxylic acid **29** (2.00 g, 7.32 mmol) in Ac_2O (40 mL) was stirred under reflux for 3 h. After being cooled to room temperature, the reaction mixture was concentrated in vacuo. The volatiles were evaporated as a toluene azeotrope to afford object product as pale brown solid (1.86 g, 99%). NMR (300 MHz, DMSO-d_6) δ ppm 1.34 (9H, s), 1.82–1.98 (2H, m), 3.03 (2H, bs), 3.18 (2H, d, J = 12.9 Hz), 4.18 (2H, d, J = 13.3 Hz).

(3*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)piperidine-3-carboxylic acid (**31**). **$(DHQD)_2AQN$ method**: To a solution of the acid anhydride **30** (400 mg, 1.57 mmol) and $(DHQD)_2AQN$ (420 mg, 0.47 mmol) in Et_2O (60 mL) and THF (20 mL) was added MeOH (0.64 mL, 15.8 mmol) at –40 °C. The reaction mixture was stirred at –40 °C for one day under Ar atmosphere (with rubber balloon), and then poured into 10% citric acid aq. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (eluted with hexane:EtOAc = 50:50–0:100) to afford object

product as pale yellow powder (255 mg, 57%) (95%ee). The ee. value was measured by chiral HPLC using chiralpak AD-H. **Quinidine method**: To a solution of the acid anhydride **30** (102 g, 398 mmol) and quinidine (142 g, 438 mmol) in THF (900 mL) was added dropwise a solution of MeOH (161 mL) in THF (700 mL) over 30 min at –40 °C. The reaction mixture was stirred at –40 °C for 7 h, concentrated in vacuo and then diluted with EtOAc. The solution was washed with 2 M HCl aq.. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 2 M HCl aq. and brine, dried over $MgSO_4$, and concentrated in vacuo to give crude mono-acid **31** as white powder (106 g, 93%) (80%ee). **Optical resolution by way of diastereomeric salt**: A mixture of the crude mono-acid **31** (216 g, 0.75 mol) and (*R*)-(+)-1-phenylethylamine (91.1 g, 0.75 mol) in EtOH (835 mL) was heated at 75 °C. Insoluble materials were removed by filtration, and the filtrate was left at room temperature for 12 h. The generated precipitations were filtered and washed with EtOAc/ hexane (1/1 v/v, 100 mL) and hexane (100 mL) successively to give mono-acid phenylethylamine salt as white powder. To a suspension of the salt thus obtained in water (1.0 L) was added sat. $KHSO_4$ aq. (1.0 L), and the mixture was extracted three times with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give object product as white powder (148 g, 69%) (95%ee). mp 170–172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (9H, s), 1.52 (1H, q, J = 12.2 Hz), 2.24 (1H, d, J = 13.3 Hz), 2.32 - 2.46 (2H, m), 2.65 (2H, bs), 3.63 (3H, s), 4.14 (2H, d, J = 8.7 Hz), 12.54 (1H, bs). MS (ESI+) *m/z*: 288 $(M+1)^+$. Anal. Calcd for $C_{13}H_{21}NO_6$: C, 54.35; H, 7.37; N, 4.88. Found: C, 54.38; H, 7.37; N, 4.88.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(benzyloxy)carbonyl]amino}piperidine-1,3-dicarboxylate (**32**). To a solution of **31** (8.3 g, 28.9 mmol) and Et_3N (4.85 mL, 34.8 mmol) in toluene (450 mL) was added dropwise DPPA (32.2 mL, 149 mmol), and the mixture was stirred at 100 °C for 1 h and then cooled to room temperature. Benzylalcohol (4.5 mL, 43.5 mmol) and Et_3N (20 mL, 143.5 mmol) were added, and the reaction mixture was stirred at 80 °C for additional 3 h. After being cooled to room temperature, the mixture was concentrated in vacuo. The residue was dissolved in EtOAc, and washed with 1 M HCl aq. and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to silica-gel column chromatography (75:25–25:75) to give crude **32** as colorless oil (8.27 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (9H, s), 1.51–1.69 (1H, m), 2.28 (1H, d, *J* = 15.1 Hz), 2.61 (1H, d, *J* = 9.5 Hz), 2.98–3.15 (1H, m), 3.59–3.73 (1H, m), 3.69 (3H, s), 3.97–4.07 (2H, m), 4.71 (1H, s), 5.10 (2H, bs), 7.24–7.40 (5H, m).

***tert*-Butyl (3*S*,5*R*)-3-{[(benzyloxy)carbonyl]amino}-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**33**). To a solution (700 mL) of 1-*tert*-butyl 3-methyl (3*R*,5*S*)-5-{[(benzyloxy)carbonyl]amino}piperidine-1,3-dicarboxylate (**32**) (115 g, 290 mmol) in methanol was added 1 M aqueous sodium hydroxide solution (350 mL, 350 mmol) under ice-cooling, and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to about 1/3 volume, and the residual aqueous solution was washed with ethyl acetate-hexane (1:1, 600 mL). The aqueous layer was neutralized with 1 M hydrochloric acid and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous

magnesium sulfate. The solvent was evaporated under reduced pressure to give the (3*R*,5*S*)-5-{[(benzyloxy)carbonyl]amino}-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (98.5 g, 90%) as colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (1H, brs), 1.40 (9H, s), 2.09 (1H, d, *J* = 12.9 Hz), 2.36–2.52 (3H, m), 3.93–4.09 (2H, m), 5.03 (2H, s), 7.28–7.43 (5H, m), 12.52 (1H, brs). (3*R*,5*S*)-5-{[(Benzyloxy)carbonyl]amino}-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (49.2 g, 130 mmol), morpholine (11.4 mL, 130 mmol), HOBt (10 g, 65.0 mmol) and triethylamine (40 mL, 287 mmol) were dissolved in DMF (250 mL), WSC (30 g, 156 mmol) was added, and the mixture was stirred at room temperature for 4 days. The reaction mixture was poured into water, and the mixture was extracted with ethyl acetate. The extract was washed successively with saturated aqueous sodium hydrogen carbonate solution and saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (62.9 g, quant.) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.69 (2H, brs), 2.04 (1H, s), 2.73 (2H, brs), 2.79–2.96 (1H, m), 3.52–3.65 (6H, m), 3.69 (2H, d, *J* = 4.2 Hz), 3.67 (1H, brs), 4.04 (1H, d, *J* = 11.7 Hz), 5.09 (2H, s), 5.40 (1H, brs), 7.25–7.41 (5H, m).

***tert*-Butyl (3*S*,5*R*)-3-[(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**35**). **33** (58.0 g, 129 mmol) and palladium(II) hydroxide-carbon (5.0 g) were suspended in MeOH (400 mL) and the mixture was stirred under a hydrogen atmosphere at room temperature for 16 h. The palladium catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The obtained residue and acetic acid (8.8 mL, 153 mmol) were dissolved in MeOH (400 mL), 2-methylpropanal

(14.0 mL, 153 mmol) was added, and the mixture was stirred at room temperature for 1 h. Sodium triacetoxyborohydride (40.4 g, 191 mmol) was added to the reaction mixture, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the concentrate was basified with 3.5 M aqueous potassium carbonate solution, and the mixture was extracted with EtOAc. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to basic silica gel column chromatography, and a fraction eluted with EtOAc-hexane (50:50) was concentrated under reduced pressure to give the object product (33.3 g, 71%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, *J* = 6.8 Hz), 1.46 (9H, s), 1.54 (1H, d, *J* = 11.4 Hz), 1.69 (1H, dt, *J* = 13.3, 6.8 Hz), 1.96–2.12 (2H, m), 2.23–2.37 (1H, m), 2.47 (3H, d, *J* = 6.8 Hz), 2.66 (1H, d, *J* = 10.6 Hz), 3.61 (1H, bs), 3.55 (2H, d, *J* = 7.2 Hz), 3.69 (5H, ddd, *J* = 9.7, 5.3, 5.0 Hz), 4.01–4.46 (2H, m).

Compound **34** was prepared following similar procedures to the synthesis of compound **35**.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (34). Yield: 65%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, *J* = 6.8 Hz), 1.22-1.38 (3H, m), 1.46 (9H, s), 1.69 (1H, dt *J* = 13.3, 6.8 Hz), 2.23–2.39 (2H, m), 2.44–2.59 (1H, m), 2.47 (2H, d, *J* = 6.8 Hz), 2.74 (1H, bs), 3.69 (3H, s), 4.18–4.34 (2H, m).

Ethyl 3-*tert*-butyl-1-(4-methoxybutyl)-1*H*-pyrazole-5-carboxylate (**37a**). To a solution of ethyl 3-*tert*-butyl-1*H*-pyrazole-5-carboxylate **36a** (981 mg, 5.0 mmol) and 4-methoxybutyl methanesulfonate (1.37 g, 10.0 mmol) in *N*,*N*-dimethylacetamide (25 mL)

was added cesium carbonate (3.26 g, 10.0 mmol), and the mixture was stirred at 65 °C for 15 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with 10% aqueous citric acid solution and saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (1.15 g, 82%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30 (9H, s), 1.37 (3H, t, *J* = 7.2 Hz), 1.49–1.66 (2H, m), 1.83–1.98 (2H, m), 3.31 (3H, s), 3.38 (2H, t, *J* = 6.6 Hz), 4.32 (2H, q, *J* = 7.3 Hz), 4.52 (2H, t, *J* = 7.2 Hz), 6.66 (1H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 14.3, 26.6, 27.4, 30.5 (3C), 31.9, 51.2, 58.5, 60.7, 72.2, 107.4, 131.8, 159.9, 160.2. HMBC (600/151 MHz, DMSO-d_6): CH_2-N/C5, ^{t}Bu-*H*/C3. MS (ESI+) *m/z* 283.1 $(M+1)^+$.

Methyl 1-(4-methoxybutyl)-3-phenyl-1*H*-pyrazole-5-carboxylate (**37b**). To a solution of **36b** (1.01 g, 5.0 mmol) and 4-methoxybutyl methanesulfonate (1.37 g, 10.0 mmol) in *N*,*N*-dimethylacetamide (25 mL) was added cesium carbonate (3.26 g, 10.0 mmol), and the mixture was stirred at 65 °C for 15 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with 10% aqueous citric acid solution and saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (1.08 g, 75%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.55–1.68 (2H, m), 1.96 (2H, t, *J* =

7.3 Hz), 3.32 (3H, s), 3.40 (2H, t, *J* = 6.6 Hz), 3.90 (3H, s), 4.63 (2H, t, *J* = 7.3 Hz), 7.11 (1H, s), 7.28–7.37 (1H, m), 7.40 (2H, t, *J* = 7.7 Hz), 7.79 (2H, d, *J* = 7.7 Hz). ^{13}C NMR (151 MHz, $CDCl_3$) δ 26.6, 27.4, 51.6, 51.9, 58.6, 72.2, 108.1, 125.5 (2C), 125.5, 128.0, 128.7 (2C), 132.6, 132.8, 149.8, 160.1. HMBC (600/151 MHz, $CDCl_3$): C*H*$_2$-N/C5, Ph-*H*/C3. MS (ESI+) *m/z* 289.0 $(M+1)^+$.

3-*tert*-Butyl-1-(4-methoxybutyl)-1*H*-pyrazole-5-carboxylic acid (**38a**). **37a** (1.15 g, 4.0 mmol) was dissolved in ethanol (8 mL) and water (4 mL), lithium hydroxide monohydrate (252 mg, 6.0 mmol) was added, and the mixture was stirred at 65 °C for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was acidified with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (985 mg, 97%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (9H, s), 1.53–1.66 (2H, m), 1.89 (2H, quin, *J* = 7.5 Hz), 3.32 (3H, s), 3.40 (2H, t, *J* = 6.6 Hz), 4.55 (2H, t, *J* = 7.2 Hz), 6.78 (1H, s), 8.30 (1H, brs). MS (ESI+) *m/z* 255.1 $(M+1)^+$.

1-(4-Methoxybutyl)-3-phenyl-1*H*-pyrazole-5-carboxylic acid (**38b**). **37b** (1.08 g, 3.75 mmol) was dissolved in ethanol (8 mL) and water (4 mL), lithium hydroxide monohydrate (236 mg, 5.63 mmol) was added, and the mixture was stirred at 65 °C for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was acidified with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (930 mg, 91%) as

colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ1.55–1.74 (2H, m), 1.88–2.10 (2H, m), 3.34 (3H, s), 3.44 (2H, t, *J* = 6.2 Hz), 4.66 (2H, t, *J* = 7.2 Hz), 5.93 (1H, brs), 7.21–7.28 (1H, m), 7.28–7.38 (1H, m), 7.38–7.48 (2H, m), 7.81 (2H, d, *J* = 6.8 Hz). MS (ESI+) *m/z* 275.1 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[3-*tert*-butyl-1-(4-methoxybutyl)-1*H*-pyrazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**39a**)**. 38a** (231 mg, 1.0 mmol) was dissolved in DCE (10 mL), **35** (370 mg, 1.0 mmol), DIEA (689 μL, 4.0 mmol) and TCFH (309 mg, 1.1 mmol) were added, and the mixture was stirred at room temperature for 15 h. Aqueous sodium bicarbonate was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The extract was washed with 10% aqueous citric acid solution saturated and brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as a white solid (400 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (6H, m), 1.29 (9H, s), 1.44 (9H, s), 1.51–1.63 (2H, m), 1.76–1.99 (3H, m), 1.95–2.47 (2H, m), 2.49–3.42 (10H, m), 3.43–3.78 (8H, m), 3.86–4.44 (5H, m), 5.91–6.44 (1H, m). MS (ESI+) *m/z* 606.5 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-3-phenyl-1*H*-pyrazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**39b**). **38b** (274 mg, 1.0 mmol) was dissolved in DCE, **35** (370 mg, 1.0 mmol), DIEA (689 μL, 4.0 mmol) and TCFH (309 mg, 1.1 mmol) were added, and the mixture was stirred at room

temperature for 15 h. Aqueous sodium bicarbonate was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The extract was washed with 10% aqueous citric acid solution saturated and brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as a white solid (610 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63–1.13 (6H, m), 1.43 (9H, s), 1.53–1.70 (3H, m), 1.72–2.34 (5H, m), 2.52–2.92 (2H, m), 3.00–3.82 (15H, m), 3.84–4.54 (5H, m), 6.38–7.05 (1H, m), 7.22–7.52 (3H, m), 7.81 (2H, brs). MS (ESI+) *m/z* 625.9 $(M+1)^{+}$.

2-*tert*-Butyl-4-chloro-1*H*-imidazole-5-carbaldehyde (**41a**). A mixture of 2,2-dimethylpropanimidamide hydrochloride (**40**) (2.00 g, 14.6 mmol), *tert*-butyl glycinate hydrochloride (2.45 g, 14.6 mmol), and triethylamine (5.10 mL, 36.6 mmol) in DMF (14 mL) was stirred at 80 °C for 18 h. After being cooled to room temperature, the reaction mixture was diluted with saturated aqueous potassium carbonate solution and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give *tert*-butyl *N*-(2,2-dimethylpropanimidoyl)glycinate (1.71 g, 55%). A solution of *tert*-butyl *N*-(2,2-dimethylpropanimidoyl)glycinate (1.70 g, 7.99 mmol) in 10% trifluoroacetic acid in DCE (20 mL, v/v) was stirred at room temperature for 3 days, and then concentrated in vacuo. The residue was suspended in toluene (20 mL) was added phosphoric trichloride (7.36 mL, 79.0 mmol), and the mixture was stirred at 80 °C for 30 min. DMF (6.12 mL, 79.0 mmol) was added, and the mixture was stirred at 100 °C for additional 12 h. After being cooled

to room temperature, volatiles were evaporated off under reduced pressure. The residue was poured into 4 M aqueous sodium hydroxide solution, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (300 mg, 22%) as pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (9H, s), 9.66 (1H, s), 9.98 (1H, brs). MS (ESI+) *m/z* 187.0 $(M+1)^+$.

2-*tert*-Butyl-4-chloro-1-(4-methoxybutyl)-1*H*-imidazole-5-carbaldehyde (**42a**). To a solution of **41a** (290 mg, 1.55 mmol) and 4-methoxybutyl methanesulfonate (425 mg, 2.33 mmol) in *N*,*N*-dimethylacetamide (5.0 mL) was added cesium carbonate (1.52 g, 4.67 mmol), and the mixture was stirred at 90 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (219 mg, 52%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.67–1.74 (2H, m), 1.77–1.84 (2H, m), 3.34 (3H, s), 3.44 (2H, t, *J* = 6.1 Hz), 4.35–4.45 (2H, m), 9.72 (1H, s). MS (ESI+) *m/z* 273.1 $(M+1)^+$.

4-Chloro-1-(4-methoxybutyl)-2-phenyl-1*H*-imidazole-5-carbaldehyde (**42b**). **41b** (commercially available) (500 mg, 2.42 mmol) and 4-methoxybutyl methanesulfonate (660

mg, 3.62 mmol) in *N*,*N*-dimethylacetamide (10 mL) was added cesium carbonate (2.4 g, 7.4 mmol), and the mixture was stirred at 90 °C for 7 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (5:95–3:7) was concentrated under reduced pressure to give the object product (702 mg, 99%) as pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43–1.58 (2H, m), 1.76–1.88 (2H, m), 3.27 (3H, s), 3.30 (2H, t, *J* = 6.2 Hz), 4.31–4.40 (2H, m), 7.52 (2H, d, *J* = 1.9 Hz), 7.41–7.56 (1H, m), 7.56–7.69 (2H, m), 9.85 (1H, s). MS (ESI+) *m/z* 293.0 $(M+1)^+$.

2-*tert*-Butyl-4-chloro-1-(4-methoxybutyl)-1*H*-imidazole-5-carboxylic acid (**43a**). To a solution of **42a** (215 mg, 0.788 mmol) in *tert*-butanol (8.0 mL) and 2-methyl-2-butene (1.0 mL) was added aqueous solution (4 mL) of sodium chlorite (85 mg, 0.94 mmol) and sodium dihydrogen phosphate (113 mg, 0.942 mmol), and the mixture was stirred at room temperature for 2days. The reaction mixture was adjusted to pH 3, by adding aqueous 1 M hydrogen chloride, and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (233 mg, quant.) as colorless viscous oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.47 (9H, s), 1.67–1.74 (2H, m), 1.77–1.84 (2H, m), 3.34 (3H, s), 3.75 (2H, t, *J* = 6.3 Hz), 4.44–4.72 (2H, m), 10.60 (1H, s). MS (ESI+) *m/z* 289.1 $(M+1)^+$.

4-Chloro-1-(4-methoxybutyl)-2-phenyl-1*H*-imidazole-5-carboxylic acid (**43b**). To a solution of **42b** (790 mg, 2.70 mmol) in *tert*-butanol (15 mL) and 2-methyl-2-butene (1.5 mL) was added aqueous solution (4 mL) of sodium chlorite (300 mg, 3.32 mmol) and sodium dihydrogen phosphate (400 mg, 3.33 mmol), and the mixture was stirred at room temperature for 12 h. The reaction mixture was adjusted to pH 3, by adding aqueous 1 M hydrogen chloride, and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (730 mg, 99%) as pale yellow viscous oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40–1.55 (2H, m), 1.75–1.88 (2H, m), 3.27 (3H, s), 3.28 (2H, t, J = 5.8 Hz), 4.30–4.42 (2H, m), 7.41–7.49 (1H, m), 7.50 (2H, d, J = 1.9 Hz), 7.55–7.70 (2H, m), 10.50 (1H, s). MS (ESI+) m/z 309.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[2-*tert*-butyl-4-chloro-1-(4-methoxybutyl)-1*H*-imidazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**44a**). **43a** (225 mg, 0.779 mmol) and **35** (288 mg, 0.779 mmol) were dissolved in DCE (6 mL), DIEA (0.21 mL, 1.18 mmol) and TCFH (265 mg, 0.944 mmol) was added and the mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) then methanol-ethyl acetate (0:100–15:85) was concentrated under reduced pressure to give the object product (417 mg, 84%) as pale yellow solid. ^{1}H NMR

(300 MHz, DMSO-d_6) δ 0.92 (6H, brs), 1.39–1.47 (18H, m), 1.67–1.84 (2H, m), 1.91–2.23 (3H, m), 2.19–3.24 (8H, m), 3.35–3.75 (8H, m), 4.44–4.70 (2H, m). MS (ESI+) *m/z* 640.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[4-chloro-1-(4-methoxybutyl)-2-phenyl-1*H*-imidazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**44b**). **43b** (309 mg, 1.0 mmol), **35** (370 mg, 1.0 mmol) and DIEA (270 μL, 1.55 mmol) were dissolved in DCE (8.0 mL), TCFH (340 mg, 1.21 mmol) was added and the mixture was stirred at room temperature for 4days. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (425 mg, 64%) as pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6H, brs), 1.39–1.47 (9H, m), 1.67–1.84 (2H, m), 1.91–2.23 (3H, m), 2.19–3.24 (8H, m), 3.35–3.75 (8H, m), 4.44–4.70 (4H, m), 7.40–7.71 (5H, m). MS (ESI+) *m/z* 660.2 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[2-*tert*-butyl-1-(4-methoxybutyl)-1*H*-imidazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**45a**). **44a** (219 mg, 0.342 mmol), palladium(II) hydroxide-carbon (10 mg) and potassium acetate (170 mg, 1.73 mmol) were suspended in methanol (5.0 ml), and the mixture was stirred under a hydrogen atmosphere at room temperature for 1day. The palladium catalyst was filtered off and the filtrate was concentrated under reduced

pressure. The residue was suspended in water, and the suspension was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (50:50–0:100) then methanol-ethyl acetate (0:100 – 20:80) was concentrated under reduced pressure to give the object product (79.4 mg, 38%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6H, brs), 1.41–1.47 (18H, m), 1.60–1.86 (2H, m), 1.91–2.20 (3H, m), 2.21–3.22 (8H, m), 3.30–3.80 (8H, m), 4.43–4.72 (2H, m), 8.12 (1H, brs). MS (ESI+) *m/z* 606.2 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-2-phenyl-1*H*-imidazol-5-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**45b**). **44b** (200 mg, 0.303 mmol), palladium(II) hydroxide-carbon (20 mg) and potassium acetate (30 mg, 0.305 mmol) were suspended in methanol (10 mL), and the mixture was stirred under a hydrogen atmosphere at room temperature for 1 day. The palladium catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was suspended in water, and the suspension was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–0:100) then methanol-ethyl acetate (0:100–20:80) was concentrated under reduced pressure to give the object product (90.2 mg, 48%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (6H, brs), 1.35–1.52 (9H, m), 1.62–1.76 (2H, m), 1.93–2.13 (3H, m), 2.18–3.22 (8H, m), 3.25–

3.70 (8H, m), 4.32–4.69 (4H, m), 7.39–7.69 (5H, m), 8.11 (1H, brs). MS (ESI+) *m/z* 626.1 $(M+1)^+$.

Ethyl 1-(4-methoxybutyl)-5-phenyl-1*H*-pyrrole-2-carboxylate (**47**). Ethyl 5-phenyl-1*H*-pyrrole-2-carboxylate (**46**) (400 mg, 2.0 mmol) and 4-methoxybutyl methanesulfonate (440 mg, 2.4 mmol) were dissolved in DMF (10 mL), cesium carbonate (780 mg, 2.4 mmol) was added and the mixture was stirred at 60 °C for 12 h. The reaction mixture was poured into water and the mixture was extracted with ethyl acetate. The extract was washed with brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (1:9–1:4) was concentrated under reduced pressure to give the object product as a white solid (430 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31–1.47 (2H, m), 1.61–1.76 (2H, m), 3.21–3.23 (5H, m), 3.83 (3H, s), 6.15 (1H, d, J = 3.96 Hz), 7.03 (1H, d, J = 3.96 Hz), 7.34–7.48 (5H, m).

1-(4-Methoxybutyl)-5-phenyl-1*H*-pyrrole-2-carboxylic acid (**48**). **47** (430 mg, 1.5 mmol) was dissolved in methanol (2 mL), 2 M aqueous sodium hydroxide solution (4 mL) was added and the mixture was stirred at 60 °C for 12 h. The reaction mixture was concentrated under reduced pressure, and the aqueous layer of the mixture was adjusted to pH 3 with 6 M hydrochloric acid, and the mixture was extracted with ethyl acetate. The extract was washed with brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give the object product as a solid (420 mg, quant.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11–1.27 (2H, m), 1.44–1.60 (2H, m), 3.01–3.13 (5H,

m), 4.31 (2H, t, *J* = 7.29 Hz), 6.15 (1H, d, *J* = 3.85 Hz), 6.91 (1H, d, *J* = 3.85 Hz), 7.34–7.50 (5H, m), 12.15 (1H, s).

***tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-5-phenyl-1*H*-pyrrol-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**49**). To a mixture of **48** (137 mg, 0.5 mmol), **35** (134 mg, 0.5 mmol), TCFH (168 mg, 0.6 mmol) and DCE (5 mL) was added DIEA (0.449 mL) and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, diluted with water, and partitioned. The aqueous layer was extracted with ethyl acetate, the organic layers were combined and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (1:1–1:0) was concentrated under reduced pressure to give the object product as a white solid (150 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85–1.02 (6H, m), 1.21–1.38 (2H, m), 1.41–1.65 (13H, m), 1.80–1.96 (1H, m), 2.08–2.33 (1H, m), 2.61–2.86 (5H, m), 2.93–3.23 (2H, m), 3.41–3.77 (10H, m), 4.02–4.44 (5H, m), 6.11 (1H, d, *J* = 3.77 Hz), 6.49 (1H, d, *J* = 3.77 Hz), 7.32–7.47 (5H, m). MS (ESI+) *m/z* 625.0 $(M+1)^+$.

Methyl 7-methoxy-3-oxoheptanoate (**51**). δ-valerolactone (**50**) (25 g, 0.25 mol) and trimethyl orthoformate (53.1 g, 0.5 mol) were dissolved in methanol (500 mL). Sulfuric acid (catalytic amount) was added to the mixture, and the resulting mixture was sttired at 65 °C for 15 h. The solvent was concentrated under reduced pressure. 8 M aqueous sodium hydroxide solution (50 mL) was added and the mixture was stirred at 95 °C for 2 h. After cooling to room temperature, the reaction mixture was acidified with hydrochloric

acid and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give 5-methoxypentanic acid (26.8 g, 81%) as colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54–1.81 (4H, m), 2.33–2.44 (2H, m), 3.34 (3H, s), 3.40 (2H, t, J = 5.9 Hz), 11.11 (1H, brs). A solution of 5-methoxypentanic acid (26.4 g, 200 mmol) in THF (250 mL) was cooled 0–5 °C, and oxalyl chloride (50.8 g, 400 mmol) was added dropwise over 30 min. The mixture was stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure. A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (28.8 g, 200 mmol) in dichloromethane (300 mL) was cooled to 0–5 °C, pyridine (31.6 g, 400 mmol) was added, and 5-methoxypentanoyl chloride/dichloromethane solution (20 mL) was added dropwise over 30 min. The mixture was stirred at 0–5 °C for 1 h. The reaction mixture was poured into 0.5 M hydrochloric acid (300 mL), washed with water, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was dissolved in methanol (450 mL) and the mixture was stirred under reflux for 15 h. The solvent was concentrated under reduced pressure, and the residue was distilled under reduced pressure. The fraction distilled under reduced pressure of 0.3 mmHg at 90–92 °C was collected to give the object product (27.4 g, 89%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52–1.74 (4H, m), 2.57 (2H, t, J = 7.1 Hz), 3.31 (3H, s), 3.37 (2H, t, J = 6.0 Hz), 3.45 (2H, s), 3.70–3.75 (3H, m).

2-Chloro-5-(4-methoxybutyl)-1-phenyl-1*H*-imidazole-4-carboxylic acid (**52a**). To a solution of **51** (5.00 g, 26.6 mmol) and 4-(acetylamino)benzenesulfonyl azide (7.02 g, 29.2 mmol) in acetonitrile (100 mL) was added triethylamine (11.1 mL, 79.6 mmol) and the

mixture was stirred at room temperature for 2 days. Insoluble materials were filtered through a pad of celite, and the filtrate was concentrated under reduced pressure. The residue was suspended in diethyl ether and the insoluble material was filtered off. The filtrate was concentrated under reduced pressure to give Methyl 2-diazo-7-methoxy-3-oxoheptanoate (6.93 g, quant.) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55–1.78 (4H, m), 2.83–2.92 (2H, m), 3.32 (3H, s), 3.39 (2H, t, *J* = 6.2 Hz), 3.84 (3H, s). Methyl 2-diazo-7-methoxy-3-oxoheptanoate (6.93 g, 26.5 mmol) and 1-phenylurea (5.41 g, 39.7 mmol) were suspended in toluene-DCE (30 mL – 30 mL), rhodium acetate dimer (230 mg, 0.52 mmol) was added and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, trifluoroacetic acid (7.5 mL) was added and the reaction mixture was stirred at room temperature for 1 day. The reaction mixture was concentrated under reduced pressure, and the residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (15:85–100:0) was concentrated under reduced pressure to give methyl 5-(4-methoxybutyl)-2-oxo-1-phenyl-2,3-dihydro-1*H*-imidazole-4-carboxylate (7.40 g, 92%) as green solid. Obtained methyl 5-(4-methoxybutyl)-2-oxo-1-phenyl-2,3-dihydro-1*H*-imidazole-4-carboxylate (1.50 g, 4.93 mmol) was dissolved in phosphorus oxychloride (18 mL) and the mixture was stirred at 100 °C for 10 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was diluted with saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography,

and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the methyl 2-chloro-5-(4-methoxybutyl)-1-phenyl-1*H*-imidazole-4-carboxylate (454 mg, 29%) as brown viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37–1.53 (4H, m), 2.72–2.88 (2H, m), 3.16–3.33 (5H, m), 3.92 (3H, s), 7.17–7.33 (2H, m), 7.51–7.57 (3H, m). MS (ESI+) *m/z* 323.1 $(M+1)^+$. Methyl 2-chloro-5-(4-methoxybutyl)-1-phenyl-1*H*-imidazole-4-carboxylate (450 mg, 1.39 mmol) was dissolved in methanol (5.0 mL), 1 M aqueous sodium hydroxide solution (4.2 mL) was added and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was acidified with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give the object product (372 mg, 87%) as yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29–1.55 (4H, m), 2.72–2.86 (2H, m), 3.02–3.39 (5H, m), 7.15–7.33 (2H, m), 7.50–7.59 (3H, m), 10.56 (1H, brs). MS (ESI+) *m/z* 309.1 $(M+1)^+$.

5-(4-Methoxybutyl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**52b**). A solution of **51** (526 mg, 2.8 mmol) and *N*,*N*-dimethylformamide dimethylacetal (0.45 mL, 3.4 mmol) in toluene (5 mL) was stirred at 80 °C for 5 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethanol (5 mL). Phenylhydrazine (0.41 mL, 4.2 mmol) was added and the mixture was stirred at 80 °C for 15 h. The reaction mixture was concentrated under reduced pressure, water was added to the residue, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

The obtained residue was subjected to silica gel chromatography, and a fraction eluted with hexane - ethyl acetate-hexane (1:9–1:3) was concentrated under reduced pressure to give methyl 5-(4-methoxybutyl)-1-phenyl-1*H*-pyrazole-4-carboxylate as brown oil (620 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44–1.54 (2H, m), 1.57–1.68 (2H, m), 2.96 (2H, d, *J* = 8.0 Hz), 3.25 (3H, s), 3.27–3.31 (2H, m), 3.86 (3H, s), 7.45–7.57 (3H, m), 8.01 (1H, s). MS (ESI+) *m/z* 289.1 $(M+1)^+$. Methyl 5-(4-methoxybutyl)-1-phenyl-1*H*-pyrazole-4-carboxylate (615 mg, 2.1 mmol) was dissolved in methanol (5 mL), 1 M aqueous sodium hydroxide solution (5 mL) was added, and the mixture was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure, and the residue was ice-cooled, neutralized with 1 M hydrochloric acid, and the precipitate was collected by filtration to give the object product as brown oil (585 mg, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43–1.74 (4H, m), 2.89–3.09 (2H, m), 3.17–3.36 (5H, m), 7.34–7.60 (5H, m), 8.10 (1H, s). MS (ESI+) *m/z* 275.2 $(M+1)^+$.

5-(4-Methoxybutyl)-1-phenyl-1*H*-1,2,3-triazole-4-carboxylic acid (**52c**). A solution of sodium hydride (60% in oil, 2.0 g, 50 mmol) in DMF (50 mL) was cooled to 0–5 °C, **51** (9.4 g, 50 mmol) was added, and the mixture was stirred at 0–5 °C for 30 min. Phenylazide (6.0 g, 50 mmol) was added, and the mixture was stirred at room temperature for 15 h. The solvent was concentrated under reduced pressure, and methanol (100 mL) was added to the residue and 4 M aqueous sodium hydroxide solution (20 mL) was further added. The mixture was stirred at 60 °C for 1 h. The solvent was evaporated under reduced pressure and water (100 mL) was added to the residue. 6 M Hydrochloric acid was added for neutralization and the mixture was extracted with ethyl acetate (100 mL×2).

The extract was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (7.2 g, 52%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46–1.72 (4H, m), 2.98–3.10 (2H, m), 3.27 (3H, s), 3.31 (3H, t, *J* = 6.1 Hz), 7.41–7.51 (2H, m), 7.54–7.68 (3H, m), 8.81 (1H, brs). MS (ESI+) *m/z* 276.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[5-(4-methoxybutyl)-1-phenyl-1*H*-imidazol-4-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**53a**). **52a** (370 mg, 1.20 mmol) and **35** (445 mg, 1.20 mmol) were dissolved in DCE (6.0 mL), DIEA (626 μL, 3.59 mmol) and TCFH (405 mg, 1.44 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give *tert*-Butyl (3*S*,5*R*)-3-[{[2-chloro-5-(4-methoxybutyl)-1-phenyl-1*H*-imidazol-4-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate (727 mg, 92%) as pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (6H, brs), 1.38–1.47 (9H, m), 1.55–1.90 (2H, m), 1.93–3.24 (11H, m), 3.39–3.75 (8H, m), 4.41–4.62 (4H, m), 7.42–7.77 (5H, m). MS (ESI+) *m/z* 660.3 $(M+1)^+$. *tert*-butyl (3*S*,5*R*)-3-[{[2-chloro-5-(4-methoxybutyl)-1-phenyl-1*H*-imidazol-4-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-

ylcarbonyl)piperidine-1-carboxylate (360 mg, 0.545 mmol), palladium(II) hydroxide-carbon (10 mg) and potassium acetate (270 mg, 2.75 mmol) were suspended in methanol (6.0 mL), and the mixture was stirred under a hydrogen atmosphere at room temperature for 14 h. The palladium catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was suspended in water, and the suspension was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (50:50–80:20) then methanol-ethyl acetate (0:100 – 20:80) was concentrated under reduced pressure to give the object product (185 mg, 54%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (6H, brs), 1.39–1.55 (9H, m), 1.60–1.81 (2H, m), 1.90–2.15 (3H, m), 2.22–3.25 (8H, m), 3.36–4.00 (8H, m), 4.25–4.55 (4H, m), 7.25–7.52 (5H, m), 8.32 (1H, s). MS (ESI+) *m/z* 626.3 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[5-(4-methoxybutyl)-1-phenyl-1*H*-pyrazol-4-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**53b**). To a solution of **52b** (300 mg, 1.10 mmol) in toluene (5 mL) were added thionyl chloride (0.11 mL) and DMF (1 drop), and the mixture was stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature, concentrated under reduced pressure, and the residue was azeotroped with toluene (5 mL). The obtained residue was dissolved in THF (5 mL), added to a solution of **35** (404 mg, 1.1 mmol) and DIEA (0.57 mL) and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure, diluted with water, and extracted with ethyl acetate. The extract

was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with acetate was concentrated under reduced pressure to give the object product as white solid (684 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (6H, d, J = 6.6 Hz), 1.47 (13H, s), 1.83–2.02 (1H, m), 2.02–2.18 (1H, m), 2.31 (1H, brs), 2.64–3.41 (12H, m), 3.47–3.80 (8H, m), 3.99–4.35 (3H, m), 7.38–7.56 (5H, m), 7.64 (1H, s). MS (ESI+) *m/z* 626.3 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[5-(4-methoxybutyl)-1-phenyl-1*H*-1,2,3-triazol-4-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**53c**). **52c** (138 mg, 0.5 mmol), **35** (185 mg, 0.5 mmol) and DIEA (345 μL, 2 mmol) were dissolved in DCE (5 mL), TCFH (154 mg, 0.55 mmol) was added and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (10:90–100:0) was concentrated under reduced pressure to give the object product (235 mg, 75%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74–1.12 (6H, m), 1.30–1.69 (13H, m), 1.82–2.31 (2H, m), 2.31–2.69 (1H, m), 2.69–3.09 (5H, m), 3.11–4.81 (18H, m), 7.39–7.52 (2H, m), 7.54–7.67 (3H, m). MS (ESI+) *m/z* 626.8 $(M+1)^+$.

Ethyl [(4-methoxybutyl)amino](thioxo)acetate (**55**). To a solution of 4-methoxybutane-1-amine hydrochloride (**54**) (1.40 g, 10.0 mmol) and triethylamine (4.18

mL, 30.0 mmol) in THF (100 mL) was added dropwise ethyl chloroglyoxylate (1.12 mL, 10.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. Water was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The extract was washed successively with 1 M hydrochloric acid, aqueous sodium bicarbonate and saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was dissolved in toluene (100 mL), Lawesson's reagent (6.07 g, 15.0 mmol) was added, and the mixture was stirred at 90 °C for 2 h. The reaction mixture was cooled to room temperature, aqueous sodium bicarbonate was added, and the mixture was stirred for 30 min and extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous sodium sulfate, and subjected to basic silica gel column chromatography. The fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as an oil (1.2 g, 55% for 2steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 1.64–1.77 (2H, m), 1.77–1.90 (2H, m), 3.38 (3H, s), 3.43 (2H, t, J = 5.7 Hz), 3.63–3.74 (2H, m), 4.37 (2H, q, J = 7.2 Hz), 9.46 (1H, brs).

Butyl 4-(4-methoxybutyl)-5-phenyl-4*H*-1,2,4-triazole-3-carboxylate (**56**). **55** (1.2 g, 5.47 mmol) and benzohydrazide (745 mg, 5.47 mmol) were dissolved in 1-butanol (10 mL), and the mixture was stirred at 140 °C for 15 h. The reaction mixture was cooled to room temperature, 10% aqueous citric acid solution was added, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with

ethyl acetate-hexane (8:2) was concentrated under reduced pressure to give the object product as an oil (80 mg, 4.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95–1.02 (3H, m), 1.42–1.57 (4H, m), 1.73–1.90 (4H, m), 3.25 (3H, m), 3.28 (2H, t, *J* = 6.2 Hz), 4.30–4.42 (2H, m), 4.45 (2H, t, *J* = 6.8 Hz), 7.47–7.67 (5H, m). MS (ESI+) *m/z* 332.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[4-(4-methoxybutyl)-5-phenyl-4*H*-1,2,4-triazol-3-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**57**). **56** (80 mg, 0.24 mmol) was dissolved in ethanol (4 mL) and water (2 mL), lithium hydroxide monohydrate (15 mg, 0.36 mmol) was added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure, and the residue was azeotroped with toluene. The residue was dissolved in acetnitrile (5 mL), **35** (89 mg, 0.24 mmol), DIEA (207 μL, 1.20 mmol) and TCFH (135 mg, 0.48 mmol) were added, and the mixture was stirred at room temperature for 15 h. Aqueous sodium bicarbonate was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (1:1) was concentrated under reduced pressure to give the object product (112 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78–1.10 (6H, m), 1.19–2.27 (16H, m), 2.34–3.05 (4H, m), 3.14–4.77 (19H, m), 7.43–7.68 (5H, m). MS (ESI+) *m/z* 627.0 $(M+1)^+$.

Ethyl 3-[(1*E*)-4-methoxybut-1-en-1-yl]imidazo[1,2-*a*]pyridine-2-carboxylate (**60**). To a suspension of (3-methoxypropyl)(triphenyl)phosphonium bromide (**59**) (3.56 g, 8.57

mmol) in THF (50 mL) was added potassium *tert*-butoxide (1.05 g, 9.35 mmol) at –78 °C, and the mixture was stirred at the same temperature for 30 min. Ethyl 3-formylimidazo[1,2-*a*]pyridine-2-carboxylate (**58**) (1.70 g, 7.79 mmol) was added to the reaction mixture, and the mixture was stirred at room temperature for 12 h. Water was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as yellow oil (256 mg, 68%). MS (ESI+) *m/z* 275.2 $(M+1)^+$.

Ethyl 3-(4-methoxybutyl)imidazo[1,2-*a*]pyridine-2-carboxylate (**61a**). **60** (530 mg, 1.93 mmol) and diphenyl sulfide (3.6 mg, 0.019 mmol) were dissolved in ethyl acetate (13 mL), 10% palladium carbon (53 mg) was added and the mixture was stirred in a hydrogen stream at room temperature and normal pressure for 2.5 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as a colorless oil (260 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (3H, t, *J* = 5.6 Hz), 1.67–1.78 (4H, m), 3.32–3.34 (2H, m), 3.33 (3H, s), 3.42 (2H, t, *J* = 6.0 Hz), 4.46 (2H, q, *J* = 5.6 Hz), 6.88 (1H, t, *J* = 6.8 Hz), 7.22 (1H, dd, *J* = 7.2, 6.8 Hz), 7.67 (1H, d, *J* = 6.8 Hz), 7.99 (1H, d, *J* = 7.2 Hz). MS (ESI+) *m/z* 277.2 $(M+1)^+$.

Ethyl 3-(4-methoxybutyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carboxylate (**61b**). **60** (1.40 g, 5.10 mmol) was dissolved in ethyl acetate (30 mL), 10% palladium carbon (50% in water) (510 mg) was added. The mixture was stirred in a hydrogen stream at ambient temperature under normal pressure for 12 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-methanol (5:1) was concentrated under reduced pressure to give the object product as a brown oil (1.14 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* = 7.2 Hz), 1.62–1.67 (4H, m), 1.89–1.92 (2H, m), 1.99–2.01 (2H, m), 2.89 (2H, t, *J* = 6.4 Hz), 2.92–2.96 (2H, m), 3.32 (3H, s), 3.38–3.41 (2H, m), 3.82-3.85 (2H, m), 4.35 (2H, q, *J* = 7.2 Hz). MS (ESI+) *m/z* 281.2 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[3-(4-methoxybutyl)imidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**62a**). **61a** (183 mg, 0.66 mmol) was dissolved in ethanol (5 mL), lithium hydroxide monohydrate (139 mg, 3.31 mmol) was added and the mixture was stirred at 60 °C for 15 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in acetonitrile (5 mL). 1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (**34**) (208 mg, 0.66 mmol), DIEA (570 μL, 3.31 mmol) and TCFH (370 mg, 1.32 mmol) were added and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated, and the residue was diluted with aqueous sodium bicarbonate, and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced

pressure. The residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give 1-*tert*-butyl 3-methyl (3*R*,5*S*)-5-[{[3-(4-methoxybutyl)imidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1,3-dicarboxylate as an oil (224 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.66–1.05 (3H, m), 1.17–1.52 (10H, m), 1.64–1.94 (5H, m), 2.10–2.89 (5H, m), 3.01–3.82 (14H, m), 4.01–4.86 (3H, m), 6.84 (1H, t, *J* = 6.6 Hz), 7.18 (1H, t, *J* = 8.1 Hz), 7.56 (1H, d, *J* = 8.7 Hz), 7.98 (1H, d, *J* = 6.8 Hz). MS (ESI+) *m/z* 545.5 $(M+1)^+$. 1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[{[3-(4-methoxybutyl)imidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (224 mg, 0.41 mmol) was dissolved in methanol (10 mL), 2 M aqueous sodium hydroxide solution (410 μL) was added dropwise at room temperature. The reaction mixture was stirred at 60 °C for 2 h. The reaction mixture was adjusted to pH 7 with 1 M hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give (3*R*,5*S*)-1-(*tert*-butoxycarbonyl)-5-[{[3-(4-methoxybutyl)imidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-3-carboxylic acid (219 mg, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65–1.06 (6H, m), 1.06–1.56 (11H, m), 1.60–2.24 (2H, m), 2.43–2.76 (3H, m), 2.79–3.14 (4H, m), 3.27–3.85 (9H, m), 4.15–4.53 (2H, m), 6.85–6.99 (1H, m), 7.27–7.35 (1H, m), 7.90-8.12 (2H, m). MS (ESI+) *m/z* 531.1 $(M+1)^+$. (3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-[{[3-(4-methoxybutyl)imidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-3-carboxylic acid (106 mg, 0.20 mmol), HOBt (38 mg, 0.28 mmol) and WSC (58 mg, 0.30 mmol) were dissolved in DMF (5 mL), morpholine (21

μL, 0.24 mmol) and DIEA (103 μL, 0.60 mmol) were added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with aqueous sodium bicarbonate. The mixture was extracted with ethyl acetate, and the extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product as a oil (102 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.64–1.06 (6H, m), 1.16–1.54(9H, m), 1.65–1.87 (4H, m), 1.89–2.51 (3H, m), 2.55–4.85 (23H, m), 6.85 (1H, t, *J* = 6.2 Hz), 7.19 (1H, t, *J* = 7.9 Hz), 7.37–7.63 (1H, m), 8.00 (1H, d, *J* = 6.4 Hz). MS (ESI+) *m/z* 600.2 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[3-(4-methoxybutyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**62b**). **61b** (56 mg, 0.2 mmol) was dissolved in ethanol (5.0 mL), lithium hydroxide monohydrate (42 mg, 1.0 mmol) was added and the mixture was stirred at 50 °C for 6 h. 8 M Aqueous sodium hydroxide solution (0.1 mL) was added to the reaction mixture, and the mixture was stirred at 60 °C for 15 h, and concentrated under reduced pressure. The residue was dissolved in acetonitrile (5 mL), **35** (74 mg, 0.2 mmol), DIEA (172 μL) and TCFH (112 mg, 0.4 mmol) were added and the mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated, and the residue was diluted with aqueous sodium bicarbonate, and extracted with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to basic silica gel column

chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (26 mg, 22%). MS (ESI+) *m/z* 604.4 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-{(1*H*-benzimidazol-2-ylcarbonyl)(2-methylpropyl)amino}-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**64a**). 2-(Trichloromethyl)-1*H*-benzimidazole (**63**) (2.00 g, 7.68 mmol) and **35** (2.84 g, 7.68 mmol) were dissolved in tetrahydrofuran-water (3:2,150 mL), sodium hydrogen carbonate (6.45 g, 76.8 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, diluted with water, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The precipitated white solid was collected by filtration, washed with ethyl acetate-hexane (1:1) and dried to give the object product as a white solid (3.03 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.01 (6H, m), 1.30–1.50 (9H, m), 1.89–2.64 (3H, m), 2.68–3.08 (2H, m), 3.22–4.01 (10H, m), 4.07–4.44 (3H, m), 5.53–6.12 (1H, m), 7.27–7.42 (2H, m), 7.52 (1H, t, *J* = 8.1 Hz), 7.61-7.86 (1H, m), 10.15-10.52 (1H, m). MS (ESI+) *m/z* 514.2 $(M+1)^+$.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[(1*H*-benzimidazol-2-ylcarbonyl)(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (**64b**). **63** (19 g, 79.5 mmol) and **34** (25 g, 79.5 mmol) were dissolved in THF (1200 mL), sodium hydrogen carbonate (67 g, 798 mmol) and water (600 mL) were added, and the mixture was stirred at room temperature for 1 h and at 50 °C for 1 h. After evaporation of the solvent, the residue was extracted three times with ethyl acetate. The extract was washed successively with 10%-aqueous citric acid solution and brine, and dried over anhydrous sodium sulfate. The

solvent was evaporated under reduced pressure. The residue was subjected to basic silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (30.6 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73–1.06 (6H, m), 1.18–1.74 (11H, m), 1.82–2.36 (2H, m), 2.40–2.90 (3H, m), 3.23–3.77 (4H, m), 4.07–4.75 (3H, m), 7.27–7.41 (2H, m), 7.51 (1H, d, *J* = 8.1 Hz), 7.72–7.89 (1H, m), 10.44–10.76 (1H, m). MS (ESI+) *m/z* 459.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**65a**). A solution of **64a** (200 mg, 0.39 mmol), 4-methoxybutyl methanesulfonate (107 mg, 0.59 mmol) and cesium carbonate (254 mg, 0.78 mmol) in *N*,*N*-dimethylacetamide (5 mL) was stirred at 60 °C for 15 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate (10 mL×2). The extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (5:95–3:7) was concentrated under reduced pressure to give the object product as a white solid (190 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63–0.80 (2H, m), 0.89–1.07 (4H, m), 1.41–1.59 (9H, m), 1.59–1.80 (2H, m), 1.87–2.23 (4H, m), 2.30–2.98 (3H, m), 3.21–3.46 (6H, m), 3.49–3.91 (10H, m), 3.95–4.47 (5H, m), 7.18–7.51 (3H, m), 7.56–7.84 (1H, m). MS (ESI+) *m/z* 600.1 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-{[(1-methyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**65b**).

64a (205 mg, 0.40 mmol) was dissolved in dimethylformamide (5 mL), methyl iodide (75 μL) and cesium carbonate (391 mg, 1.2 mmol) were added and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, diluted with aqueous sodium bicarbonate, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was subjected to silica gel chromatography, and a fraction eluted with ethyl acetate-hexane (6:4) was concentrated under reduced pressure to give the object product (184 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61–1.06 (6H, m), 1.17–1.52 (9H, m), 1.65–2.24 (2H, m), 2.27–3.05 (3H, m), 3.13–4.52 (17H, m), 7.23–7.49 (3H, m), 7.52–7.86 (1H, m). MS (ESI+) *m/z* 528.0 $(M+1)^+$.

Compound **65c** was prepared following similar procedures to the synthesis of compound **65b**.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(1-ethyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}piperidine-1,3-dicarboxylate (**65c**). Yield: 87%, a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63–1.08 (6H, m), 1.19–1.62 (13H, m), 1.72–1.95 (1H, m), 2.12–2.39 (1H, m), 2.41–2.69 (2H, m), 2.81 (1H, d, *J* = 12.1 Hz), 3.23–3.81 (6H, m), 4.08–4.57 (4H, m), 7.28–7.41 (2H, m), 7.42-7.47 (1H, m), 7.80 (1H, dd, *J* = 7.0, 1.3 Hz).MS (ESI+) *m/z* 487.4 $(M+1)^+$.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (**65d**). **64b** (30 g, 65.4 mmol) and 4-methoxybutyl methanesulfonate (12.5 g, 68.7 mmol) were dissolved in DMA (600 mL), cesium carbonate (32 g, 98.1 mmol) was added, and the mixture was

stirred at 70 °C for 12 h. The reaction mixture was poured into ice water, and the mixture was extracted twice with ethyl acetate. The extract was washed with brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (1:4 - 1:1) was concentrated under reduced pressure to give the object product (28.7 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70–1.05 (6H, m), 1.29–1.53 (9H, m), 1.58–2.04 (6H, m), 2.12–2.95 (4H, m), 3.24–3.85 (11H, m), 4.06–4.56 (4H, m), 7.27–7.48 (3H, m), 7.74–7.85 (1H, m). MS (ESI+) *m/z* 545.0 $(M+1)^+$.

***tert*-Butyl (3*S*,5*R*)-3-{[(1-ethyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate** (**66**). **65c** (900 mg, 1.85 mmol) was dissolved in methanol (5 mL) and THF (2 mL), 1 M aqueous sodium hydroxide solution (410 μL) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was acidified with aqueous $KHSO_4$, and extracted with ethyl acetate. The extract was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give (3*R*,5*S*)-1-(*tert*-butoxycarbonyl)-5-{[(1-ethyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}piperidine-3-carboxylic acid (870 mg, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.67–1.06 (6H, m), 1.08–1.30 (2H, m), 1.41–1.58 (9H, m), 1.74–1.96 (1H, m), 2.13–3.18 (5H, m), 3.31–3.96 (4H, m), 4.22–4.51 (4H, m), 7.31–7.51 (3H, m), 7.94–8.14 (1H, m). (3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(1-ethyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}piperidine-3-carboxylic acid (320 mg, 0.68 mmol), HOBt (156 mg, 0.99 mmol) and WSC (195 mg, 0.99 mmol) were dissolved

in acetonitrile (5 mL), morpholine (59 μL, 0.68 mmol) and triethylamine (283 μL, 2.03 mmol) were added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo and diluted with aqueous sodium bicarbonate. The mixture was extracted with ethyl acetate, and the extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate-hexane (9:1) was concentrated under reduced pressure to give the object product as a white solid (233 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.58–1.08 (6H, m), 1.15–1.63 (13H, m), 1.66–2.25 (2H, m), 2.28–3.00 (3H, m), 3.11–3.90 (10H, m), 3.96–4.54 (5H, m), 7.27–7.51 (3H, m), 7.57–7.86 (1H, m). MS (ESI+) *m/z* 542.3 $(M+1)^+$.

tert-Butyl (3*R*,5*S*)-3-(hydroxymethyl)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1-carboxylate (**67**). Sodium borohydride (4.45 g, 118 mmol) was suspended in THF (25 mL)–ethanol (75 mL), and calcium chloride (6.5 g, 58.72 mmol) was added. After stirring at 0 °C for 1 h, a solution of **65d** (4.0 g, 7.34 mmol) in THF (50 mL) was added. After stirring at room temperature for 12 h, ethyl acetate and water were slowly added, and the mixture was extracted with ethyl acetate. The extract was washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (1.8 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.67–1.07 (6H, m), 1.30–1.50 (9H, m), 1.53–2.87 (11H, m), 3.24–3.77 (10H, m),

4.03–4.45 (4H, m), 7.27–7.39 (2H, m), 7.40–7.46 (1H, m), 7.72–7.85 (1H, m). MS (ESI+) *m/z* 517.2 $(M+1)^+$.

***tert*-Butyl (3*R*,5*S*)-3-(1-hydroxy-1-methylethyl)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1-carboxylate** (**68**).

A solution of **65d** (330 mg, 0.61 mmol) in THF (5.0 mL) was cooled to –40 °C, 3 M methyl magnesium bromide in diethyl ether (1.0 mL, 3.0 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into saturated aqueous ammonium chloride solution, and the mixture was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction eluted with ethyl acetate was concentrated under reduced pressure to give the object product (180 mg, 54%). MS (ESI+) *m/z* 417.0 $(M+1)^+$.

Compound **69** was prepared following similar procedures to the synthesis of compound **67**.

***tert*-Butyl (3*S*,5*R*)-3-{[(1-ethyl-1*H*-benzimidazol-2-yl)carbonyl](2-methylpropyl)amino}-5-(hydroxymethyl)piperidine-1-carboxylate** (**69**). Yield: 63%, a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63–1.05 (6H, m), 1.23–1.93 (16H, m), 2.02–2.83 (3H, m), 3.21–3.76 (5H, m), 4.05–4.48 (4H, m), 7.28–7.40 (2H, m), 7.41–7.47 (1H, m), 7.75–7.91 (1H, m). MS (ESI+) *m/z* 459.4 $(M+1)^+$.

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]-1*H*-indole-2-carboxamide (**71**). Methyl 1*H*-indole-2-carboxylate (**70**) (0.67 g, 3.8 mmol), cesium carbonate (1.9 g, 5.8 mmol) and 4-

methoxybutyl methanesulfonate (0.70 g, 3.8 mmol) were suspended in DMA (20 mL), and the suspension was stirred at 60 °C for 4 h. The reaction mixture was concentrated under reduced pressure, the residue was diluted with water, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The obtained residue was subjected to silica gel chromatography, and a fraction eluted with ethyl acetate-hexane (0:10 – 4:6) was concentrated under reduced pressure. The obtained residue was dissolved in methanol (10 mL), 4 M aqueous sodium hydroxide solution (5.0 mL) was added, and the mixture was heated at 80 °C for 2 h. The mixture was allowed to cool to room temperature, acidified with 1 M aqueous hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the object product (0.77 g, 82%). ^{1}H NMR (DMSO-d_6) δ 1.32–1.53 (2H, m), 1.63–1.79 (2H, m), 3.18 (3H, s), 3.28 (2H, t, J = 6.4 Hz), 4.59 (2H, t, J = 7.2 Hz), 7.11 (1H, s), 7.23 (1H, d), 7.32 (1H, s), 7.58 (1H, dd, J = 8.5, 0.9 Hz), 7.67 (1H, d, J = 7.9 Hz). MS (ESI+) *m/z* 248.1 $(M+1)^+$.

***N*-(4-methoxybutyl)benzene-1,2-diamine** (**73**). A suspention of 1,2-phenylenediamine (**72**) (10.81 g, 100 mmol), 4-methoxybutyl methanesulfonate (9.11 g, 50 mmol) and potassium carbonate (20.7 g, 150 mmol) in acetonitrile (100 mL) was refluxed for 14 h. After cooling to room temperature, water was added to the reaction mixture, and the mixture was extracted twice with ethyl acetate. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a

fraction eluted with ethyl acetate-hexane (35:65) was concentrated under reduced pressure to give the object product (5.44 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65–1.81 (4H, m), 3.08–3.17 (2H, m), 3.33 (3H, brs), 3.35 (3H, s), 3.39–3.47 (2H, m), 6.62–6.75 (3H, m), 6.81 (1H, dd, *J* = 7.2, 3.3 Hz). MS (ESI+) *m/z* 195.1 $(M+1)^+$.

1-(4-Methoxybutyl)-2-(trichloromethyl)-1*H*-benzimidazole (**74**). To a solution of **73** (1.2 g, 6.18 mmol) in AcOH (30 mL) was added methyl 2,2,2-trichloroethanimidate (770 μL) at room temperature. After being stirred for 1 h, the mixture was poured into water and extracted with diisopropyl ether. the extract was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give the object product (1.9 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60–1.79 (2H, m), 1.84–2.02 (2H, m), 3.25 (3H, s), 3.41 (2H, t, *J* = 6.2 Hz), 4.52–4.65 (2H, m), 7.31–7.39 (1H, m), 7.45 (1H, td, *J* = 7.8, 1.1 Hz), 7.71 (1H, d, *J* = 8.0 Hz), 7.80 (1H, d, *J* = 8.0 Hz). MS (ESI+) *m/z* 321.1 $(M+1)^+$.

***tert*-Butyl (3*S*)-3-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1-carboxylate** (**76**). **74** (470 mg, 1.46 mmol) and *tert*-butyl (3*S*)-3-[(2-methylpropyl)amino]piperidine-1-carboxylate (**75**) (400 mg, 1.56 mmol) were dissolved in acetonitrile (30 mL) and water (15 mL), potassium carbonate (2.02 g, 14.6 mmol) was added, and the mixture was stirred at 80 °C for 15 h. The reaction mixture was cooled to room temperature and diluted with saturated brine. The mixture was extracted with ethyl acetate, and the extract was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography, and a fraction

eluted with ethyl acetate was concentrated under reduced pressure to give the object product (446 mg, 63%). MS (ESI+) *m/z* 487.0 $(M+1)^+$.

General procedures for assays of biological activities.

Rh-renin inhibition assay (enzyme-linked immunosorbent assay (ELISA)). The inhibitory potency of the compounds against recombinant human renin was determined by the following protocol. In 384-well plates (ABgene), 1 μL of test compound in 100 % DMSO was incubated with 14 μL of enzyme (at a final concentration of 40 pM recombinant human renin） in buffer (20 mM Phosphate buffer, 1 mM EDTA, pH 7.4, with 0.004 % Tween 20 (HSA–) or 2 % human serum albumin (HSA+)) at 37 °C. After 10 min, 5 μL of recombinant human angiotensinogen was added to a final concentration of 1.5 μM and incubated at 37 °C for 30 min. The enzymatic reaction was terminated by adding 20 μL of stop solution (1 μM CGP-29287 in diluent buffer (20 mM Tris-HCl, pH7.4, 150 mM NaCl, 0.1 % BSA, 0.05 % Tween20)). The diluent buffer was used for diluting each reagent in ELISA. Angiotensin I generated during the incubation was measured by ELISA. 10 μL of the incubates or angiotensin I peptide standards were transferred to 384-well immuno plates which were previously coated with anti-angiotensinI antibody (Peninsula Laboratories) and incubated with 15 μL of 1.6 nM biotin-conjugated angiotensin I (AnaSpec) at room temperature for 1 h. After washing the plates 5 times with wash buffer (0.05 % Tween20 in PBS), 25 μL of 100 ng/mL streptavidin-HRP (Pierce) was incubated at room temperature for 30 min. After washing, 25 μL of substrate of HRP (Pierce) was added and chemiluminescence was detected using a microplate reader.

Human PRA inhibition assay. The inhibitory effect of each compound on the human plasma renin activity was tested using the radioimmunoassay kit (TFB, Tokyo, Japan). IC_{50} values were calculated from concentration-response curves with SAS software (SAS Institute Japan Ltd., Tokyo, Japan).

Pharmacokinetic Analysis in Rat Cassette Dosing. Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg) by cassette dosing to rats. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

Pharmacokinetic analysis in monkey cassette dosing: Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg) by cassette dosing to fed monkey. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

Mesurement of LogD. LogD, which is a partition coefficient between 1-octanol and aqueous buffer pH 7.4, of the compounds was measured on the chromatographic procedure whose condition was developed based on a published method.[22]

PAMPA membrane permiability test. Donor solutions were prepared by filtration of mixture of compound solutions in DMSO and system solutions, pH 7.4 (pION Inc.). Donor solutions were set in a well plate at the bottom across a GIT Lipid (pION Inc.) containing membrane filter from Acceptor Sink Buffer pH 7.4 (pION Inc.). After 3 hours incubation, the concentrations of compound in both sides were quantified by UV

absorption measurement, and the membrane permeability rates were calculated by the analysis software.

Cynomolgus monkey in vivo test. All adult male cynomolgus monkeys were purchased from Keari Co., Ltd. (Wakayama, Japan), and all animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Compound **13** or compound **2** dissolved in 0.5% methylcellulose solution was orally administered at a volume of 2 mL/kg. Blood was collected through the femoral vein using a 1 mL syringe containing EDTA-2Na, as an anticoagulant, at a final concentration of approximately 3.0 mmol/L before the drug administration and at 4 and 24 hours after the drug administration. Blood samples were centrifuged (13,000 g, 4°C, 10 min) to obtain plasma. Plasma samples were dispensed to tubes and stored at –80°C until measurement. PRA was measured using a RIA kit (TFB, INC., Tokyo, Japan). The PRA inhibition rate of each sample (% inhibition) was calculated using the following formula:

PRA (ng/mL/h) = $PRA_{37°C} – PRA4_{°C}$

PRA inhibition (%) = $100 – PRA_x/PRA_{pre} \times 100$

PRA_{pre}: PRA before drug administration, PRA_x: PRA at 4 or 24 hours after drug administration

General procedure for X-ray crystallography of inhibitors with rh-renin. Crystallization of mature rh-renin (1–340) was carried out by the sitting drop vapor diffusion method (Nanovolume CrystallizationTM methods[23]). Conditions for the reservoir solution were 19.95–40.95% PEG600, 100 mM citric acid buffer pH 4.5–6.0, or 24–45%

PEG600, citric acid buffer pH 4.5–6.0, 50 mM NaH_2PO_4 aq. A mixture of the reservoir solution and a solution of rh-renin (ca. 6 mg/mL in 25 mM Tris pH 7.9 and 150 mM NaCl aq.) was left at 20 °C until crystals of apoprotein were generated. Co-crystal of inhibitors and rh-renin were prepared by soaking. The apoprotein crystals were placed into a soaking buffer, which was prepared by adding inhibitors to the reservoir solution to 1–10 mM, for 30 min to 1 day. To this was then added ethylene glycol to give a 0–12% solution, and the mixture was frozen. X-ray diffraction analysis was carried out by using beamline 5.0.3 at the Advanced Light Source. The data obtained was processed by HKL2000[24] to generate initial complex structures, carrying out molecular replacement with MOLREP of CCP4 (ver.4.0). Structure refinements of the models, which were generated by Xfit[25] based on the initial complex structures, were carried out by using REFMAC[26].

Accession Codes

Atomic coordinates and structure factors have been deposited in the Protein Data Bank with codes 5TMK for **9**, 5TMG for **10c** and 5KOT for **13**. Authors will release the atomic coordinates and experimental data upon article publication.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS BA, bioavailability; RAAS, renin–angiotensin–aldosterone system; BP, blood pressure; Ang I, angiotensin I; ACE, Angiotensin-converting enzyme; Ang II, angiotensin II; AT1, Ang II type I receptor; DRI, direct renin inhibitor; TPSA, topological polar surface area; TCFH, *N*,*N*,*N*',*N*'-tetramethylchloroformamidinium hexafluorophosphate; DIEA, *N*,*N*-diisopropylethylamine; DMF-DMA, *N*,*N*-dimethylformamide dimethyl acetal; rh-renin, recombinant human renin; hPRA, human plasma renin activity; HSA, human serum albumin; DCE, 1,2-dichloroethane; ELISA, enzyme-linked immunosorbent assay

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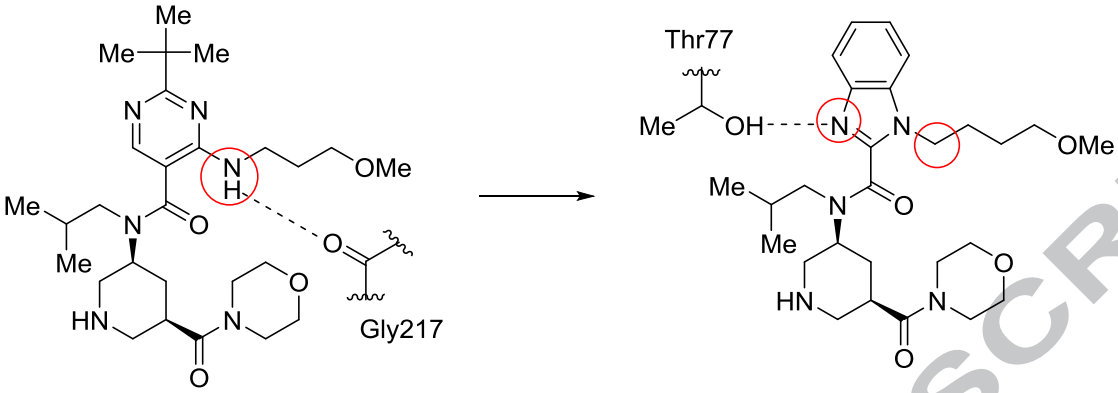
Graphical abstract



3: hPRA: IC_{50} = 0.79 nM
TPSA: 109 $Å^2$
LogD: 2.99
PAMPA: 390 nm/sec
BA: <1%

13: hPRA: IC_{50} = 1.4 nM
TPSA: 88.9 $Å^2$
LogD: 2.11
PAMPA: 345 nm/sec
BA: 14%