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Novel 3-phenylpiperidine-4-carboxamides as highly potent and orally long-acting neurokinin-1 receptor antagonists with reduced CYP3A induction

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ABSTRACT

The synthesis and biological evaluation of a series of novel 3-phenylpiperidine-4-carboxamide derivatives are described. These compounds are generated by hybridization of the substructures from two types of tachykinin NK_1 receptor antagonists. Compound **42** showed high metabolic stability and excellent efficacy in the guinea-pig GR-73637-induced locomotive activity assay at 1 and 24 h after oral administration. It also exhibited good pharmacokinetic profiles in four animal species, and a low potential in a pregnane X receptor induction assay.

1. Introduction

Substance P (SP) is one of the most abundant neuropeptides in the mammalian nervous system, and plays an important role in neurotransmission.[1] The binding of SP to the tachykinin NK_1 receptor causes various physiological responses in both the central nervous system (CNS) and peripheral tissues, including the transmission of pain and stress signals, neurogenic inflammation, and the contraction of smooth muscles. Animal studies with tachykinin NK_1 receptor antagonists (NK_1 antagonists) indicate prospective treatments for several diseases such as overactive bladder (OAB), gastrointestinal disorders, emesis, pain, and CNS disorders.[2]

In our previous study, we found that the 3-phenyl-4-benzylaminopiperidine NK_1 antagonist **1** had a high potential for antagonizing the centrally mediated effects of an SP analog, inducing locomotive activity in guinea pigs (Fig. 1).[3] We also reported that NK_1 antagonists improve OAB symptoms by reducing the bladder sensory system in the spinal cord.[4] These observations indicated that compound **1** could be a useful drug with a novel mode of action for the treatment of OAB patients.

However, in the clinical phase-I trials, compound **1** showed drug–drug interaction (DDI) via the pregnane X receptor (PXR)-mediated induction of human CYP3A (96% induction at 10 μM). A structure–activity relationship (SAR) study revealed that the 3-phenyl-4-{2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}piperidine subunit in compound **1** was an essential motif for the CYP3A induction. This subunit was also crucial for recognizing the tachykinin NK_1 receptor. Thus, replacement of the subunit with an alternative group was needed in order to identify a drug with reduced CYP3A induction.

In addition to DDI, the clinical trials revealed an insufficient duration of compound **1** in plasma. From the viewpoint of developing an anti-OAB drug, a long duration as well as high potency is required to allow dosing at a rate of once per day. Therefore, our efforts were focused on the discovery of an NK_1 antagonist with high potency, long duration, and minimized CYP3A induction.

These pharmacokinetic issues could be circumvented by structural modification of compound **1**. We assumed that the benzylamine moiety in compound **1** could be replaced by a benzylcarboxamide subunit. Previously, we had discovered a naphthyridine-6-carboxamide derivative **2** with high tachykinin NK_1 receptor affinity and a preferable pharmacokinetic profile.[5] Moreover, we found that the benzylcarboxamide moiety was important for effective binding (Fig. 1). Thus, we decided to synthesize a series of derivatives based on compound **3**, derived from the structural hybridization of the 3-phenylpiperidine framework of compound **1** and the benzylcarboxamide moiety of compound **2**. In this paper, we describe the synthesis and SAR of this series of compounds, addressing the issues of pharmacokinetic profiles.

2. Chemistry

The designed 3-phenylpiperidine-4-carboxamides **12**–**17** were synthesized as illustrated in Scheme 1. The addition of phenylmagnesium bromide to compound **4** in the presence of copper(I) iodide

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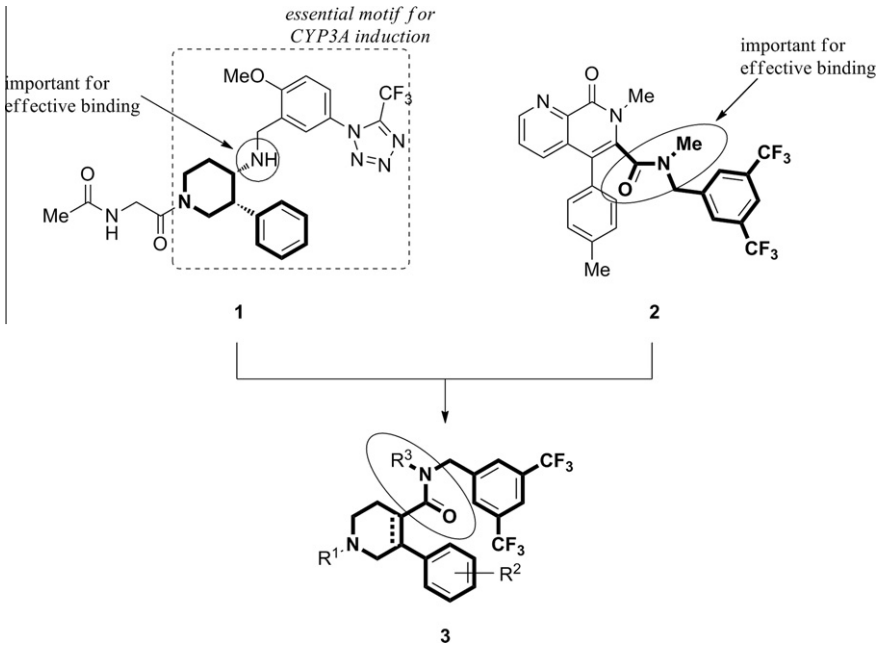
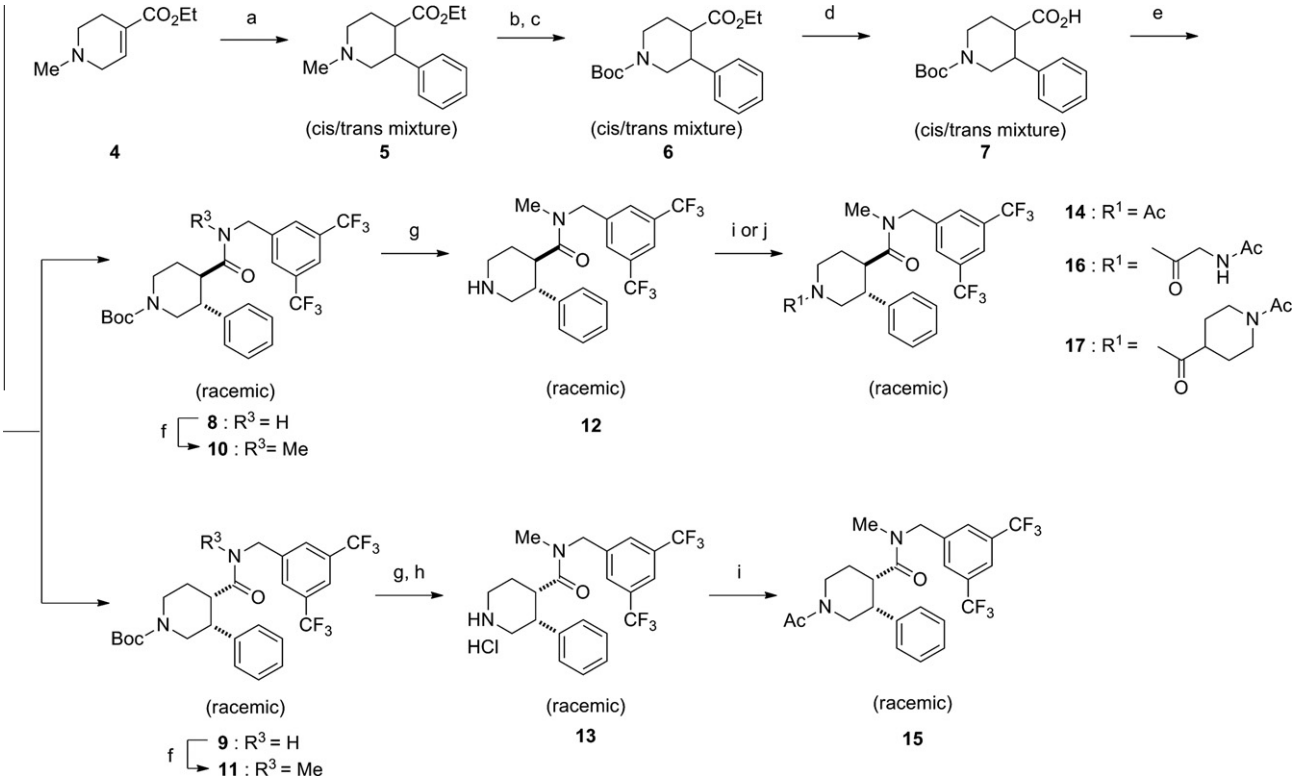


Figure 1. Drug design based on structural combination of 3-phenylpiperidine **1** and benzylcarboxamide **2**.

yielded ethyl 3-phenylpiperidine-4-carboxylate **5**[7] as a mixture of cis and trans isomers. Transformation of the N-methyl group of compound **5** into a Boc group followed by hydrolysis gave the *N*-Boc-protected 3-phenylpiperidine-4-carboxylic acid **7**, which was coupled with 3,5-bis(trifluoromethyl)benzylamine to form the separable *trans*-3-phenylpiperidine-4-carboxamide **8** and the *cis*-isomer **9**. These were converted to the *N*-methyl carboxamides **10** or **11**, respectively, after which the deprotection of the Boc group



Scheme 1. Preparation of 3-phenylpiperidine-4-carboxamides **12**–**17**. Reagents and conditions: (a) phenylmagnesium bromide, copper(I) iodide, Et_2O, −20 °C; (b) α-chloroethyl chloroformate, 1,2-dichloroethane, 100 °C then MeOH, 80 °C; (c) Boc_2O, Et_3N, MeCN, rt; (d) 2 N KOH, EtOH, 50 °C; (e) 3,5-bis(trifluoromethyl)benzylamine, WSC, HOBt, Et_3N, DMF, rt then separation of diastereoisomers by silica gel chromatography; (f) NaO^{t}Bu, methyl iodide, THF, 5 °C to rt; (g) TFA, THF, 5 °C then aqueous NaOH; (h) 4 N HCl–EtOAc; (i) acetyl chloride, Et_3N, THF, 5 °C; (j) acetylglycine or 1-acetylpiperidine-4-carboxylic acid, WSC, HOBt, MeCN, rt.

afforded compounds **12** or **13** without epimerization. Further conversion of compound **12** led to three *N*-acyl derivatives, **14**, **16**, and **17**, while compound **13** led to the acetamide **15**.

Compounds **17a**–**e** and **24**–**31** with various substituents on the phenyl ring and at the piperidine nitrogen were prepared, as shown in Scheme 2. The corresponding Grignard reagents were used for coupling with compound **4**. The following synthesis was carried out in a similar manner to that described in Scheme 1.

A coupling reaction between the aryl boronic acids and piperidin-3-one **20** was adopted as another route for the synthesis of compounds **17f** and **17g** (Scheme 3). The piperidin-3-one **20** was transformed into its enol triflate by treatment of *N*-phenylbis (trifluoromethanesulfonimide) in the presence of sodium hydride, followed by a Pd-catalyzed coupling reaction to obtain the 5-phenyltetrahydropyridines **21f**–**h**. For the synthesis of compounds **21f** and **21g**, removal of the benzyl group and reduction of the tetrahydropyridine ring were carried out by hydrogenation, followed by treatment under basic conditions and hydrolysis to give the trans acids **22f** and **22g**. Further steps were conducted in a similar manner to Scheme 1 to obtain the target compounds **17f** and **17g**.

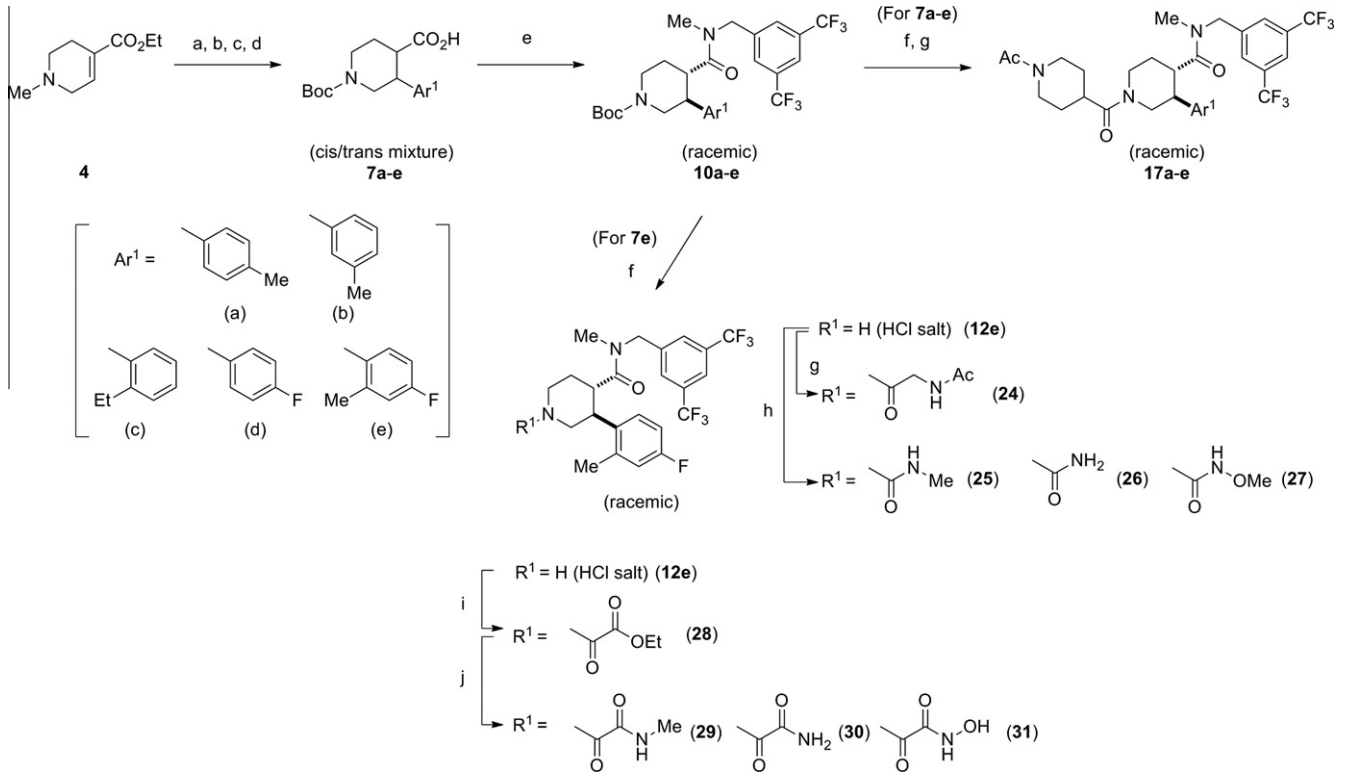
The N-nonsubstituted carboxamide **18** and 5-phenyltetrahydropyridine **19** were synthesized as shown in Scheme 4, in order to examine the effect of an N-methyl group at the carboxamide and the stereochemical requirements in the piperidine ring for the activity. The removal of the Boc group in compound **8** and the introduction of a 1-acetylpiperidinecarbonyl group afforded **18**. Acid-catalyzed hydrolysis of compound **21h** (prepared as in Scheme 3) gave 1,2,3,6-tetrahydropyridine-4-carboxylic acid **23**, which was condensed with an *N*-methylbenzylamine group using thionyl chloride, subjected to the removal conditions for the benzyl group, and coupled with 1-acetylpiperidine-4-carboxylic acid to furnish compound **19**.

The optically active compound **35** with (3*R*,4*R*)-configuration was synthesized as shown in Scheme 5. Optical resolution of the racemic *trans*-piperidine-4-carboxylic acid **22e**[8] derived from compound **4** was successful, using (*R*)-1-phenylethylamine as the chiral resolving agent to afford the diastereomeric salt **32** in 99%*de*. The absolute stereochemistry for the salt **32** was determined by single crystal X-ray analysis to be the (3*R*,4*R*)-configuration. Double decomposition of the salt **32** gave the (3*R*,4*R*)-acid **33**, which led to the target compound **35** as described above, without loss of optical purity.

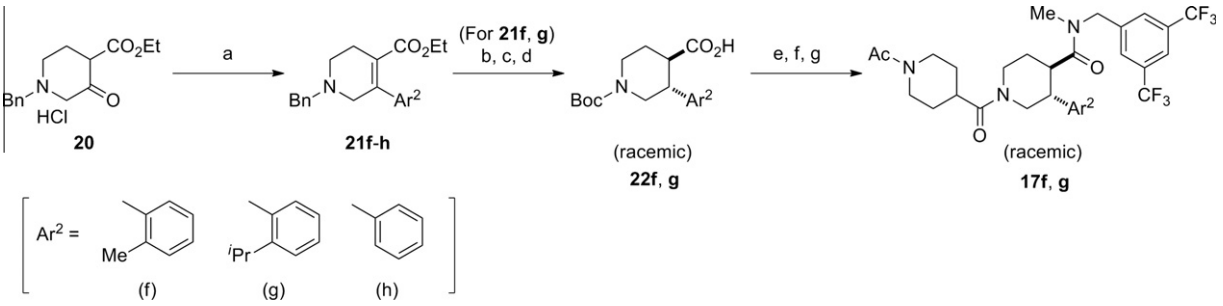
Similarly, the optically active compounds **41–43** with (3*S*,4*S*)-configuration were synthesized as shown in Scheme 6. The (3*S*,4*S*)-acid **37** was obtained by optical resolution of the racemic carboxylic acid **22e** using (*S*)-1-phenylethylamine followed by double decomposition. The introduction of an *N*-methylbenzylamine or methyl-substituted *N*-methylbenzylamines into **37** afforded the carboxamides **38–40**, which led to the target compounds **41–43** as described in Scheme 5.

3. Results and discussion

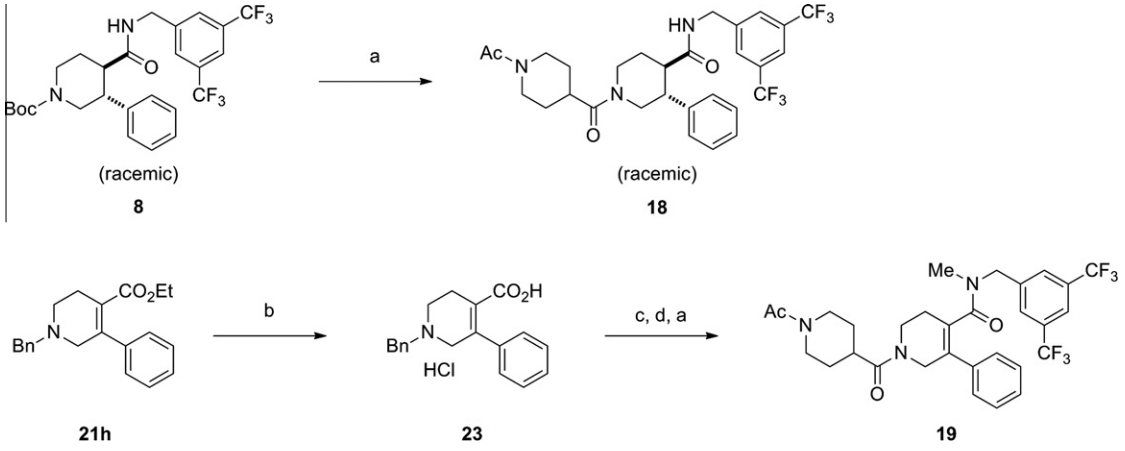
The tachykinin NK_1 antagonistic activities in the CNS of the compounds thus synthesized were first evaluated for their in vitro binding affinity against [^{125}I]Bolton–Hunter (BH)–SP binding in human IM-9 cells. Subsequently, in vivo screening was carried out and the inhibitory activities were measured by their locomotive activity induced by the intracerebroventricularly infused tachykinin NK_1 receptor agonist (NK_1 agonist) GR73637 in guinea pigs. When the compounds were administered intravenously, the inhibition rate (%inh.) was calculated by comparison with a control group after 0–30 min after administration. When the compounds were administered orally, %inh. and ID_{50} values



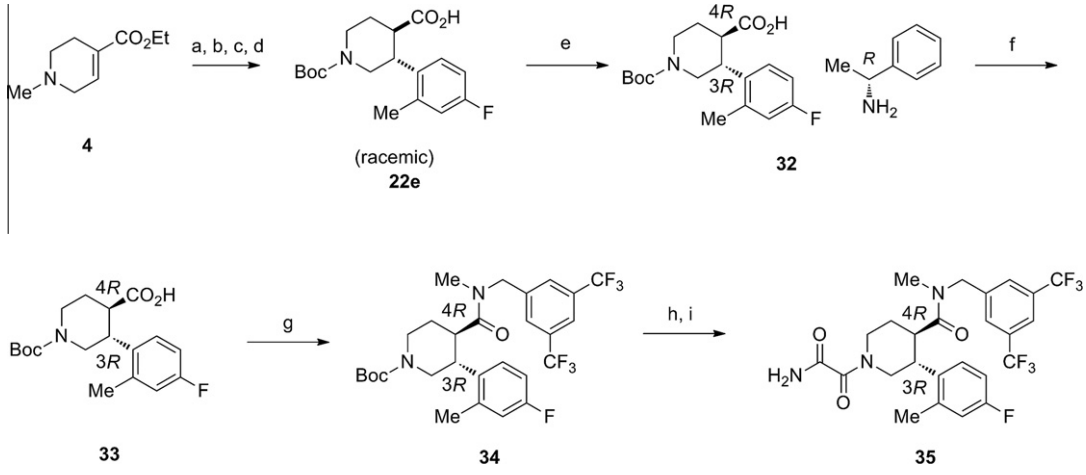
Scheme 2. Preparation of 3-phenylpiperidine-4-carboxamide **17a–e** and **24–31**. Reagents and conditions: (a) Ar^1MgBr, copper(I) iodide, Et_2O, −20 °C; (b) α-chloroethyl chloroformate, 1,2-dichloroethane, 100 °C then MeOH, 80 °C; (c) Boc_2O, Et_3N, MeCN, rt; (d) 2 N KOH, EtOH, 50 °C; (e) *N*-methyl-3,5-bis(trifluoromethyl)benzylamine, WSC, HOBt, Et_3N, DMF, rt and separation of diastereoisomers by silica gel chromatography; (f) 4 N HCl–EtOAc, 50 °C; (g) carboxylic acids, WSC, HOBt, Et_3N, MeCN, rt; (h) bis(trichloromethyl)carbonate, Et_3N, THF, 0 °C then amines; (i) ethyl oxalylchloride, Et_3N, CH_2Cl_2, 0 °C; (j) amines, Et_3N.



Scheme 3. Preparation of 3-phenylpiperidine-4-carboxamides **17f** and **17g**. Reagents and conditions: (a) (i) NaH, $PhNTf_2$, DMF, 0 °C; (ii) $Ar^2B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, H_2O, 100 °C; (b) (i) 4 N HCl–EtOAc, (ii) H_2, 10% Pd–C, HCl, EtOH; (c) Boc_2O, Et_3N, CH_3CN, rt; (d) NaOMe, MeOH, reflux then 2 N NaOH, THF, reflux; (e) *N*-methyl-3,5-bis(trifluoromethyl)benzylamine hydrochloride, WSC, HOBt, Et_3N, DMF, rt; (f) 4 N HCl–EtOAc, 50 °C; (g) 1-acetylpiperidine-4-carboxylic acid, WSC, HOBt, Et_3N, MeCN, rt.



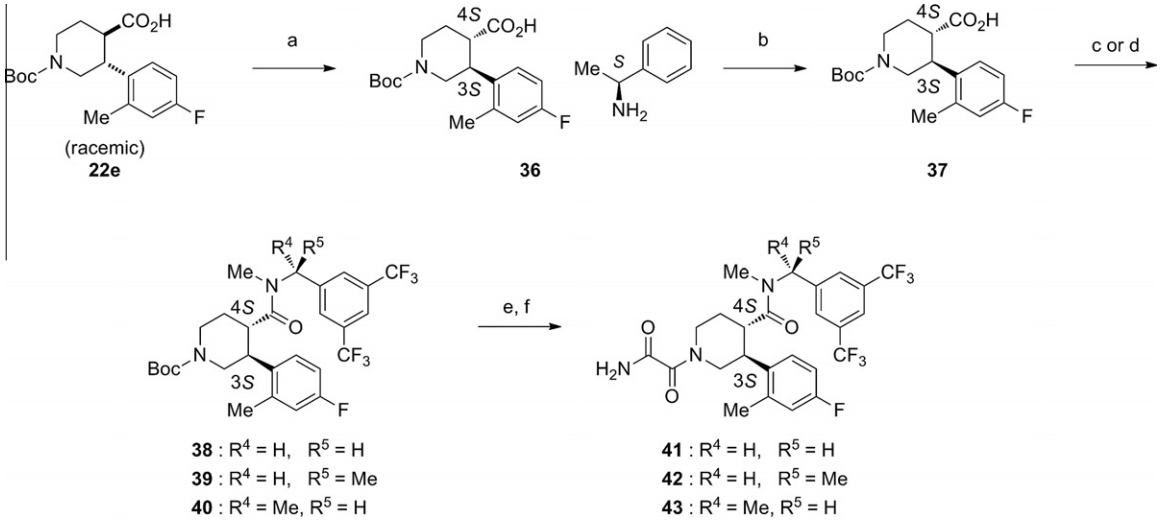
Scheme 4. Preparation of N-nonsubstituted carboxamide **18** and 5-phenyl-1,2,3,6-tetrahydropyridine-4-carboxamide **19**. Reagents and conditions: (a) (i) 4 N HCl–EtOAc, 50 °C (ii) 1-acetylpiperidine-4-carboxylic acid, WSC, HOBt, Et_3N, DMF, rt; (b) HCl, AcOH, 100 °C; (c) (i) $SOCl_2$, 90 °C, (ii) *N*-methyl-3,5-bis(trifluoromethyl)benzylamine hydrochloride, Et_3N, CH_3CN, 0 °C to rt; (d) (i) α-chloroethyl chloroformate, 1,2-dichloroethane, 100 °C then MeOH, 80 °C, (ii) Boc_2O, Et_3N, MeCN, rt.



Scheme 5. Preparation of (3*R*,4*R*)-isomer **35**. Reagents and conditions: (a) 4-fluoro-2-methylphenylmagnesium bromide, copper(I) iodide, Et_2O, −20 °C; (b) α-chloroethyl chloroformate, 1,2-dichloroethane, 100 °C then MeOH, 80 °C; (c) Boc_2O, Et_3N, CH_3CN, rt; (d) NaOMe, MeOH, reflux then 2 N NaOH, THF, 55 °C; (e) (*R*)-1-phenylethylamine, EtOAc; (f) citric acid then extraction and crystallization; (g) (i) oxalyl chloride, DMF, THF, 0 °C, (ii) 1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylmethanamine hydrochloride, $^iPr_2NEt$, THF, rt; (h) 4 N HCl–EtOAc, rt; (i) oxamic acid, WSC, HOBt, Et_3N, MeCN, rt.

were calculated after 0.75–1.25 h or 23.75–24.25 h after administration. The compounds were evaluated for their metabolic stability in human liver microsomes by HPLC measurement of the remaining parent compound after incubation periods of 15 and 30 min under standard conditions.

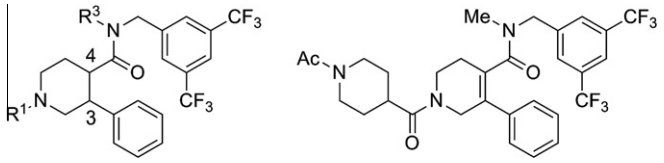
First, the in vitro binding affinities of the designed compounds **12**–**17** were evaluated in order to examine the potential of the 3-phenylpiperidine-4-carboxamide skeleton for antagonizing the tachykinin NK_1 receptor (Table 1). Compounds **12** and **13** with a hydrogen atom, and compounds **14** and **15** with an acetyl group at the 1-position showed strong affinities at the nanomolar level, indicating that this skeleton provided effective binding to the tachykinin NK_1 receptor. A comparison of compounds **12**–**15** demonstrated that the *trans* isomer was more potent than the *cis*



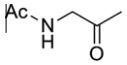
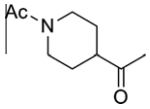
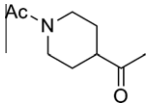
Scheme 6. Preparation of (3*S*,4*S*)-isomer **41**–**43**. Reagents and conditions: (a) (*S*)-1-phenylethylamine, acetone; (b) citric acid then extraction and crystallization; (c) 1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylmethanamine hydrochloride, WSC, HOBt, Et_3N, MeCN, rt; (d) $(COCl)_2$, DMF, THF, 5 °C then (*S*) or (*R*)-1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylethanamine, $^iPr_2NEt$, THF, rt; (e) 4 N HCl–EtOAc, rt; (f) oxamic acid, WSC, HOBt, Et_3N, MeCN, rt.

isomer. Therefore, further evaluations were conducted with the trans analogs. The introduction of an acetylglycyl group (**16**), which is a characteristic substituent in compound **1**, enhanced the affinity slightly, whereas a remarkable enhancement was observed in the compound bearing an acetylpiperidine-carbonyl group (**17**). A comparison of compounds **17**–**19** revealed that the introduction of a methyl group at the carboxamide moiety and saturation at the 3- and 4-positions in the piperidine ring contributed to the strong affinities observed at a subnanomolar (or lower) level. Since compound **17** showed the strongest affinity among the compounds in Table 1, it was evaluated for its antagonistic activity in the CNS. Upon intravenous administration, compound **17** showed strong inhibitory activity, suggesting that it should be considered for further optimization.

Table 1
Binding affinity and in vivo CNS NK_1 antagonistic activity of 3-phenylpiperidine-4-carboxamides

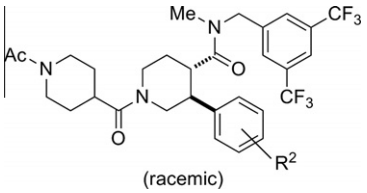


12-18 (racemic) **19**

Compound[a]	Stereochemistry at the 3- und 4-position	R^1	R^3	Binding affinity[b] IC_{50} (nM)	In vivo CNS NK, antagonistic activity[c] iv[d] %inh. (0.3 mg kg)
12	trans	H	Me	2.6	NT[e]
13	cis	H	Me	5.6	NT[e]
14	trans	Ac	Me	2.6	NT[e]
15	cis	Ac	Me	3.2	NT[e]
16	trans		Me	12	NT[e]
17	trans		Me	0.098	72.1%
18	trans		H	2.7	NT[e]
19	—	—	—	0.17	NT[e]

[a] All of the compounds arc racemic except for **19**.
[b] Inhibition of [^{125}I]Bolton–Hunter-SP binding in human IM-9 cells.
[c] Inhibition GR73632-induced increase in locomotoractivity in guinea pigs. The values of %inh. are averages of 5–10 independent experiments.
[d] Alter 5 min of intraveneous administration of the compounds.
[e] NT means 'not tested'.

Table 2
Effects of the substituents on the phenyl at the 3-position

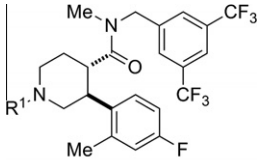


(racemic)

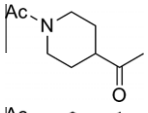
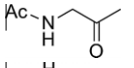
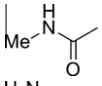
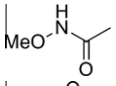
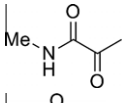
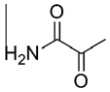
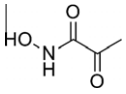
Compound[a]	R^2	Binding affinity[b] IC_{50} (nM)	In vivo CNS NK_1 antagonistic activity[c] iv[d] %inh. (0.3 mg/kg)
17	H	0.098	72.1%
17a	4-Me	0.18	NT[e]
17b	3-Me	0.14	NT[e]
I7f	2-Me	0.032	60.1%
17c	2-Et	0.064	NT[e]
I7g	2-Pr	0.21	NT[e]
17d	4-F	0.052	41.4%
17e	2-Me, 4-F	0.047	105.0%

[a] All of the compounds are racemic.
[b] Inhibition of [^{125}I]Bolton–Hunter-SP binding in human IM-9 cells.
[c] Inhibition GR73632-induced increase in locomotoractivity in guinea pigs. The values of %inh. are averages of 5–10 independent experiments.
[d] Alter 5 min of intraveneous administration of the compounds.
[e] NT means 'not tested'.

Table 3
Effects of the 1-position substituents on antagonistic activity and metabolic stability



(racemic)

Compound[a]	R^1	Binding affinity[b] IC_{50} (nM)	In vivo CNS NK_1 antagonistic activity[c] iv[d] %inh. (0.3 mg/kg)	Metabolic stability in microsomes[e] Human (μL/mg/min)
17e		0.047	105.0%	111
24		0.043	48.9%	59
25		0.048	80.0%	199
26		0.035	80.3%	112
27		0.073	−23.9%[f]	−12
29		0.11	51.5%	115
30		0.052	86.7%	40
31		0.046	−15.5%[f]	2

[a] All of the compounds are racemic except for **19**.
[b] Inhibition of [^{125}I]Bolton–Hunter-SP binding in human IM-9 cells.
[c] Inhibition GR73632-induced increase in locomotoractivity in guinea pigs. The values of %inh. are averages of 5–10 independent experiments.
[d] After 5 min of intraveneous administration of the compounds.
[e] Measured at 1 μM.
[f] At 0.1 mg/kg.

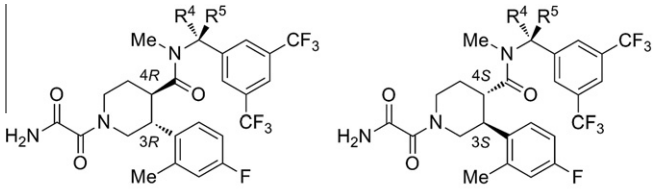
Next, the effects of substituents on the phenyl group at the piperidine 3-position were examined as shown in Table 2. The introduction of a methyl group to the 4- and 3-positions (compounds **17a** and **17b**) slightly reduced the binding affinities in comparison with the phenyl analog **17**. In contrast, the 2-methylphenyl analog **17f** exhibited an affinity three times as strong as its phenyl analog **17**. However, in vivo screening upon intravenous administration of the 2-methylphenyl analog **17f** showed a drop in activity compared with **17**. The transformation of the methyl moiety into more bulky groups, such as ethyl and isopropyl groups, resulted in a decrease in the affinity (compounds **17c** and **17g**). The introduction of a fluorine atom at the 4-position (compound **17d**) increased the affinity but reduced the in vivo activity as compared with **17**. Interestingly, a combination of 2-methyl and 4-fluorine introduction increased both the affinity and the in vivo activity (compound **17** versus **17e**). Therefore, in this series of compounds, the 4-fluoro-2-methylphenyl group is one of the best substituents at the 3-position, having potential to induce both strong affinity and high CNS activity. According to a PXR induction assay, compound **17e** also had a much reduced potency for CYP3A induction (2% induction at 10 μM). These observations urged us to refine the 4-fluoro-2-methylphenyl series.

Compound **17e** had significant reduced CYP3A induction; however, it showed low metabolic stability in human microsomes (111 μL/mg/min). Optimization was conducted to obtain a compound with high metabolic stability (<50 μL/mg/min). In general, metabolism can be reduced by decreasing the lipophilicity of the compound, or by incorporation of stable functional groups (i.e., blocking groups) at metabolically vulnerable sites.[6] Our approaches for enhancing metabolic stability involved (1) the introduction of a polar substituent at the 1-position, at which various functional groups were expected to be tolerated, and (2) the blocking of a possible metabolic site.

A reduction in lipophilicity through the introduction of a polar substituent would result in an improvement in metabolic stability. However, an increase in molecular weight might cause a drop in CNS activity, presumably because of poor CNS penetration. We therefore envisaged that the introduction of a 'small' and 'polar' substituent at the 1-position would allow us to identify an NK_1 antagonist with a good balance between high metabolic stability and good CNS activity.

Table 3 shows the effects of the 1-position substituents on antagonistic activity and metabolic stability. As expected, all of the compounds displayed sub-nanomolar or better affinities. Upon transformation of the *N*-acetylpiperidine-carbonyl group (**17e**) into an *N*-acetylglycyl group (**24**), the affinity was maintained but the CNS activity was reduced. However, the metabolic stability was improved by this transformation. This result encouraged us to explore smaller and more polar substituents, as illustrated in compounds **25**–**27** and **29**–**31**. We found that compounds with

Table 4
Stereochemical effects of the 3- and 4-position and introduction of a methyl group at the benzylic position



35 **41 - 43**

Compound[a]	R^4	R^5	Binding affinity[a]	In vivo CNS NK_1 antagonistic activity[b]			Metabolic stability in microsomes[e]
			IC_{50} (nM)	iv[c] %inh. (0.1 mg/kg)	po (1 h)[f] ID_{50}[h] (mg/kg)	po (24 h)[g] ID_{50}[h] (mg/kg)	Human (μL/mg/min)
35	H	H	>100	NT[d]	NT[d]	NT[d]	NT[d]
41	H	H	0.020	88.8%	0.14	0.72	30
42	H	Me	0.017	78.9%	0.10	0.57	2
43	Me	H	0.20	43.1%	NT[d]	NT[d]	57

[a] All of the compounds are chiral.
[b] Inhibition of [^{125}I]Bolton–Hunter-SP binding in human IM-9 cells.
[c] Inhibition GR73632-induced increase in locomotoractivity in guinea pigs. The values of %inh. are averages of 5–10 independent experiments.
[d] Alter 5 min of intraveneous administration of the compounds.
[e] NT means 'not tested'.
[f] 0.75 h after oral administration of the compounds.
[g] 23.75 h after oral administration of the compounds.
[h] ID_{50} values are compound doses causing 50% inhibition in locomotoractivity.

Table 5
Pharmacokinetic profiles of **42**

		Rat	Guinea pig	Dog	Monkey
$AUC(po)_{0-24h}$[a,b]	ng·h/mL	521	1075	2146	1039
MRT[a,c]	h	4.5	7.5	6.5	6.4
BA[a,d]	%	29	83	40	38
Metabolic stability in microsomes	μL/mg/min	13	3	0	14

[a] 1 mg/kg, po.
[b] Area under the blood concentration time curve.
[c] Mean residence time.
[d] Bioavailability.

N-methylcarboxamide (**25**), carboxamide (**26**), and oxamide (**30**) groups displayed high CNS activities. Among these, compound **30** exhibited a relatively high metabolic stability (40 μL/mg/min).

Then, we moved our efforts to the blocking of a possible metabolic site, the benzylic position. To avoid structural complications with the generation of a new chiral center in compound **30**, we examined metabolic stabilization in a stepwise manner. First, we determined the absolute stereochemistry of a eutomer of compound **30**, and then we examined the effect of blocking the benzylic position of that eutomer.

As shown in Table 4, a comparison of optically active compounds revealed that the (3*S*,4*S*)-isomer (**41**) was more potent than the (3*R*,4*R*)-isomer (**35**). Additionally, a slight enhancement in metabolic stability was observed in the (3*S*,4*S*)-isomer (**41**) (30 μL/mg/min) compared to the racemic compound **30** (40 μL/mg/min). The introduction of an (*S*)-methyl group (R^5 = Me) at the benzylic position in compound **41** almost completely restricted the metabolism, and furthermore improved its in vivo potency (po at 1 h). Compound **42** also proved to have a long duration of activity in the CNS at 24 h after oral administration. On the other hand, the introduction of an (*R*)-methyl group (R^4 = Me) at that position caused a decrease in both metabolic stability and affinity.

Table 5 shows the pharmacokinetic profiles and metabolic stabilities of compound **42** in four animal species. In rats, compound **42** displayed acceptable area under the blood concentration time curve (AUC), mean residence time (MRT), and bioavailability (BA) upon oral administration at 1 mg/kg. The MRT value was improved considerably, especially in guinea pigs, dogs, and monkeys. Since the metabolic stability in human microsomes was comparable to that of the four animal species, we would expect a long duration of activity seen in humans.

Compound **42** also had a minimized potential for human CYP3A induction, as examined by the PXR induction assay (3% induction at 10 μM), which suggested a reduced potential for DDI of compound **42** in humans.

4. Conclusion

In summary, the hybridization of substructures (a 3-phenylpiperidine framework and a benzylcarboxamide moiety from two types of NK_1 antagonists) allowed us to generate a novel series of 3-phenylpiperidine-4-carboxamide derivatives with efficacious affinity for the tachykinin NK_1 receptor and reduced CYP3A induction. Although compound **17** had high CNS activity in the in vivo assay, it showed low metabolic stability in human microsomes. We examined two possible approaches for the improvement of its metabolic stability: the reduction of the lipophilicity, and the blocking of a possible metabolic site. We found that compound **42** exhibited a good balance between high metabolic stability and high CNS activity. Compound **42** displayed excellent efficacy in the guinea-pig GR73637-induced locomotive activity assay at 1 and 24 h after oral administration. It was also observed to have good pharmacokinetic profiles across four different animal species with a low potential for PXR induction as seen in the PXR induction assay results. These results suggest that it should have a sufficient duration of activity in humans along with little to no CYP3A induction which would minimize the potential for DDI.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini-200 or a Varian Mercury-300 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Reactions were followed by TLC on Silica gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Chromatographic separations were carried out on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100-200 mesh, Fuji Silysia Chemical Ltd) using the indicated eluents. Preparative HPLC was performed by a Gilson's NEBULA™ Series Preparative HPLC System using an YMC™ CombiRep ODS-AS-5 μM 50 × 20 mm column. A gradient from 5–95% acetonitrile/water containing 0.1% TFA was used to elute samples. The detector wavelength was set at 220 nm. Compounds **4** and **20**, and solvents were commercially available and used as received. Compound **5**[7] and **22e**[8] were prepared in the same or similar manner as reported previously. $MgSO_4$ was used as a drying agent. Yields are not optimized. Chemical intermediates were characterized by ^{1}H NMR. Low-resolution mass spectra (MS) and chemical purity (detection at 220 nm) were determined using a Waters Liquid Chromatography-Mass Spectrometer System with 0.05% TFA containing water/acetonitrile mobile phase. Elemental analyses (C, H, N) were carried out by the Analytical Department of Takeda Chemical Industries.

5.1.1. Ethyl 1-methyl-3-phenylpiperidine-4-carboxylate (5)

To a cooled stirred solution of phenylmagnesium bromide, which was prepared from magnesium (6.46 g, 266 mmol), bromobenzene (41.8 g, 266 mmol) and a catalytic amount of iodine (0.050 g) in Et_2O (170 mL), was added cupper(I) iodide (3.38 g, 17.7 mmol) at between −10 and 0 °C, and then the mixture was cooled to −20 °C. A solution of **4** (25.0 g, 148 mmol) in Et_2O (20 mL) and THF (30 mL) was slowly added thereto at between −50 and 10 °C. After stirring for 30 min, the reaction was quenched by aqueous saturated NH_4Cl, and the product was extracted with EtOAc. The insoluble material was removed by filtration through a pad of Cerite, and then the filtrate was washed with brine, dried over $MgSO_4$, and concentrated. The residual oil was fractionally distilled under reduced pressure to give **5** (12.9 g, 52.2 mmol, 35%) as colorless oil, bp 90–110 °C (1–0.8 mmHg). ^{1}H NMR ($CDCl_3$, δ): 7.45–7.10 (m, 5H), 4.00–3.82 (m, 2H), 3.20–1.80 (m, 11H), 1.04–0.94 (m, 3H). LC–MS *m/z* (ion): 258 $(M+H)^+$.

5.1.2. 1-*tert*-Butyl 4-ethyl 3-phenylpiperidine-1,4-dicarboxylate (6)

To a solution of **5** (9.45 g, 38.2 mmol) in 1,2-dichloroethane (30 mL) was added α-chloroethyl chloroformate (5.50 g, 38.4 mmol) at room temperature, and then the mixture was heated at 100 °C under reflux for 1 h, cooled, and concentrated in vacuo. The residue was dissolved in MeOH (50 mL), and then the solution was heated at 80 °C for 2 h, and concentrated in vacuo. The residue was dispersed in a mixture of EtOAc and aqueous NaOH. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give a pale yellow oil, which was used in the next step without further purification.

This obtained oil was dissolved in CH_3CN (70 mL), and followed by addition of Et_3N (3.9 g, 38.5 mmol) and Boc_2O (12.5 g, 57.3 mmol). After the mixture was stirred for 14 h, the solvent was evaporated, and then the residue was dispersed in a mixture of EtOAc and water. The organic layer was washed with 10% aqueous citric acid and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with an elution of 10% EtOAc/hexanes to give **6** (8.14 g, 24.4 mmol, 64%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, δ):7.35–7.17 (m, 5H), 4.40–3.45 (m, 4H), 3.20–2.69 (m, 4H), 2.05–1.35 (m, 11H), 1.04–0.94 (m, 3H). Chemical purity: 87.6%.

5.1.3. 1-(*tert*-Butoxycarbonyl)-3-phenylpiperidine-4-carboxylic acid (7)

A mixture of **7** (7.40 g, 31.7 mmol) and 2 N KOH (25 mL) in EtOH (25 mL) was heated at 50 °C for 2 h and poured into water and EtOAc. The aqueous layer was made slightly acidic with 2 N HCl and the product was extracted twice with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated to give **7** (5.45 g, 17.8 mmol, 56%) as white powder. ^{1}H NMR ($CDCl_3$, δ):7.34–7.18 (m, 5H), 4.40–4.00 (br, 2H), 3.00–2.60 (m, 4H), 2.05–1.65 (m, 2H), 1.50–1.40 (m, 9H), The CO_2H peak was not observed. Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.54; H, 7.48; N, 4.40. LC–MS *m/z* (ion): 232 (M–^{t}BuO)$^+$.

5.1.4. 1-(*tert*-Butoxycarbonyl)-3-(4-methylphenyl)piperidine-4-carboxylic acid (7a)

This compound was prepared from **4** and 4-methylphenylmagnesium bromide in a manner similar to that described for **7** as white crystals, mp 138–140 °C. ^{1}H NMR ($CDCl_3$, δ): 7.12–7.00 (m, 4H), 4.40–2.60 (m, 7H), 2.35–2.30 (m, 3H), 2.10–1.60 (m, 2H), 1.60–1.30 (m, 9H). Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.64; H, 7.64; N, 4.29. LC–MS *m/z* (ion): 246 (M–^{t}BuO)$^+$.

5.1.5. 1-(*tert*-Butoxycarbonyl)-3-(3-methylphenyl)piperidine-4-carboxylic acid (7b)

This compound was prepared from **4** and 3-methylphenylmagnesium bromide in a manner similar to that described for **7** as white crystals, mp 121–123 °C. ^{1}H NMR ($CDCl_3$, δ):10.50–9.50 (br, 1H), 7.25–6.98 (m, 4H), 4.40–3.40 (m, 3H), 3.16–2.60 (m, 3H), 2.32–2.27 (m, 3H), 2.10–1.65 (m, 2H), 1.60–1.41 (m, 9H). Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.72; H, 7.65; N, 4.46. LC–MS *m/z* (ion): 246 (M–^{t}BuO)$^+$.

5.1.6. 1-(*tert*-Butoxycarbonyl)-3-(2-ethylphenyl)piperidine-4-carboxylic acid (7c)

This compound was prepared from **4** and 2-ethylphenylmagnesium bromide in a manner similar to that described for **7** as white crystals, mp 133–135 °C. ^{1}H NMR ($CDCl_3$, δ):10.50–9.50 (br, 1H), 7.20–7.13 (m, 4H), 4.40–4.00 (m, 2H), 3.16 (dt, J = 11.7, 3.9 Hz, 1H), 2.95–2.60 (m, 5H), 2.10–2.00 (m, 1H), 1.85–1.65 (m, 1H), 1.44 (s, 9H), 1.21 (t, J = 7.5 Hz, 3H). Anal. Calcd for $C_{19}H_{27}NO_4$: C, 68.44; H, 8.16; N, 4.20. Found: C, 68.29; H, 8.08; N, 3.98. LC–MS *m/z* (ion) 260 (M–^{t}BuO)$^+$.

5.1.7. 1-(*tert*-Butoxycarbonyl)-3-(4-fluorophenyl)piperidine-4-carboxylic acid (7d)

This compound was prepared from **4** and 4-fluorophenylmagnesium bromide by the same procedure as described for the synthesis of **7** as white crystals, mp 142–144 °C. ^{1}H NMR ($CDCl_3$, δ): 7.22–7.13 (m, 2H), 7.05–6.88 (m, 2H), 4.40–2.60 (m, 7H), 2.10–1.60 (m, 2H), 1.60–1.30 (m, 9H). Anal. Calcd for $C_{17}H_{22}NO_4F$: C, 63.14; H, 6.86; N, 4.33. Found: C, 63.24; H, 6.97; N, 4.18. LC–MS *m/z* (ion): 250 (M–^{t}BuO)$^+$.

5.1.8. 1-(*tert*-Butoxycarbonyl)-3-(2-methyll-4-fluorophenyl) piperidine-4-carboxylic acid (7e)

This compound was prepared from **4** and 2-methyl-4-fluorophenylmagnesium bromide by the same procedure as described for the synthesis of **7** as white crystals, mp 149–151 °C. ^{1}H NMR ($CDCl_3$, δ): 7.11–7.05 (m, 1H), 6.87–6.80 (m 2H), 4.40–3.40 (m, 3H), 3.18–2.95 (m, 1H), 2.90–2.70 (m, 2H), 2.64–2.45 (m, 1H), 2.36–2.30 (m, 3H), 2.10–2.00 (m, 1H), 1.84–1.66 (m, 1H), 1.55–1.45 (m, 9H). Anal. Calcd for $C_{18}H_{24}FNO_4$: C, 64.08; H, 7.17; N, 4.15. Found: C, 63.98; H, 7.27; N, 4.19. LC–MS *m/z* (ion): 264 (M–^{t}BuO)$^+$.

5.1.9. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl] carbamoyl}-3-phenylpiperidine-1-carboxylate (8)

See Section 5.1.10.

5.1.10. *tert*-Butyl (3*RS*,4*SR*)-4-{[3,5-bis(trifluoromethyl)benzyl] carbamoyl}-3-phenylpiperidine-1-carboxylate (9)

To a mixture of **7** (2.87 g, 9.40 mmol), 3,5-bis(trifluoromethyl)benzylamine (2.74 g, 11.3 mmol) in DMF (20 mL) were added WSC (2.70 g, 14.1 mmol) and HOBt (2.16 g, 14.1 mmol) at room temperature. The mixture was stirred for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc and IPE to give **8** (2.78 g, 5.41 mmol, 58%) as white powder. ^{1}H NMR ($CDCl_3$, δ): 7.72 (s, 1H), 7.38 (s, 2H), 7.25–7.13 (m, 5H), 5.45–5.35 (m, 1H), 4.42–4.10 (m, 3H), 4.15 (dd, J = 15.9 and 5.7 Hz, 1H), 2.93 (td, J = 11.4 and 3.9 Hz, 1H), 3.00–2.65 (br, 2H), 2.44 (td, J = 11.1 and 5.1 Hz, 1H), 2.00–1.86 (m, 2H), 1.46 (s, 9H). Anal. Calcd for $C_{26}H_{28}N_2O_3F_6$: C, 58.86; H, 5.32; N, 5.28. Found: C, 58.71; H, 5.30; N, 5.17. LC–MS *m/z* (ion): 457 (M–^{t}BuO)$^+$.

The filtrate was concentrated and the residue was purified by silica gel chromatography with a gradient elution of 10–20% EtOAc/hexane to give **9** (0.86 g, 1.62 mmol, 33%) as pink powder. ^{1}H NMR ($CDCl_3$, δ): 7.47 (s, 1H), 7.45 (s, 2H), 7.24–7.10 (m, 5H), 5.40–5.20 (br, 1H), 4.30–3.70 (m, 6H), 3.08 (dt, J = 9.6, 4.5 Hz, 1H), 2.72 (dd, J = 9.3, 4.5 Hz, 1H), 2.00–1.90 (m, 2H), 1.45 (s, 9H). Anal. Calcd for $C_{26}H_{28}N_2O_3F_6$: C, 58.86; H, 5.32; N, 5.28. Found: C, 58.71; H, 5.57; N, 5.17. LC–MS *m/z* (ion): 457 (M–^{t}BuO)$^+$.

5.1.11. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl] (methyl)carbamoyl}-3-phenylpiperidine-1-carboxylate (10)

To a cooled solution of **8** (1.48 g, 2.80 mmol) in THF (14 mL) at 5 °C was added sodium *tert*-butoxide (0.54 g, 5.62 mmol) followed by methyl iodide (0.35 mL, 5.62 mmol). The mixture was allowed to warm to room temperature and stirred for 1 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc and hexane to give **10** (1.09 g, 2.00 mmol, 72%) as white powder. ^{1}H NMR ($CDCl_3$, δ): 7.73 (s, 1H), 7.39 (s, 2H), 7.25–7.10 (m, 5H), 4.90–4.68 (m, 1H), 4.50–4.10 (m, 4H), 3.24–2.74 (m, 6H), 2.00–1.70 (m, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{27}H_{30}N_2O_3F_6$: C, 59.55; H, 5.55; N, 5.14. Found: C, 59.26; H, 5.61; N, 5.05. LC–MS *m/z* (ion): 471 (M–^{t}BuO)$^+$.

5.1.12. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl] (methyl)carbamoyl}-3-(4-methylphenyl)piperidine-1-carboxylate (10a)

This compound was prepared from **7a** in a manner similar to that described for **10** in 31% yield as white crystals, mp 160–162 °C. ^{1}H NMR ($CDCl_3$, δ): 7.80–7.72 (m, 1H), 7.41–7.38 (m, 2H), 7.10–6.99 (m, 4H), 4.90–4.10 (m, 4H), 3.20–2.70 (m, 7H), 1.46 (s, 9H), 2.40–2.25 (m, 3H), 2.00–1.70 (m, 2H). Anal. Calcd for $C_{28}H_{32}F_6N_2O_3$: C, 60.21; H, 5.77; N, 5.02. Found: C, 60.19; H, 5.76; N, 5.09. LC–MS *m/z* (ion): 485 (M–^{t}BuO)$^+$.

5.1.13. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl] (methyl)carbamoyl}-3-(3-methylphenyl)piperidine-1-carboxylate (10b)

This compound was prepared from **7b** in a manner similar to that described for **10** as white crystals, mp 111–113 °C. ^{1}H NMR ($CDCl_3$, δ): 7.78–7.72 (m, 1H), 7.39–7.35 (m, 2H), 7.15–6.90 (m, 4H), 5.00–4.00 (m, 6H), 3.20–3.00 (m, 1H), 3.00–2.27 (m, 7H), 2.00–1.75 (m, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{28}H_{32}F_6N_2O_3{\cdot}0.5H_2O$: C, 59.25; H, 5.86; N, 4.94. Found: C, 59.33; H, 5.66; N, 5.03. LC–MS *m/z* (ion): 485 (M–^{t}BuO)$^+$.

5.1.14. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(2-ethylphenyl)piperidine-1-carboxylate (10c)

This compound was prepared from **7c** in a manner similar to that described for **10** as white crystals, mp 129–131 °C. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.70 (m, 1H), 7.41–7.33 (m, 2H), 7.20–7.05 (m, 5H), 4.90–4.10 (m, 4H), 3.30–3.15 (m, 1H), 3.15–2.55 (m, 7H), 2.00–1.80 (m, 2H), 1.46 (s, 9H), 1.25 (t, J = 7.5 Hz, 3H). Anal. Calcd for $C_{29}H_{34}F_6N_2O_3$: C, 60.83; H, 5.99; N, 4.89. Found: C, 60.84; H, 5.84; N, 4.90. LC–MS m/z (ion): 499 (M–^{t}BuO)$^+$.

5.1.15. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(4-fluorophenyl)piperidine-1-carboxylate (10d)

This compound was prepared from **7d** in a manner similar to that described for **10** as white crystals, mp 159–161 °C. ^{1}H NMR ($CDCl_3$, δ): 7.74 (s, 1H), 7.40 (s, 2H), 7.20–7.10 (m, 2H), 7.00–6.89 (m, 2H), 5.00–4.00 (m, 4H), 3.20–3.10 (m, 1H), 3.05–2.90 (m, 1H), 2.90–2.65 (m, 5H), 2.00–1.80 (m, 2H), 1.46 (s, 9H). Anal. Calcd for $C_{27}H_{29}F_7N_2O_3$: C, 57.65; H, 5.20; N, 4.98. Found: C, 57.65; H, 5.33; N, 4.91. LC–MS m/z (ion): 507 (M–^{t}Bu+2H)$^+$.

5.1.16. *tert*-Butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(4-fluoro-2-methylphenyl)piperidine-1-carboxylate (10e)

This compound was prepared from **7e** in a manner similar to that described for **10** as white crystals, mp 154–156 °C. ^{1}H NMR ($CDCl_3$, δ): 7.84–7.72 (m, 1H), 7.45–7.36 (m, 2H), 7.05–6.68 (m, 3H), 4.90–3.90 (m, 4H), 3.39 (dt, J = 11.1 and 4.2 Hz, 1H), 3.25–2.55 (m, 6H), 2.50–2.35 (m, 3H), 1.95–1.75 (m, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{28}H_{31}F_7N_2O_3$: C, 58.33; H, 5.42; N, 4.86. Found: C, 58.35; H, 5.43; N, 4.70. LC–MS m/z (ion): 521 (M–^{t}Bu+2H)$^+$.

5.1.17. *tert*-Butyl (3*RS*,4*SR*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-phenylpiperidine-1-carboxylate (11)

This compound was prepared from **9** in a manner similar to that described for **10** in 65% yield as white powder. ^{1}H NMR ($CDCl_3$, δ): 7.77 (m, 1H), 7.53 (s, 2H), 7.35–7.28 (m, 1H), 7.22–7.10 (m, 4H), 4.70–3.40 (m, 7H), 3.38–3.30 (m, 1H), 3.15–2.95 (m, 1H), 2.54 (brs, 2H), 1.95–1.75 (m, 2H), 1.46 (s, 9H). Anal. Calcd for $C_{27}H_{30}N_2O_3F_6$: C, 59.55; H, 5.55; N, 5.14. Found: C, 59.38; H, 5.49; N, 5.14. LC–MS m/z (ion): 471 (M–^{t}BuO)$^+$.

5.1.18. (3*RS*,4*RS*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide (12)

To a cooled solution of **10** (1.05 g, 1.93 mmol) in THF (5 mL) was added TFA (10 mL) at 5 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h and concentrated. The residue was taken into aqueous NaOH and EtOAc, and then the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give **12** (0.50 g, 1.12 mmol, 58%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.78–7.71 (m 1H), 7.44–7.34 (m, 2H), 7.30–7.14 (m, 5H), 4.76 (d, J = 15.0 Hz, 1H), 4.19 (d, J = 15.0 Hz, 1H), 3.60–3.10 (m, 7H), 2.80–2.72 (m, 3H), 2.30–1.80 (m, 2H). LC–MS m/z (ion): 445 (M+H)$^+$. Chemical purity: 100.0%.

5.1.19. (3*RS*,4*RS*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide hydrochloride (12e)

A mixture of **10e** (13.5 g, 23.4 mmol) in 4 N HCl–EtOAc (23.4 mL) was heated at 50 °C for 2 h and concentrated. The residue was crystallized from EtOAc and IPE to give **12e** (10.2 g, 19.8 mmol, 84.6%) as white crystals, mp 229–233 °C (dec). ^{1}H NMR (DMSO-d_6, δ): 9.43–8.76 (br, 2H), 8.21–7.83 (m, 1H), 7.77–7.50 (m, 2H), 7.50–6.58 (m, 3H), 5.06–4.09 (m, 2H), 3.75–2.69 (m, 10H), 2.69–2.20 (m, 2H), 2.16–1.96 (m, 1H), 1.96–1.68 (m, 1H), NH and HCl peaks were not observed. Anal. Calcd for $C_{23}H_{24}F_7N_2OCl$: C, 53.86; H, 4.72; N, 5.46. Found: C, 53.82; H, 4.64; N, 5.44. LC–MS m/z (ion): 477 (M–HCl+H)$^+$. Chemical purity: 100.0%.

5.1.20. (3*RS*,4*SR*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide hydrochloride (13)

To a cooled solution of **11** (0.49 g, 0.90 mmol) in THF (1 mL) was added TFA (5 mL) at 5 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h and concentrated. The residue was taken into aqueous NaOH and EtOAc, and then the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give colorless oil. ^{1}H NMR ($CDCl_3$, δ): 7.80–7.15 (m, 8H), 4.52 (d, J = 22 Hz, 1H), 4.42 (d, J = 22 Hz, 1H), 4.20–2.40 (m, 10H), 2.20–1.80 (m, 2H). The residue was treated with 1 equivalent of 4 N HCl–EtOAc to give **13** (0.40 g, 0.81 mmol, 90%) as white powder. LC–MS m/z (ion): 445 (M–HCl+H)$^+$. Chemical purity: 82.4%.

5.1.21. (3*RS*,4*RS*)-1-Acetyl-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide (14)

To a cooled solution of **12** (0.15 g, 0.34 mmol) and Et_3N (0.10 mL, 0.72 mmol) in THF (5 mL) was added acetyl chloride (0.048 mL, 0.68 mmol) at 5 °C, and the mixture was allowed to warm to room temperature and stirred for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 67–80% EtOAc/hexanes then 0 to 10% MeOH/EtOAc to give **14** (0.11 g, 0.23 mmol, 67%) as white crystals, which was crystallized from EtOAc and IPE, mp 153–155 °C. ^{1}H NMR ($CDCl_3$, δ): 7.80–7.72 (m, 1H), 7.42–7.32 (m, 2H), 7.30–7.13 (m, 5H), 4.90–3.90 (m, 4H), 3.30–2.60 (m, 7H), 2.20–2.10 (m, 3H), 2.00–1.80 (m, 2H). Anal. Calcd for $C_{24}H_{24}F_6N_2O_2$: C, 59.26; H, 4.97; N, 5.76. Found: C, 59.06; H, 4.85; N, 5.71. LC–MS m/z (ion): 487 (M+H)$^+$.

5.1.22. (3*RS*,4*SR*)-1-Acetyl-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide (15)

This compound was prepared from **13** in a manner similar to that described for **14** in 66% yield as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.77–7.10 (m, 8H), 4.70–4.30 (m, 4H), 4.00–2.00 (m, 10H), 2.00–1.80 (m, 2H). LC–MS m/z (ion): 487 (M+H)$^+$. Chemical purity: 99.9%.

5.1.23. (3*RS*,4*RS*)-1-(*N*-Acetylglycyl)-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide (16)

To a solution of **12** (0.24 g, 0.50 mmol), acetylglycine (0.090 g, 0.76 mmol) and Et_3N (0.070 mL, 0.75 mmol) in DMF (5 mL) were added WSC (0.144 g, 0.75 mmol) and HOBt (0.115 g, 0.75 mmol) at room temperature, and the mixture was stirred for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by preparative HPLC to give **16** (0.26 g, 0.478 mmol, 96%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.81–7.74 (m, 1H), 7.39–7.35 (m, 2H), 7.30–7.10 (m, 5H), 6.68–6.58 (m, 1H), 4.90–4.70 (m, 2H), 4.40–3.10 (m, 7H), 2.90–2.70 (m, 4H), 2.10–1.80 (m, 5H). LC–MS m/z (ion): 544 (M+H)$^+$. Chemical purity: 97.2%.

5.1.24. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-phenylpiperidine-4-carboxamide (17)

This compound was prepared from **12** and 1-acetylpiperidine-4-carboxylic acid in a manner similar to that described for **16** in 94% yield as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.74 (m, 1H), 7.45–7.34 (m, 2H), 7.29–7.10 (m, 5H), 5.00–3.80, 3.30–2.60, and 2.20–1.60 (m, total 25H). LC–MS m/z (ion): 598 (M+H)$^+$.

5.1.25. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-(4-methylphenyl) piperidine-4-carboxamide (17a)

This compound was prepared from **10a** in a manner similar to that described for **17** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.81–7.73 (m, 1H), 7.48–7.34 (m, 2H), 6.98–7.14 (m, 4H), 5.00–3.75, 3.30–2.60, and 2.40–1.60 (m, total 28H). LC–MS *m/z* (ion): 628 $(M+H)^+$. Chemical purity: 100.0%.

5.1.26. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-(3-methylphenyl) piperidine-4-carboxamide (17b)

This compound was prepared from **10b** in a manner similar to that described for **17** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.80–7.72 (m, 1H), 7.44–7.30 (m, 2H), 7.20–6.92 (m, 4H), 4.90–3.80, 3.30–2.60, and 2.40–1.60 (m, total 28H). LC–MS *m/z* (ion): 612 $(M+H)^+$. Chemical purity: 98.9%.

5.1.27. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-(2-ethylphenyl)-*N*-methylpiperidine-4-carboxamide (17c)

This compound was prepared from **10c** in a manner similar to that described for **17** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.70 (m, 1H), 7.42–7.28 (m, 2H), 7.20–7.00 (m, 4H), 5.00–2.50 and 2.15–1.55 (m, total 27H), 1.30–1.15 (m, 3H). LC–MS *m/z* (ion): 626 $(M+H)^+$. Chemical purity: 99.7%.

5.1.28. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-(4-fluorophenyl)-*N*-methylpiperidine-4-carboxamide (17d)

This compound was prepared from **10d** in a manner similar to that described for **17** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.74 (m, 1H), 7.45–7.34 (m, 2H), 7.25–7.10 (m, 2H), 7.04–6.88 (m, 4H), 5.00–3.80, 3.30–2.60 and 2.20–1.60 (m, total 25H). LC–MS *m/z* (ion): 616 $(M+H)^+$. Chemical purity: 99.1%.

5.1.29. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (17e)

This compound was prepared from **10e** in a manner similar to that described for **17** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.50–7.30 (m, 2H), 7.15–6.75 (m, 3H), 4.90–3.70, 3.50–2.30 and 2.20–1.60 (m, total 28H). LC–MS *m/z* (ion): 630 $(M+H)^+$. Chemical purity: 94.6%.

5.1.30. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-(2-methylphenyl) piperidine-4-carboxamide (17f)

To a mixture of **22f** (2.40 g, 7.51 mmol), *N*-methyl-3,5-bis(trifluoromethyl)benzylamine hydrochloride (2.52 g, 9.01 mmol) and Et_3N (1.26 mL, 9.01 mmol) in CH_3CN (30 mL) were added WSC (1.73 g, 9.01 mmol) and HOBt (0.69 g, 4.51 mmol) and then the mixture was stirred for 18 h and poured into aqueous saturated $NaHCO_3$ and EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 10–50% EtOAc/hexanes to give *tert*-butyl (3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(2-methylphenyl) piperidine-1-carboxylate (2.87 g, 5.14 mmol, 68%) as white powder, which was crystallized from EtOAc, IPE and hexanes, mp 121–122 °C. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.70 (m, 1H), 7.44–7.35 (m, 2H), 7.20–6.90 (m, 4H), 4.90–4.00 (m, 4H), 3.43 (dt, J = 11.1, 3.6 Hz, 1H), 3.30–3.15 (m, 1H), 3.00–2.60 (m, 5H), 2.47–2.41 (m, 3H), 2.00–1.80 (m, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{28}H_{32}F_6N_2O_3$: C, 60.21; H, 5.77; N, 5.02. Found: C, 60.26; H, 5.75; N, 5.09. LC–MS *m/z* (ion): 485 $(M-{}^tBuO)^+$.

A mixture of the obtained powder (2.37 g, 4.24 mmol) in 4 N HCl–EtOAc (16 mL) was stirred at room temperature for 14 h and concentrated. The residue was crystallized from EtOAc and IPE to give (3*RS*,4*RS*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-(2-methylphenyl)piperidine-4-carboxamide hydrochloride (2.08 g, 4.20 mmol, 99%) as white powder, mp 245–247 °C. ^{1}H NMR (DMSO-d_6, δ): 9.20–8.50 (br, 2H), 8.07–7.96 (m, 1H), 7.72–7.59 (m, 2H), 7.40–6.90 (m, 3H), 5.02–4.59 (m, 1H), 4.31–4.23 (m, 1H), 4.15–2.90 (m, 10H), 2.40–2.30 (m, 3H), 2.20–1.75 (m, 2H). Anal. Calcd for $C_{23}H_{25}ClF_6N_2O$: C, 55.82; H, 5.09; N, 5.66. Found: C, 55.57; H, 4.92; N, 5.64. LC–MS *m/z* (ion): 459 $(M-HCl+H)^+$.

To a mixture of the obtained powder (0.24 g, 0.485 mmol), 1-acetylpiperidine-4-carboxylic acid (0.125 g, 0.728 mmol) and Et_3N (0.070 mL, 0.485 mmol) in CH_3CN (5 mL) were added WSC (0.190 g, 0.970 mmol) and HOBt (0.110 g, 0.728 mmol) and then the mixture was stirred for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by basic silica gel chromatography with a gradient elution of 50–100% EtOAc/hexanes to give **17f** (0.210 g, 0.343 mmol, 71%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.82–7.71 (m, 1H), 7.42–7.36 (m, 2H), 7.70–7.18 (m, 4H), 5.00–3.70, 3.55–2.30 and 2.15–1.60 (m, total 28H). LC–MS *m/z* (ion): 612 $(M+H)^+$. Chemical purity: 99.7%.

5.1.31. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-3-[2-(1-methylethyl) phenyl]piperidine-4-carboxamide (17g)

This compound was prepared from **22f** in a manner similar to that described for **17f** as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.81–7.70 (m, 1H), 7.40–7.00 (m, 6H), 4.95–2.50 and 2.15–1.60 (m, total 26H), 1.40–1.20 (m, 6H). LC–MS *m/z* (ion): 640 $(M+H)^+$. Chemical purity: 100.0%.

5.1.32. (3*RS*,4*RS*)-1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-phenylpiperidine-4-carboxamide (18)

A mixture of **8** (0.39 g, 0.73 mmol) and 4 N HCl–EtOAc (0.73 mL) in MeOH (5 mL) was stirred at 50 °C for 2 h and concentrated. The residue was crystallized from EtOAc–MeOH to give white powder (0.34 g).

To a mixture of the powder (0.20 g), 1-acetylpiperidine-4-carboxylic acid (0.11 g, 0.64 mmol) and Et_3N (0.060 mL, 0.643 mmol) in DMF (10 mL) were added WSC (0.123 g, 0.64 mmol) and HOBt (0.098 g, 0.64 mmol) and then the mixture was stirred at room temperature for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by preparative HPLC to give **18** (0.22 g, 0.38 mmol, 88%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.73 (s, 1H), 7.38 (s, 2H), 7.28–7.10 (m, 5H), 5.60–5.40 (m, 1H), 4.90–4.70 (m, 1H), 4.90–3.80 (m, 5H), 3.30–2.50 (m, 7H), 2.10–1.60 (m, 9H). LC–MS *m/z* (ion): 584 $(M+H)^+$. Chemical purity: 100.0%.

5.1.33. 1-[(1-Acetylpiperidin-4-yl)carbonyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenyl-1,2,3,6-tetrahydropyridine-4-carboxamide (19)

A mixture of **23** (0.50 g, 1.52 mmol) in $SOCl_2$ (3 mL) was heated at 90 °C for 1 h and concentrated. The residue was dissolved in CH_3CN (5 mL). The solution was added to a cooled solution of *N*-methyl-3,5-bis(trifluoromethyl)benzylamine hydrochloride (0.67 g, 2.28 mmol) and Et_3N (0.96 mL, 6.88 mmol) in CH_3CN (15 mL) at 0 °C. The mixture was allowed to warm to room temperature, stirred for 1 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica

gel chromatography with a gradient elution of 50–80% EtOAc/hexanes then 10% MeOH/EtOAc to give pale brown amorphous (0.70 g, 1.31 mmol, 86%). ^{1}H NMR ($CDCl_3$, δ): 7.84–7.58 and 7.50–7.40 (m, total 7H), 7.34–7.14 (m, 6H), 4.84–4.05 (m, 4H), 3.60–3.40 (m, 2H), 2.70–3.20 (m, 7H). LC–MS *m/z* (ion): 533 $(M+H)^+$.

A mixture of the amorphous (2.05 g, 3.85 mmol) and 1-chloroethyl chloroformate (1.64 mL, 15.2 mmol) in 1,2-dichloroethane (20 mL) was heated at 100 °C for 1 h. Additional 1-chloroethyl chloroformate (1.64 mL, 15.2 mmol) was added thereto, and the mixture was heated at 100 °C for 1 h and concentrated. The residue was dissolved in MeOH (30 mL) and then the mixture was heated at 90 °C for 1 h and concentrated. The residue was suspended in CH_3CN (20 mL) at room temperature followed by Et_3N (0.64 mL, 8.72 mmol) and Boc_2O (4.58 mmol). The mixture was stirred at room temperature for 14 h and poured into water and EtOAc. The organic layer was washed with 10% aqueous citric acid and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 20–50% EtOAc/hexanes to give pale brown amorphous (1.24 g, 2.28 mmol, 75%). ^{1}H NMR ($CDCl_3$, δ): 7.74 (s, 1H), 7.46 (s, 2H), 7.34–7.14 (m, 5H), 5.00–2.20 (m, 11H), 1.41 (s, 9H). LC–MS *m/z* (ion): 487 $(M-{}^t\text{Bu}+2H)^+$.

A mixture of the amorphous (1.24 g, 2.28 mmol) and 4 N HCl–EtOAc (2.3 mL) in MeOH (15 mL) was heated at 50 °C for 3 h and concentrated to give amorphous (1.06 g, 2.21 mmol, 97%). LC–MS (*m/z*): 443 $(M-HCl+H)^+$.

A mixture of the amorphous (0.20 g, 0.42 mmol), 1-acetylpiperidinecarboxylic acid (0.11 g, 0.63 mmol), WSC (0.12 g, 0.63 mmol), HOBt (0.096 g, 0.63 mmol) and Et_3N (0.088 mL, 0.63 mmol) in DMF (10 mL) was stirred at room temperature for 14 h and poured into water and EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by preparative HPLC to give **19** (0.19 g, 0.31 mmol, 75%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.55–7.15 (m, 8H), 5.00–2.20 (m, 16H), 2.20–1.60 (m, 7H). LC–MS *m/z* (ion): 596 $(M+H)^+$. Chemical purity: 93.9%.

5.1.34. Ethyl 1-benzyl-5-(2-methylphenyl)-1,2,3,6-tetrahydropyridine-4-carboxylate (21f)

To a mixture of sodium hydride (7.36 g, 0.184 mol) in DMF (150 mL) was added a mixture for **20** (35.3 g, 0.123 mol) in DMF (50 mL) at 5 °C. After the mixture was stirred at 5 °C for 5 min, *N*-phenylbis(trifluoromethanesulfonimide) (49.2 g, 0.135 mol) was added thereto. After stirred at 5 °C for 2 h, the mixture was poured into water and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO_2 with an elution of 9% EtOAc/hexane to give an oil.

The oil was added to a mixture of 2-methylphenylboronic acid (25.0 g, 0.184 mol), $Pd(PPh_3)_4$ (7.08 g, 0.0061 mol) and K_2CO_3 (17.0 g, 0.123 mol) in water (25 mL) and toluene (420 mL). The mixture was degassed with argon, and heated at 100 °C for 14 h and concentrated. The residue was poured into water and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO_2 with an elution of 9% EtOAc/hexane to give **21f** (39.6 g, 0.11 mmol, 96%) as a colorless oil. ^{1}H NMR ($CDCl_3$, δ): 7.38–7.21 (m, 5H), 7.16–7.07 (m, 3H), 6.97–6.92 (m, 1H), 3.84 (q, J = 6.9 Hz, 2H), 3.64 (dd, J = 24.9 and 13.2 Hz, 2H), 3.11 (q, J = 2.3 Hz, 2H), 2.72 (q, J = 5.3 Hz, 2H), 2.63–2.56 (m, 2H), 2.18 (s, 3H), 0.82 (t, J = 7.2 Hz, 3H). LC–MS *m/z* (ion): 336 $(M+H)^+$. Chemical purity: 97.6%.

5.1.35. Ethyl 1-benzyl-5-[2-(1-methylethyl)phenyl]-1,2,3,6-tetrahydropyridine-4-carboxylate (21g)

This compound was prepared from **20** and 2-(1-methylethyl)phenylboronic acid in a manner similar to that described for **21f** as an oil. ^{1}H NMR ($CDCl_3$, δ): 7.19 (m, 7H), 7.10–7.03 (m, 1H), 6.91–6.88 (m, 1H), 3.92–3.75 (m, 2H), 3.74 (d, J = 13.2 Hz, 1H), 3.53 (d, J = 13.2 Hz, 1H), 3.20–2.90 (m, 2H), 2.90–2.80 (m, 2H), 2.70–2.60 (m, 3H), 1.13 (d, J = 6.9 Hz, 3H), 1.06 (d, J = 6.9 Hz, 3H), 0.75 (t, J = 7.1 Hz, 3H). LC–MS *m/z* (ion): 364 $(M+H)^+$. Chemical purity: 94.4%.

5.1.36. Ethyl 1-benzyl-5-phenyl-1,2,3,6-tetrahydropyridine-4-carboxylate (21h)

This compound was prepared from **20** and phenylboronic acid in a manner similar to that described for **21f** as an oil. ^{1}H NMR ($CDCl_3$, δ): 7.38–7.20 (m, 8H), 7.13–7.07 (m, 2H), 3.87 (q, J = 7.1 Hz, 2H), 3.66 (s, 2H), 3.26 (t, J = 2.7 Hz, 2H), 2.72–2.67 (m, 2H), 2.60–2.55 (m, 2H), 0.84 (t, J = 7.1 Hz, 3H). LC–MS *m/z* (ion): 322 $(M+H)^+$. Chemical purity: 77.6%.

5.1.37. (3*RS*,4*RS*)-1-(*tert*-Butoxycarbonyl)-3-(4-fluoro-2-methylphenyl)piperidine-4-carboxylic acid (22e)[8]

5.1.37.1. Ethyl 3-(4-fluoro-2-methylphenyl)-1-methylpiperidine-4-carboxylate. This compound was prepared from **4** in a manner similar to that described for **5** as an oil, bp 70–115 °C (1 mmHg). ^{1}H NMR ($CDCl_3$, δ): 7.25–6.75 (m, 3H), 3.95–3.75 (m, 2H), 3.20–1.80 (m, 14H), 1.01–0.85 (m, 3H). LC–MS *m/z* (ion): 280 $(M+H)^+$.

5.1.37.2. 1-*tert*-Butyl 4-ethyl 3-(4-fluoro-2-methylphenyl)piperidine-1,4-dicarboxylate. This compound was prepared from ethyl 3-(4-fluoro-2-methylphenyl)-1-methylpiperidine-4-carboxylate in a manner similar to that described for **6** as an oil. ^{1}H NMR ($CDCl_3$, δ): 7.21–7.13 (m, 1H), 6.90–7.01 (m, 2H), 4.30–3.40 and 3.20–2.64 (m, 8H), 2.40–2.34 (m, 3H), 2.00–1.60 (m, 2H), 1.55–1.35 (m, 9H), 1.06–0.96 (m, 3H).

5.1.37.3. (3*RS*,4*RS*)-1-(*tert*-Butoxycarbonyl)-3-(4-fluoro-2-methylphenyl)piperidine-4-carboxylic acid. To a solution of 1-*tert*-butyl 4-ethyl 3-(4-fluoro-2-methylphenyl)piperidine-1,4-dicarboxylate (44.3 g, 121 mmol) in MeOH (180 mL) was added 28% NaOMe in MeOH (37 mL). After the mixture was heated under reflux for 3 h, 2 N NaOH (91 mL) and THF (90 mL) were added. The mixture was stirred at 50 °C for 3 h, cooled to room temperature and made neutral with 1 M $KHSO_4$. The product was extracted twice with EtOAc and the extract was washed with water and brine, dried (Na_2SO_4) and filtered through a pad of silica gel. The filtrate was concentrated and crystallized from EtOAc, IPE and hexane to give **22e** (36.0 g, 107 mmol, 88%) as white crystals. ^{1}H NMR ($CDCl_3$, δ): 7.09–6.81 (m, 3H), 4.26 (m, 1H), 4.04 (m, 1H), 3.12–3.05 (m, 1H), 2.86–2.79 (m, 2H), 2.53 (m, 1H), 2.35 (s, 3H), 2.04–2.00 (br, 1H), 1.78–1.68 (m, 1H), 1.46 (br, 9H), The CO_2H peak was not observed. Anal. Calcd for $C_{18}H_{24}NO_4F{\cdot}0.25H_2O$: C, 63.23; H, 7.22; N, 4.10. Found: C, 63.42; H, 7.11; N, 4.01. Chemical purity: 99.9%.

5.1.38. (3*RS*,4*RS*)-1-(*tert*-Butoxycarbonyl)-3-(2-methylphenyl)piperidine-4-carboxylic acid (22f)

To a solution of **21f** (39.6 g, 0.118 mol) in EtOAc (400 mL) was added 4 N HCl–EtOAc (32.5 mL, 0.130 mol), and then the precipitate was collected by filtration to give white powder. The obtained powder was dissolved in EtOH (1000 mL), and followed by 10% Pd–C (12 g). The mixture was stirred at room temperature under H_2 (3 atm) for 7 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was dissolved in CH_3CN (1000 mL), and followed by Boc_2O (39.2 g, 0.18 mol) and Et_3N (30.4 mL, 0.218 mol). After stirred at room temperature for 14 h, the mixture was poured into water and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO_2 to a colorless oil (34.4 g, 0.099 mol, 84%). The

obtained oil (22.9 g, 0.066 mol) was dissolved in MeOH (210 mL), and followed by 28% NaOMe in MeOH (25.2 mL). After the mixture was heated under reflux for 3 h, 2 N NaOH (165 mL) and THF (84.1 mL) were added. The mixture was stirred at 60 °C for 14 h, cooled to room temperature and made neutral with 2 N HCl. The product was extracted with EtOAc and the extract was washed with water and brine, dried (Na_2SO_4) and concentrated to give **22f** (18.8 g, 0.059 mol, 89%) as white crystals, mp 172–174 °C. ^{1}H NMR ($CDCl_3$, δ): 10.50–9.50 (br, 1H), 7.19–7.08 (m, 4H), 4.35–4.22 (m, 1H), 4.16–4.03 (m, 1H), 3.15 (dt, *J* = 11.5 and 4.0 Hz, 1H), 2.90 (dt, *J* = 11.6 and 3.6 Hz, 1H), 2.79 (t, J = 12.9 Hz, 1H), 2.57 (t, *J* = 12.3 Hz, 1H), 2.38 (s, 3H), 2.08–1.99 (m, 1H), 1.83–1.65 (m, 1H), 1.45 (s, 9H). Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.48; H, 7.85; N, 4.32.

5.1.39. (3*RS*,4*RS*)-1-(*tert*-Butoxycarbonyl)-3-[2-(1-methylethyl)phenyl]piperidine-4-carboxylic acid (22g)

This compound was prepared from **21g** in a manner similar to that described for **22f** as white crystals, mp 163–165 °C. ^{1}H NMR ($CDCl_3$, δ): 7.28–7.10 (m, 4H), 4.40–4.00 (m, 2H), 3.40–3.20 (m, 2H), 3.00–2.55 (m, 4H), 2.10–1.95 (m, 1H), 1.85–1.65 (m, 1H), 1.44 (s, 9H), 1.24 (d, *J* = 6.9 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 3H). Anal. Calcd for $C_{20}H_{29}NO_4$: C, 69.14; H, 8.41; N, 4.03. Found: C, 68.93; H, 8.37; N, 3.91.

5.1.40. 1-Benzyl-5-phenyl-1,2,3,6-tetrahydropyridine-4-carboxylic acid hydrochloride (23)

A mixture of **21h** (2.00 g, 6.22 mmol) in acetic acid (5 mL) and hydrochloric acid (5 mL) was heated at 100 °C for 14 h and concentrated. The residue was crystallized from EtOAc, IPE and hexane to give **23** (1.03 g, 3.12 mmol, 50%) as white crystals. ^{1}H NMR ($CDCl_3$, δ): 11.23 (br, 1H), 7.70–7.60 (m, 2H), 7.50–7.30 (m, 6H), 7.24–7.15 (m, 2H), 4.39 (m, 2H), 3.91 (s, 2H), 3.37 (m, 2H), 2.52 (m, 2H), HCl peak was not observed. LC–MS *m/z* (ion): 294 $(M-HCl+H)^+$. Chemical purity: 94.8%.

5.1.41. (3*RS*,4*RS*)-1-(*N*-Acetylglycyl)-*N*-[3,5-bis(trifluoro methyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (24)

This compound was prepared from **12e** and *N*-acetylglycine by the same procedure as described for the synthesis of **16** in 76% yield as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.46–7.34 (m, 2H), 7.10–6.60 (m, 4H), 4.90–4.45 (m, 2H), 4.25–2.35 (m, 14H), 2.10–1.80 (m, 5H). Anal. Calcd for $C_{27}H_{28}N_3O_3F_7$: C, 56.35; H, 4.90; N, 7.30. Found: C, 56.23; H, 5.03; N, 7.14. LC–MS *m/z* (ion): 576 $(M+H)^+$.

5.1.42. (3*RS*,4*RS*)-N^4-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-N^1,N^4-dimethylpiperidine-1,4-dicarboxamide (25)

To a cooled solution of **12e** (0.15 g, 0.29 mmol) and Et_3N (0.082 mL, 0.59 mmol) in THF (5 mL) was added bis(trichloromethyl)carbonate (0.17 g, 0.57 mmol) at 0 °C, and the mixture was allowed to warm to room temperature. After stirring for 1 h, 40% aqueous $MeNH_2$ (1 mL, 12.9 mmol) was added thereto. The mixture was stirred for 14 h and poured into H_2O and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 50% EtOAc/hexanes then 10% MeOH/EtOAc to give **25** (0.11 g, 0.21 mmol, 72%) as white crystals, mp 118–120 °C. ^{1}H NMR ($CDCl_3$, δ): 7.83–7.71 (m, 1H), 7.46–7.32 (m, 2H), 7.09–6.98 (m, 1H), 6.94–6.70 (m, 2H), 4.82–4.40 (m, 2H), 4.30–4.05 (m, 2H), 4.00–3.80 (m, 1H), 3.50–3.40 (m, 1H), 3.25–2.70 (m, 9H), 2.50–2.35 (m, 3H), 2.00–1.80 (m, 2H). Anal. Calcd for $C_{25}H_{26}N_3O_2F_7$: C, 56.28; H, 4.91; N, 7.88. Found: C, 56.24; H, 4.94; N, 7.85. LC–MS *m/z* (ion): 534 $(M+H)^+$.

5.1.43. (3*RS*,4*RS*)-N^4-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-N^1-methylpiperidine-1,4-dicarboxamide (26)

This compound was prepared from **12e** and 28% ammonia solution by the similar procedure as described for the synthesis of **25** in 67% yield as white crystals, mp 187–189 °C. ^{1}H NMR ($CDCl_3$, δ): 7.84–7.73 (m, 1H), 7.45–7.35 (m, 2H), 7.08–7.00 (m, 1H), 6.86–6.70 (m, 2H), 4.82 (d, *J* = 15.0 Hz, 1H), 4.60–4.40 (m, 2H), 4.21 (d, *J* = 15.0 Hz, 1H), 4.30–4.10 (m, 1H), 3.90–3.80 (m, 1H), 3.55–3.40 (m, 1H), 3.20–2.75 (m, 6H), 2.47–2.34 (m, 3H), 2.10–1.90 (m, 2H). Anal. Calcd for $C_{24}H_{24}N_3O_2F_7{\cdot}0.5H_2O$: C, 54.55; H, 4.77; N, 7.95. Found: C, 54.37; H, 4.54; N, 7.92. LC–MS *m/z* (ion): 520 $(M+H)^+$.

5.1.44. (3*RS*,4*RS*)-N^4-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-N^1-methoxy-N^4-methylpiperidine-1,4-dicarboxamide (27)

This compound was prepared from **12e** and *O*-methylhydroxylamine hydrochloride by the similar procedure as described for the synthesis of **25** in 56% yield as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.83–7.72 (m, 1H), 7.42–7.35 (m, 2H), 6.98–6.70 (m, 4H), 4.84–4.45 (m, 1H), 4.30–3.90 (m, 3H), 3.72 (s, 3H), 3.54–2.30 (m, 10H), 2.00–1.85 (m, 2H). Anal. Calcd for $C_{25}H_{26}N_3O_3F_7{\cdot}0.1$diisopropyl ether: C, 54.94; H, 4.93; N, 7.51. Found: C, 54.75; H, 5.02; N, 7.31. LC–MS *m/z* (ion): 550 $(M+H)^+$.

5.1.45. [(3*RS*,4*RS*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(4-fluoro-2-methylphenyl)piperidin-1-yl](oxo)acetate (28)

To a cooled solution of **12e** (0.30 g, 0.59 mmol) and Et_3N (0.082 mL, 0.59 mmol) in CH_2Cl_2 (10 mL) was added ethyl oxalyl chloride (0.10 mL, 0.89 mmol) at 0 °C, and the mixture was allowed to warm to room temperature. After stirring for 14 h, additional ethyl oxalyl chloride (0.10 mL, 0.89 mmol) was added, and the mixture was stirred for further 14 h and poured into H_2O and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated to give **28** (0.33 g, 0.57 mmol, 97%) as white crystals, mp 184–186 °C. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.34 (m, 1H), 7.10–7.00 (m, 2H), 6.96–6.75 (m, 2H), 4.92–2.35 (m, 16H), 2.05–1.90 (m, 2H), 1.45–1.30 (m, 3H). Anal. Calcd for $C_{27}H_{27}N_2O_4F_7$: C, 56.25; H, 4.72; N, 4.86. Found: C, 56.00; H, 4.75; N, 4.57. LC–MS *m/z* (ion): 577 $(M+H)^+$.

5.1.46. (3*RS*,4*RS*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methyl-1-[(methylamino)(oxo) acetyl]piperidine-4-carboxamide (29)

A solution of **28** (0.10 g, 0.17 mmol) and 40% aqueous $MeNH_2$ (1 mL) in EtOH (1.5 mL) was heated in a sealed tube at 100 °C for 4 h and poured into H_2O and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 50% EtOAc/hexanes then 10% MeOH/EtOAc to give **29** (0.028 g, 0.050 mmol, 29%) as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.46–7.35 (m, 2H), 7.30–6.70 (m, 2H), 5.42–5.09 (m, 1H), 4.84 (d, *J* = 15.0 Hz, 1H), 4.80–4.50 (m, 1H), 4.20 (d, *J* = 15.0 Hz, 1H), 3.65–2.60 (m, 11H), 2.50–2.40 (m, 3H), 2.10–1.80 (m, 2H), The NH peak was not observed. LC–MS *m/z* (ion): 562 $(M+H)^+$. Chemical purity: 92.1%.

5.1.47. (3*RS*,4*RS*)-1-[Amino(oxo)acetyl]-*N*-[3,5-bis(trifluoro methyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (30)

A solution of **28** (0.53 g, 0.92 mmol) and 28% ammonia solution (10 mL) in EtOH (5 mL) was stirred at room temperature for 6 h and poured into H_2O and EtOAc. The organic layer was washed with aqueous saturated NH_4Cl and brine, dried over $MgSO_4$, and

concentrated. The residue was purified by silica gel chromatography with a gradient elution of 50 to 100% EtOAc/hexanes to give **30** (0.46 g, 0.84 mmol, 91%) as white crystals, mp 111–113 °C. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.46–7.35 (m, 2H), 7.10–6.70 (m, 3H), 4.84 (d, J = 15.0 Hz, 1H), 5.66–4.50 (m, 3H), 4.20 (d, J = 15.0 Hz, 1H), 3.70–2.30 (m, 11H), 2.10–1.80 (m, 2H). Anal. Calcd for $C_{25}H_{24}N_3O_3F_7$: C, 54.85; H, 4.42; N, 7.68. Found: C, 54.51; H, 4.40; N, 7.46. LC–MS m/z (ion): 548 $(M+H)^+$.

5.1.48. (3*RS*,4*RS*)-*N*^{4}-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*^{1}-hydroxy-*N*^{4}-methylpiperidine-1,4-dicarboxamide (31)

This compound was prepared from **28** and 50% aqueous hydroxylamine by the similar procedure as described for the synthesis of **30** in 59% yield as white amorphous. ^{1}H NMR ($CDCl_3$, δ): 7.83–7.72 (m, 1H), 7.41–7.34 (m, 2H), 7.06–6.70 (m, 4H), 6.58 (brs, 1H), 4.85–4.45 (m, 1H), 4.30–3.90 (m, 3H), 3.50–2.30 (m, 10H), 2.00–1.85 (m, 2H). Anal. Calcd for $C_{24}H_{24}F_7N_3O_3$: C, 53.82; H, 4.52; N, 7.85. Found: C, 53.72; H, 4.65; N, 7.71. LC–MS m/z (ion): 536 $(M+H)^+$.

5.1.49. (3*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-3-(4-fluoro-2-methylphenyl)piperidine-4-carboxylic acid (*R*)-1-phenylethylamine complex (32)

A mixture of **22e** (8.43 g, 25.0 mmol) in EtOAc (250 mL) was added to a solution of (*R*)-1-phenylethylamine (1.51 g, 12.5 mmol) in EtOAc (150 mL) over a period of 2 h at room temperature. After the mixture was stirred at room temperature for 1 h, the precipitate was collected by filtration with EtOAc. The precipitate was washed with EtOAc to give **32** (4.50 g, 9.81 mmol, 39%). For further information, See Section 5.2.

5.1.50. (3*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-3-(4-fluoro-2-methylphenyl)piperidine-4-carboxylic acid (33)

Compound **32** (4.50 g, 9.81 mmol) was taken into aqueous citric acid and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated to give **33** (3.12 g, 9.25 mmol, 94%) as white powder, whose optical purity was determined as 99.5%*ee* by HPLC, mp 138–140 °C. ^{1}H NMR ($CDCl_3$, δ): 7.09–6.81 (m, 3H), 4.26 (m, 1H), 4.04 (m, 1H), 3.12–3.05 (m, 1H), 2.86–2.79 (m, 2H), 2.53 (m, 1H), 2.35 (s, 3H), 2.04–2.00 (br, 1H), 1.78–1.68 (m, 1H), 1.46 (br, 9H), The CO_2H peak was not observed.

5.1.51. *tert*-Butyl (3*R*,4*R*)-4-{[3,5-Bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(4-fluoro-2-methylphenyl)piperidine-1-carboxylate (34)

To a cooled solution of **33** (2.0 g, 5.93 mmol) and DMF (31 μL) in THF (18 mL) was added oxalyl chloride (0.61 mL, 7.11 mmol) at 0 °C. After stirred at 0 °C for 1 h, the reaction mixture was concentrated in vacuo at below 10 °C. The residue was dissolved in THF (20 mL), and then a cooled solution of 1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylmethanamine hydrochloride (1.91 g, 6.52 mmol) and ^{i}Pr$_2$NEt (2.28 mL, 13.1 mmol) in THF (20 mL) was added thereto at 0 °C. After stirred at room temperature for 14 h, the reaction mixture was poured into water and extracted with EtOAc. The extract was washed with aqueous saturated NH_4Cl and brine, dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient elution of 5–40% EtOAc/hexane and crystallized from EtOAc–IPE to give **34** (3.03 g, 5.26 mmol, 89%) as white crystals, mp 175–177 °C. ^{1}H NMR ($CDCl_3$, δ): 7.84–7.72 (m, 1H), 7.45–7.36 (m, 2H), 7.05–6.68 (m, 3H), 7.05–6.68 (m, 3H), 4.90–3.90 (m, 4H), 3.39 (dt, J = 11.1 and 4.2 Hz, 1H), 1.47 (s, 9H), 3.25–2.55 (m, 6H), 2.50–2.35 (m, 3H), 1.95–1.75 (m, 2H). Anal. Calcd for $C_{28}H_{31}F_7N_2O_3$: C, 58.33; H, 5.42; N, 4.86. Found: C, 58.34; H, 5.42; N, 4.89. LC–MS m/z (ion): 521 $(M-^t$Bu$+2H)^+$.

5.1.52. (3*R*,4*R*)-1-[Amino(oxo)acetyl]-*N*-[3,5-bis(trifluoromethyl)benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (35)

This compound was prepared from **34** and oxamic acid in a manner similar to that described for **24** as white crystals, mp 131–133 °C. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.46–7.35 (m, 2H), 7.10–6.70 (m, 3H), 4.84 (d, J = 15.0 Hz, 1H), 5.66–4.50 (m, 3H), 4.20 (d, J = 15.0 Hz, 1H), 3.70–2.30 (m, 11H), 2.10–1.80 (m, 2H). Anal. Calcd for $C_{25}H_{24}N_3O_3F_7$: C, 54.85; H, 4.42; N, 7.68. Found: C, 54.62; H, 4.44; N, 7.72. LC–MS m/z (ion): 548 $(M+H)^+$. $[\alpha]_D^{25}$ +19.5 (c 1.0, methanol).

5.1.53. (3*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-3-(4-fluoro-2-methylphenyl)piperidine-4-carboxylic acid (37)

A mixture of **22e** (180.2 g, 0.534 mol) in acetone (2140 mL) was added to a solution of (*S*)-1-phenylethylamine (32.4 g, 0.267 mol) in acetone (535 mL) over a period of 2.5 h at room temperature. After the mixture was stirred at room temperature for 2 h, the precipitate was collected by filtration using acetone (445 mL). The precipitate was washed with acetone (445 mL) to give **36** (82.1 g, 0.179 mol, 34%).

Compound **36** (82.1 g, 0.179 mol) was taken into water (360 mL), EtOAc (360 mL) and citric acid (37.8 g, 0.197 mol), and then the mixture was stirred at room temperature for 1 h. The organic layer was washed with brine, dried over $MgSO_4$. The solution was passed through a pad of silica gel (50 g) with EtOAc. The elution was concentrated under reduced pressure until the precipitation started. Hexane (595 mL) was slowly added thereto and then the precipitate was collected by filtration with hexane (200 mL) to give **37** (56.4 g, 0.167 mol, 93%) as white powder, whose optical purity was determined as 99.6%*ee* by HPLC, mp 138–140 °C. ^{1}H NMR ($CDCl_3$, δ): 7.09–6.81 (m, 3H), 4.26 (m, 1H), 4.04 (m, 1H), 3.12–3.05 (m, 1H), 2.86–2.79 (m, 2H), 2.53 (m, 1H), 2.35 (s, 3H), 2.04–2.00 (br r, 1H), 1.78–1.68 (m, 1H), 1.46 (br, 9H), The CO_2H peak was not observed. Anal. Calcd for $C_{18}H_{24}NO_4F$: C, 64.08; H, 7.17; N, 4.15. Found: C, 64.12; H, 7.28; N, 4.21.

5.1.54. *tert*-Butyl (3*S*,4*S*)-4-{[3,5-bis(trifluoromethyl)benzyl](methyl)carbamoyl}-3-(4-fluoro-2-methylphenyl)piperidine-1-carboxylate (38)

A mixture of **37** (46.7 g, 138 mmol), 1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylmethanamine hydrochloride (46.4 g, 166 mmol), WSC (31.7 g, 166 mmol), HOBt (21.1 g, 138 mmol) and Et_3N (23.1 mL, 166 mmol) in acetonitrile (550 mL) was stirred at room temperature for 3 h and concentrated. The residue was poured into EtOAc and aqueous saturated $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography with an elution of 50% EtOAc/hexane, and crystallized form EtOAc and IPE to give **38** (61.6 g, 107 mmol, 77%) as white solid. ^{1}H NMR ($CDCl_3$, δ): 7.89–7.67 (m, 1H), 7.55–7.31 (m, 2H), 7.11–6.65 (m, 3H), 5.01–3.88 (m, 5H), 3.50–2.32 (m, 9H), 2.00–1.72 (m, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{28}H_{31}F_7N_2O_3$: C, 58.33; H, 5.42; N, 4.86. Found: C, 58.35; H, 5.43; N, 4.70. Chemical purity: 100.0%.

5.1.55. *tert*-Butyl (3*S*,4*S*)-4-[{(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethyl}(methyl)carbamoyl]-3-(4-fluoro-2-methylphenyl)piperidine-1-carboxylate (39)

To a cooled stirred solution of **37** (800 mg, 2.37 mmol) and DMF (8 μL) in THF (20 mL) was added oxalyl chloride (244 μL, 2.84 mmol) at 5 °C under argon. After stirred at 5 °C for 2 h, the mixture was concentrated in vacuo and dissolved in THF (8 mL). A solution of (1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylethanamine hydrochloride (771 mg, 2.84 mmol) and Et_3N (462 μL, 3.32 mmol) in THF (2 mL) was added dropwise thereto at 5 °C. The mixture was stirred at room temperature for 4 h and poured

into aqueous saturated $NaHCO_3$ and EtOAc. The organic layer was washed with water and 1 M $KHSO_4$, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 10–50% EtOAc/hexane to give **39** (1.30 g, 2.20 mmol, 93%) as white crystals, which were crystallized from EtOAc and hexanes, mp 173–174 °C. ^{1}H NMR ($CDCl_3$, δ): 7.89–7.66 (m, 1H), 7.64–7.28 (m, 2H), 7.18–6.65 (m, 3H), 5.91 (q, J = 7.0 Hz, 1H), 4.68–4.22 (m, 1H), 4.22–3.88 (m, 1H), 3.55–3.30 (m, 1H), 3.23–2.98 (m, 1H), 2.97–2.33 (m, 2H), 2.69 (s, 3H), 2.45 (s, 3H), 1.79–1.75 (m, 2H), 1.54–1.37 (m, 12H). Anal. Calcd for $C_{29}H_{33}N_2O_3F_7$: C, 58.98; H, 5.63; N, 4.74. Found: C, 59.00; H, 5.58; N, 4.77. LC–MS m/z (ion): 517 (M–^{t}BuO)$^+$.

5.1.56. *tert*-Butyl (3*S*,4*S*)-4-[{(1*S*)-1-[3,5-bis(trifluoromethyl) phenyl]ethyl}(methyl)carbamoyl]-3-(4-fluoro-2-methylphenyl)piperidine-1-carboxylate (40)

To a cooled stirred solution of **37** (800 mg, 2.37 mmol) and DMF (8 μL) in THF (20 mL) was added oxalyl chloride (244 μL, 2.84 mmol) at 5 °C under argon. After stirred at 5 °C for 2 h, the mixture was concentrated in vacuo and dissolved in THF (8 mL). A solution of (1*S*)-1-[3,5-bis(trifluoromethyl)phenyl]-*N*-methylethanamine hydrochloride (771 mg, 2.84 mmol) and Et_3N (462 μL, 3.32 mmol) in THF (2 mL) was added dropwise thereto at 5 °C. The mixture was stirred at room temperature for 4 h and poured into aqueous saturated $NaHCO_3$ and EtOAc. The organic layer was washed with water and 1 M $KHSO_4$, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography with a gradient elution of 10–50% EtOAc/hexane to give **40** (1.19 g, 2.34 mmol, 85%) as white crystals, which were crystallized from EtOAc and hexanes, mp 126–127 °C. ^{1}H NMR ($CDCl_3$, δ): 7.88–7.72 (m, 1H), 7.64–7.30 (m, 2H), 7.12–6.57 (m, 3H), 5.93 (q, J = 7.0 Hz, 1H), 4.61–4.25 (m, 1H), 4.25–3.81 (m, 1H), 3.65–3.32 (m, 1H), 3.29–2.96 (m, 1H), 2.96–2.37 (m, 8H), 2.09–1.75 (m, 2H), 1.48 (s, 9H), 1.72–1.10 (m, 3H). Anal. Calcd for $C_{29}H_{33}N_2O_3F_7$: C, 58.98; H, 5.63; N, 4.74. Found: C, 59.02; H, 5.56; N, 4.76. LC–MS m/z (ion): 517 (M–^{t}BuO)$^+$.

5.1.57. (3*S*,4*S*)-1-[Amino(oxo)acetyl]-*N*-[3,5-bis(trifluoromethyl) benzyl]-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (41)

This compound was prepared from **38** and oxamic acid in a manner similar to that described for **24** as white crystals, which was crystallized from methanol and water. mp 124 °C. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.73 (m, 1H), 7.46–7.35 (m, 2H), 7.10–6.70 (m, 3H), 4.84 (d, J = 15.0 Hz, 1H), 5.66–4.50 (m, 3H), 4.20 (d, J = 15.0 Hz, 1H), 3.70–2.30 (m, 11H), 2.10–1.80 (m, 2H). Anal. Calcd for $C_{25}H_{24}N_3O_3F_7$: C, 54.85; H, 4.42; N, 7.68. Found: C, 54.64; H, 4.39; N, 7.66. LC–MS m/z (ion): 548 (M+H)$^+$. $[\alpha]_D^{25}$ −19.1 (c 1.0, methanol).

5.1.58. (3*S*,4*S*)-1-[Amino(oxo)acetyl]-*N*-{(1*S*)-1-[3,5-bis(trifluoro methyl)phenyl]ethyl}-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (42)

This compound was prepared from **39** and oxamic acid in a manner similar to that described for **24** as white crystals, which were crystallized from EtOAc and IPE, mp 109–110 °C. ^{1}H NMR ($CDCl_3$, δ): 7.90–7.67 (m, 1H), 7.64–7.21 (m, 2H), 7.21–6.68 (m, 4H), 5.91 (q, J = 6.0 Hz, 1H), 5.77–5.39 (m, 1H), 5.33–4.47 (m, 2H), 3.76–2.36 (m, 10H), 2.13–1.75 (m, 2H), 1.54–1.16 (m, 3H). Anal. Calcd for $C_{26}H_{26}N_3O_3F_7$: C, 55.62; H, 4.67; N, 7.48. Found: C, 55.72; H, 4.82; N, 7.43. LC–MS m/z (ion): 562 (M+H)$^+$. $[\alpha]_D^{25}$ −73.0 (c 1.0, methanol).

5.1.59. (3*S*,4*S*)-1-[Amino(oxo)acetyl]-*N*-{(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethyl}-3-(4-fluoro-2-methylphenyl)-*N*-methylpiperidine-4-carboxamide (43)

This compound was prepared from **40** and oxamic acid in a manner similar to that described for **24** as white crystals, which were crystallized from EtOAc and IPE, mp 107–109 °C. ^{1}H NMR ($CDCl_3$, δ): 7.85–7.71 (m, 1H), 7.66–7.22 (m, 2H), 7.19–6.52 (m, 4H), 5.92 (q, J = 6.0 Hz, 1H), 5.76–5.42 (m, 1H), 5.42–4.48 (m, 2H), 3.79–2.39 (m, 10H), 2.22–1.79 (m, 2H), 1.25–1.10 (m, 3H). Anal. Calcd for $C_{26}H_{26}N_3O_3F_7$: C, 55.62; H, 4.67; N, 7.48. Found: C, 55.59; H, 4.72; N, 7.51. LC–MS m/z (ion): 562 (M+H)$^+$. $[\alpha]_D^{25}$ +85.4 (c 1.0, methanol).

5.2. Single-crystal X-ray analysis of 32

A crystal of **32** was obtained by recrystallization from water and MeOH. A diffractometer R-AXIS RAPID was used with graphite monochromated Mo Kα radiation. The crystal data, intensity measurements and structure solution and refinement were summarized in Table 6.

5.3. Biological evaluation

5.3.1. [^{125}I]Bolton–Hunter (BH) substance P binding in human IM-9 cells, preparation of receptors

The tachykinin NK_1 receptors from human lymphoblast cells (IM-9) were prepared according to the protocol with minor modification.[9] IM-9 cells (2×10^5 cells/mL) were inoculated and incubated for 3 days (1 L) and then subjected to centrifugation for 5 min at 500 g to obtain a cell pellet. The pellet was washed once with PBS crushed using a Polytron homogenizer (Kinematika, Germany) in 30 mL of 50 mM Tris–HC1 buffer (pH 7.4) containing NaCl (120 mM), KC1 (5 mM), chymostatin (2 μg/mL), bacitracin (40 μg/mL), (*p*-amidinophenyl)methanesulfonyl fluoride (40 μg/mL), and ethylenediaminetetraacetic acid (EDTA) (1 mM), and then centrifuged at 40,000 g for 20 min. The residue was suspended in 30 mL of a reaction buffer [50 mM Tris–HC1 buffer (pH 7.4), 0.02% bovine serum albumin, (*p*-amidinophenyl)methanesulfonyl fluoride (40 μg/mL), chymostatin (2 μg/mL), bacitracin (40 μg/mL) and $MnCl_2$ (3 mM)] and then preserved frozen (−80 °C) as a receptor specimen.

5.3.2. Radioligand binding assay

The above specimen was suspended in the reaction buffer, and a 50 μL portion of the suspension was used in the reaction. After addition of the sample and [^{125}I]BH-SP (final concentration 130 pM), the reaction was allowed to proceed in 0.2 mL of reaction mixture at room temperature for 30 min. The amount of nonspecific binding was determined by adding SP at a final concentration of 2×10^{-6} M. After the reaction, a cell harvester (Filtermate Harvester PerkinElmer, USA) was used, and the reaction was terminated by rapid filtration through a glass filter (GF/C) (PerkinElmer, USA). After washing three times with 50 mM Tris–HC1 buffer (pH 7.4) containing 0.02% bovine serum albumin, the radioactivity remaining on the filter was measured with TopCount Microplate Scintillation Counter (Packard BioScience). Before use, the filter was immersed in 0.3% poly(ethy1enimine) for 2–24 h.

5.3.3. Inhibitory activity on hypermotility induced by intracerebroventricularly infused an NK_1 agonist, GR73637, in guinea pigs

Experiments were performed according to the methods reported by Rupniak et al. with a minor modification.[10] Male Hartley guinea pigs were used. Under ether anesthesia, an animal was fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). An incision was made in the midline of the scalp and a 27-gauge needle connected to a syringe was inserted vertically to the third cerebral ventricle (a depth of 7.5–8 mm at bregma) through a burr hole. Physiological saline or a selective NK_1 agonist, GR73632 (0.1 nmol, Bachem AG, Bubendorf, Switzerland) dissolved in physiological saline was infused in a volume of 5 μL over a

Table 6
Crystal data and summary of data collection

Empirical Formula	$[C_{18}H_{23}FNO_4]^-$ $[C_8H_{12}N]^+$
Formula Weight	458.57
Crystal Color, Habit	Colorless, needle
Crystal Dimensions	0.75 × 0.20 × 0.10 mm
Crystal System	Orthorhombic
Lattice Parameters	a = 5.870 Å b = 11.636 Å c = 27.148 Å V = 2537.5 Å^3
Space Group	$P2(1)2(1)2(1)$ (#19)
Z value	4
D_{calc}	1.200 g/cm^3
Diffractometer	Rigaku RAXIS-RAPID Imaging Plate
Radiation	Mo Kα (λ = 0.71075 Å^3) Graphite monochromated
Residuals: R; Rw	0.033; 0.075

period of 1 min. Immediately after recovery from anesthesia, an animal was placed in an observation cage equipped with a locomotor activity counter (Animex Auto® MK-110, Muromachi Kikai, Inc., Tokyo, Japan) under the cage and the 30-minute measurement of the locomotor activity was started. A 0.5% methylcellulose solution or test compounds suspended in 0.5% methylcellulose solution in a volume of 2 mL/kg was administered orally either 23.75 or 0.75 h before the infusion of the NK_1 agonist. A mixture of DMA-PEG400 (1:1, v/v) or test compounds in a mixture of DMA-PEG400 (1:1, v/v) in a volume of 0.5 mL/kg was intravenously administered immediately before the infusion of the NK_1 agonist. An animal infused with saline was served as a sham-infusion control. The number of animals per group was 6–8. The inhibition rate in percentage in the group was obtained from the following formula:

[1 – (activity counts in the drug-treated group
– activity counts in the sham-infused group)/(activity counts in the vehicle
– treated control group – activity counts in the sham – infused group)]
× 100

In addition, using the inhibition rates (%inh.), the 50% inhibitory dose (ID_{50}) and its 95% confidence interval (CI) for each drug was determined by a linear regression analysis of the dose–response curve, if possible.

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