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Design, structure–activity relationship, and highly efficient asymmetric synthesis of 3-phenyl-4-benzylaminopiperidine derivatives as novel neurokinin-1 receptor antagonists

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ABSTRACT

We synthesized a series of novel 3-phenyl-4-benzylaminopiperidine derivatives that were identified as potent tachykinin NK_1 receptor antagonists by structural modification of the 3-benzhydrylpiperidone derivative through high-throughput screening. *N*-{2-[(3*R*,4*S*)-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}acetamide ((+)-**39**) was found to be one of the most potent tachykinin NK_1 receptor antagonists with high metabolic stability. Highly efficient asymmetric synthesis of (+)-**39** was achieved via dynamic kinetic resolution.

1. Introduction

Substance P (SP), an endogenous undecapeptide belonging to the tachykinin peptide family, is widely distributed in the body of mammals and involved in several physiological functions as a neurotransmitter or neuromodulator mediated by tachykinin NK_1 receptor. Antagonists of the tachykinin NK_1 receptor induce various biological responses in both the central nervous system (CNS) and peripheral tissues, including pain transmission, stress signals, neurogenic inflammation, and contraction of smooth muscles. Therefore, many pharmaceutical companies have paid much attention to the development of tachykinin NK_1 receptor antagonists for treatment of several diseases in clinics such as overactive bladder, gastrointestinal disorders, emesis, pain, and CNS disorders.[1]

In our previous study, we have identified **1** (TAK-637, Fig. 1) that can be used for the treatment of bladder function disorders.[2] In an attempt to investigate a new class of tachykinin NK_1 receptor antagonists, we recently identified a series of 3-benzhydryl-4-piperidone derivatives derived from high-throughput screening (HTS) hit **2**, which exhibited strong affinity in the subnanomolar range.[3]

However, further optimization for **2** was required because it possessed several pharmacokinetic shortcomings including low solubility and metabolic instability, which were mainly attributed to the physical properties of the scaffold of **2**. Compound **2** was precipitated even at 30 μM under physiological conditions, and most of the compounds related to **2** displayed high metabolic rates in human liver microsomes (>100 μL/mg/min). Thus, our main focus in this study was to find low-molecular weight scaffolds with good chemical properties by lead-hopping using **2**. In addition to enhancing metabolic stability, which is a key approach to improve pharmacokinetic properties, various strategies including blockade/modification of metabolic labile site and/or reducing lipophilicity can be also used.[4]

On the basis of the structure–activity relationship (SAR) study of the 3-benzhydryl-4-piperidone derivatives, we found that the relative location of three components, namely, two phenyl rings of benzhydryl and benzyl group, and a piperidone nitrogen, is crucial for their activity.[3] Therefore, it is necessary to preserve the relative locations of these functional groups during the course of scaffold hopping. As shown in Figure 2a, reconstruction of the piperidone ring in 3-benzhydryl-4-piperidone (type A) generates type B compound. Subsequent replacement of the benzhydryl group with the phenyl group affords a 3-phenyl-4-benzylaminopiperidine pharmacophore (type C) in the form of two diastereoisomers. A molecular modeling study using Molecular Operating Environment (MOE) software revealed that each diastereoisomer was well superimposed on type A compound

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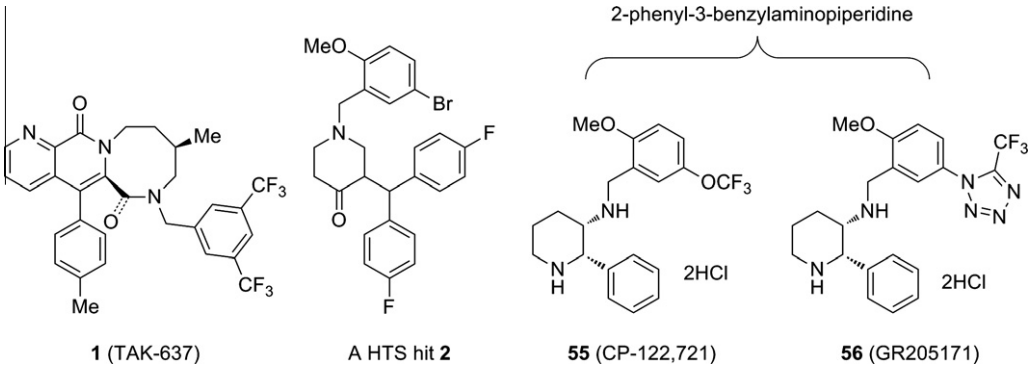


Figure 1. Structure of **1** (TAK-637), a HTS hit **2**, **55** (CP-122,721), and **56** (GR205171).

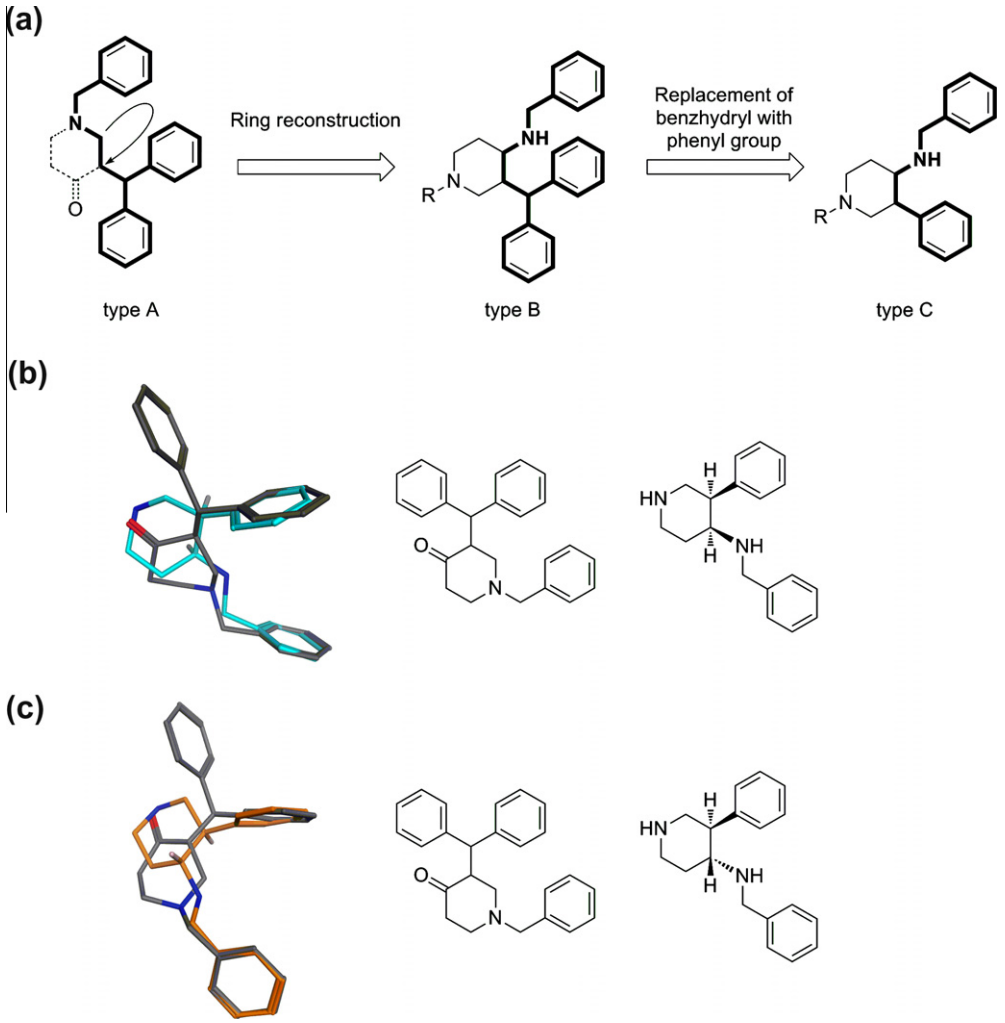


Figure 2. (a) Structural transformation of type A to type C, (b) overlapping between the most stable conformers of 3-benzhydryl-4-piperidone (gray) with *cis*-3-phenyl-4-benzylaminopiperidine (light blue), (c) overlapping between the most stable conformers of 3-benzhydryl-4-piperidone (gray) with *trans*-3-phenyl-4-benzylaminopiperidine (orange).

at the three fragments that were essential for its activity (Fig. 2b and c).[5]

As illustrated in Figure 1, the 2-phenyl-3-benzylaminopiperidine skeleton is similar to that of a prototype tachykinin NK_1 receptor antagonist such as **55** (CP-122,721)[6] and **56** (GR205171).[7] However, the structure of 3-phenyl-4-benzylaminopiperidine, which is a type C compound (Fig. 2), has not been extensively investigated.

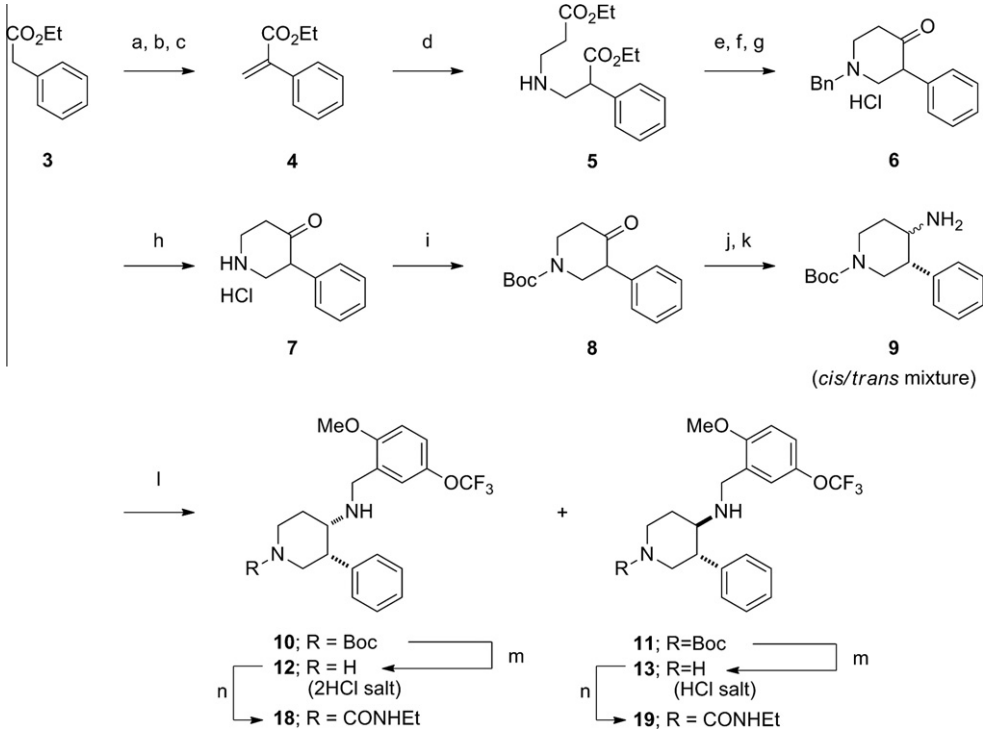
To evaluate the potential of type C compounds as tachykinin NK_1 receptor antagonists, we introduced the benzylamine moieties of **55** and **56** in the type C compounds. Most of the 2-phenyl-3-benzylaminopiperidine tachykinin NK_1 receptor antagonists have no substituents on the piperidine nitrogen.[8] Our main interest was to evaluate the similarity in SAR at the piperidine nitrogen in these different substitution patterns. Herein, we describe the

synthesis and the SAR of a series of 3-phenyl-4-benzylaminopiperidine derivatives. In addition, we wish to report the stereochemical requirements and asymmetric synthesis of the 3-phenyl-4-benzylaminopiperidine series.

2. Chemistry

The 3-phenyl-4-benzylaminepiperidine key intermediates **12** (3,4-*cis*) and **13** (3,4-*trans*) bearing a 2-methoxy-5-trifluoromethoxyphenyl group were synthesized as illustrated in Scheme 1. Treatment of ethyl phenylacetate **3** with sodium hydride and diethyl oxalate followed by the addition of formalin and aqueous potassium carbonate provided ethyl phenylacrylate **4**.[9] Then, compound **4** was converted to 1-benzyl-4-piperidone **6** in five sequential steps: Michael addition with ethyl β-alanine, N-benzylation, Dieckmann cyclization, hydrolysis, and decarboxylation.[9] Modification of the *N*-benzyl group in **6** afforded 1-Boc-4-piperidone **8**, which was subjected to an imine formation condition by using hydroxylamine and subsequent reduction with Raney nickel to yield a *cis*/*trans* mixture of 4-amino-3-phenylpiperidine **9**. Reductive alkylation of **9** with 2-methoxy-5-(trifluoromethoxy)benzaldehyde yielded the benzylamines **10** and **11**, which were easily separated by silica gel chromatography. Deprotection of Boc groups in **10** and **11** afforded the key intermediates **12** and **13**, respectively.

To determine the relative stereochemistry at the 3- and 4-positions of the key intermediates **12** and **13**, *N*-ethylcarboxamides **18** and **19** were prepared by reacting **12** and **13** with ethyl isocyanate in the presence of a base (Scheme 1). Both stereoisomers **18** and **19** had chair conformation by ^{1}H NMR analysis (data not shown). Significant nuclear Overhauser effect (NOE) correlations of **19** were observed between 5-CH equatorial and 4-CH axial protons and between 5-CH equatorial and 16-CH_2 protons, whereas no NOE correlations of **18** between the corresponding protons were detected (Fig. 3). These observations suggested that **19** is a 3,4-*trans* isomer, and **18** is a 3,4-*cis* isomer. Coupling constants (*J*) between



Scheme 1. Synthesis of key intermediates **12** and **13** and *N*-ethylcarboxamide **18** and **19** for relative stereochemistry determination. Reagents and conditions: (a) NaH, $(CO_2Et)_2$, xylene, EtOH, 0 °C; (b) 37% aqueous HCHO, H_2O; (c) K_2CO_3; (d) β-alanine ethyl ester·HCl, Et_3N; (e) BnBr, Na_2CO_3, MeCN, 70 °C; (f) NaOEt, toluene, 75 °C; (g) concentrated HCl, AcOH, 125 °C; (h) H_2, Pd-C, EtOH, THF; (i) Boc_2O, Et_3N, MeCN; (j) NH_2OH·HCl, NaOAc, EtOH, H_2O, 75 °C; (k) H_2 (5 atm), Raney Ni, EtOH, 75 °C; (l) 2-methoxy-5-(trifluoromethoxy)benzaldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 then separation of diastereoisomers on SiO_2; (m) TFA, THF, 0 °C then 4 N HCl–EtOAc; (n) ethyl isocyanate, Et_3N, MeCN, 0 °C.

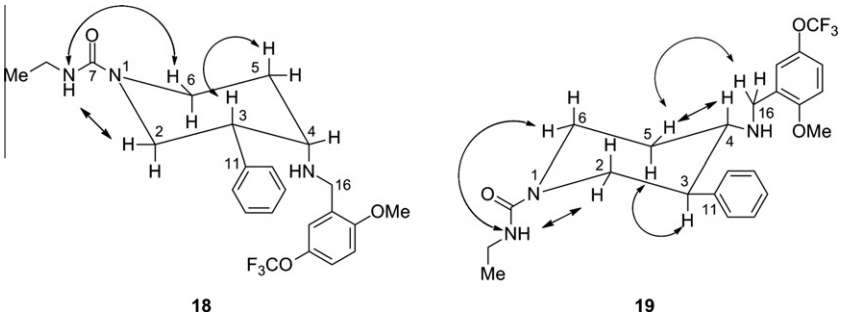


Figure 3. NOE correlations of **18** and **19**.

3-CH and 4-CH observed in this experiment were also consistent with this identification (Table 1). Therefore, we concluded that **12** and **13** are 3,4-*cis* and 3,4-*trans*, respectively.

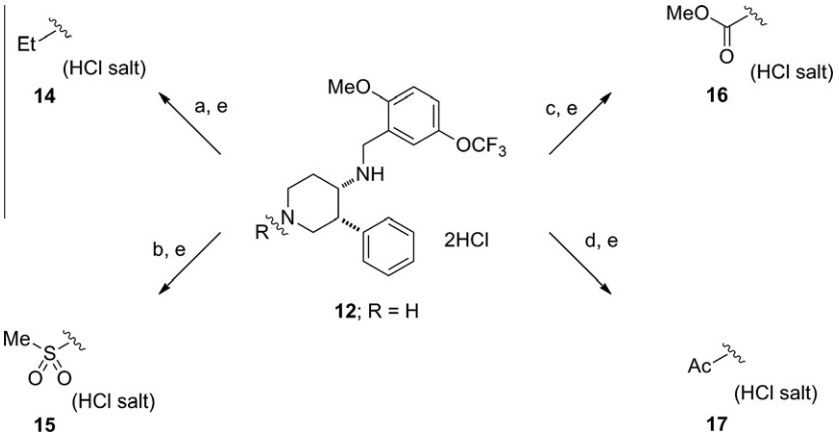
After obtaining the key intermediate **12**, we synthesized its N-substituted derivatives **14**–**17** by using a variety of electrophiles such as ethyl iodide, methanesulfonyl chloride, acetyl chloride, and methyl chloroformate, as illustrated in Scheme 2.

For the synthesis of a series of benzylamine derivatives bearing a 5-(trifluoromethyl)tetrazolyl group at the benzylamine tether, benzaldehyde intermediates **25** and **27** were prepared as shown in Scheme 3. 2,3-Dihydrobenzofuran **20** was converted to known 5-benzofuranamine (**22**)[10] by sequential nitration and Pd-catalyzed hydrogenation, and followed by acetylation using trifluoacetic anhydride to afford trifluoroacetamide **23**. Then, the

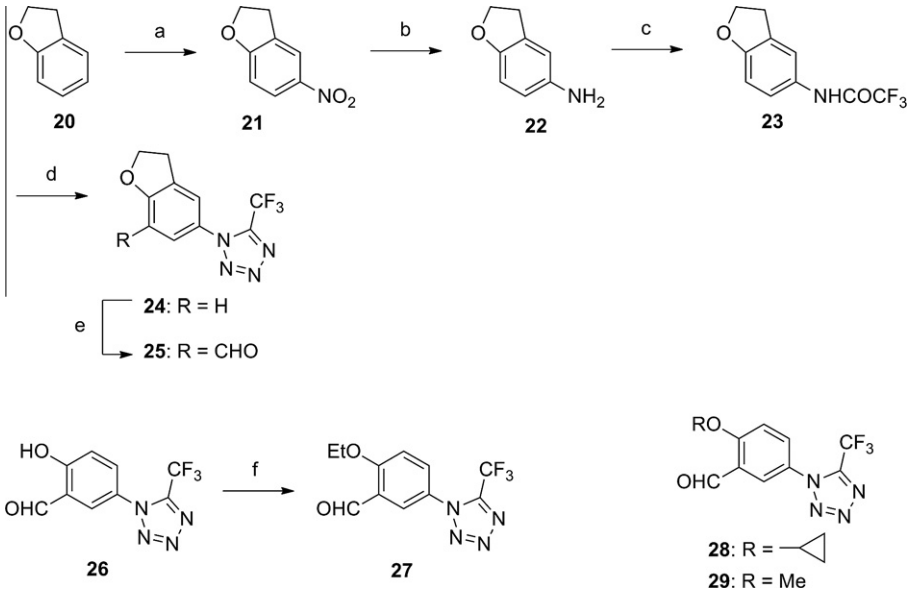
Table 1
Selected chemical shifts (δ), multiplicity, and coupling constants (J) for **18** and **19**[a]

Proton	**18**			**19**		
	δ	Multiplicity	J (Hz)	δ	Multiplicity	J (Hz)
3-CH	3.001	dt	13.5,4.0	2.600	td	10.6,4.0
4-CH	2.930-2.950	m	<3	2.690	td	10.6,4.0
5-CH_2 axial	1.708	m		1.430	m	
5-CH_2 equatrial	1.903	ddt	13.9,3.5,3.8	2.145	m	

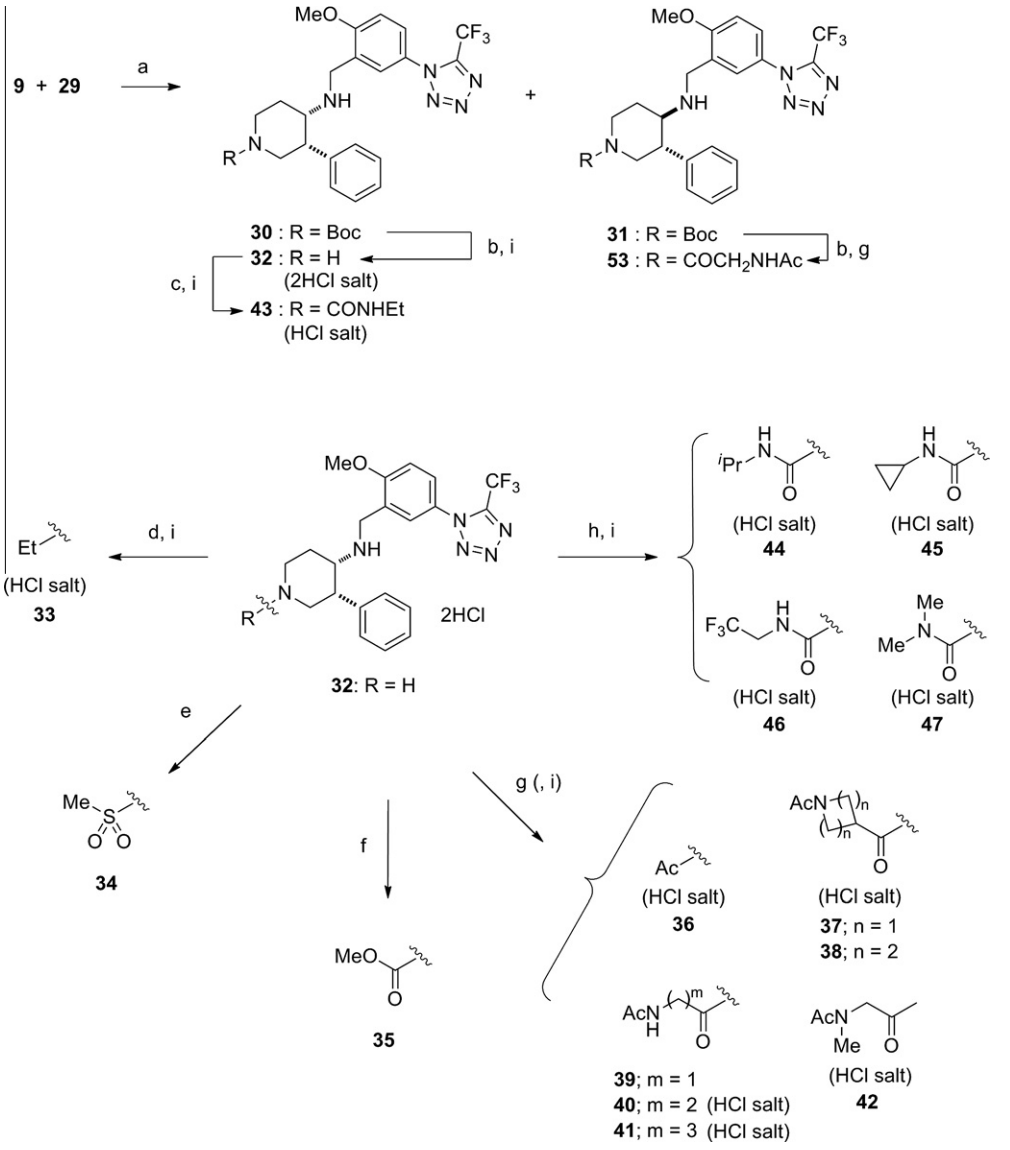
[a] Data were at 300 K in $CDCl_3$.



Scheme 2. Synthesis of N-substituted 3-phenyl-4-benzylaminopiperidines **14**–**17**. Reagents and conditions: (a) Ethyl iodide, Et_3N, THF, DMF, rt; (b) $MeSO_2Cl$, Et_3N, THF, DMF, 0 °C; (c) MeOCOCl, Et_3N, THF, DMF, 0 °C; (d) AcCl, Et_3N, THF, 0 °C; (e) 4 N HCl–EtOAc.



Scheme 3. Synthesis of benzaldehydes **25** and **27** and chemical structure of benzaldehyde **28** and **29**. Reagents and conditions: (a) HNO_3, AcOH, rt; (b) H_2, 10% Pd-C, EtOH, THF, rt; (c) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0 °C; (d) PPh_3, CCl_4, 85 °C then NaN_3, DMF, rt; (e) hexamethylenetetramine, methanesulfonic acid, 80 °C; (f) ethyl iodide, K_2CO_3, DMF, 85 °C.

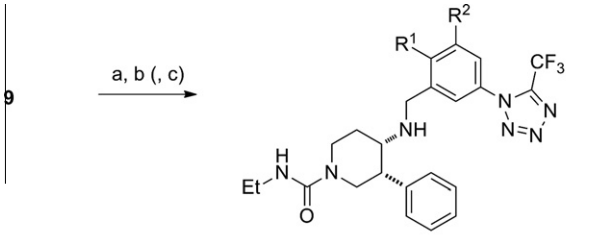


Scheme 4. Synthesis of key intermediates **32**, and N-substituted derivatives **33**–**47** and **53**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (b) TFA, 0 °C then aqueous NaOH; (c) ethyl isocyanate, Et_3N, MeCN, 0 °C; (d) ethyl iodide, Et_3N, THF, DMF, rt; (e) $MeSO_2Cl$, Et_3N, THF, DMF, 0 °C; (f) MeOCOCl, Et_3N, THF, DMF, 0 °C; (g) acid chlorides, Et_3N, THF, 0 °C or acids, WSC, HOBt, Et_3N, DMF; (h) alkyl isocyanate or 4-nitrophenyl alkylcarbamate or alkylcarbamyl chloride, Et_3N, DMF or MeCN, rt; (i) 4 N HCl–EtOAc.

trifluoroacetamide group in **23** was transformed into the 5-(trifluoromethyl)tetrazolyl group by a known procedure[7] to provide **24**, which was converted to benzaldehyde **25** under Duff reaction conditions. Ethoxybenzaldehyde **27** was prepared by alkylation of a known hydroxybenzaldehyde **26**[11] by treatment with ethyl iodide in the presence of potassium carbonate. The cyclopropyloxy analog **28** and methoxy analog **29** were obtained by a known procedure.[12,13]

As illustrated in Scheme 4, reductive coupling of 4-amino-3-phenylpiperidine **9** with benzaldehyde **29** produced separable *cis*-3-phenyl-4-aminopiperidine **30** and *trans* isomer **31**. The *cis* isomer **30** was used to synthesize a key intermediate **32**, to which various substituents were introduced at the piperidine nitrogen by a method similar to that described in Scheme 2. The *trans* isomer **31** was also used to synthesize acetamide **53** by the same procedure as that of **39**.

The benzaldehydes **25**, **27**, and **28** shown in Scheme 3 were coupled with 4-amino-3-phenylpiperidine **9**, followed by separation of



Scheme 5. Synthesis of **48**–**50**. Reagents and conditions: (a) Benzaldehyde **25**, **27** or **28**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 then separation of diastereoisomers on SiO_2; (b) TFA, THF, 0 °C then aqueous NaOH then 4 N HCl–EtOAc; (c) ethyl isocyanate, Et_3N, MeCN, 0 °C.

stereoisomers, deprotection, and urea formation with ethyl isocyanate to afford *N*-ethylcarboxamides **48**–**50**, as shown in Scheme 5.

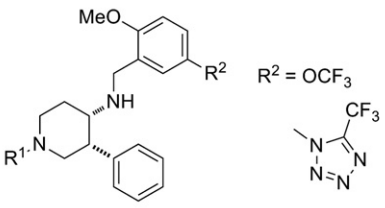
3. Results and discussion

In this study, we evaluated the antagonistic activities of the synthesized compounds in the CNS. We conducted preliminary examination for evaluating the in vitro inhibitory activity of these compounds against [^{125}I]Bolton–Hunter (BH)–SP binding in human IM-9 cells.[14] Subsequently, we performed in vivo screening and analyzed the inhibitory activity based on locomotive activity in guinea pigs induced by intracerebroventricular infusion of a tachykinin NK_1 receptor agonist, namely, GR73637, at 1 h after oral administration (po) of the compounds.[15] To evaluate the metabolic stability of these compounds in humans, we used liver microsomes and analyzed the remaining parent compound by high-performance liquid chromatography (HPLC) after 15 and 30 min incubation periods under standard conditions.

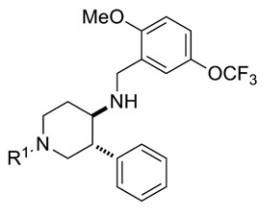
The biological data of two series of 3-phenyl-4-benzylamine derivatives bearing several substituents on the piperidine nitrogen is shown in Table 2. Compared with **55** (CP-122,721) and **56** (GR205171), the unsubstituted compounds (R^1 = H; **12**, **32**) exhibited much lower affinities than expected. These results indicated that the piperidine nitrogen in **55** and **56** plays an important role in recognizing the tachykinin NK_1 receptor.[16] We hypothesized that the binding affinity of the compounds can be improved by appropriate incorporation of hydrogen bonding acceptor around the piperidine nitrogen. As shown in Table 2a, this modification had almost no effect on the affinity of the ethyl (**14**, **33**) analogs, while significant enhancement was observed in the methanesulfonyl (**15**, **34**), methoxycarbonyl (**16**, **35**), acetyl (**17**, **36**), and ethylcarbamoyl (**18**, **43**) analogs, with 7 to 49-fold higher affinity as

Table 2
Comparison of 2 series of 3-phenyl-4-benzylaminopiperidines: (a) binding affinity; (b) in vivo CNS NK_1 antagonistic activity

(a)



12, **14-18**, **32-36**, **43**



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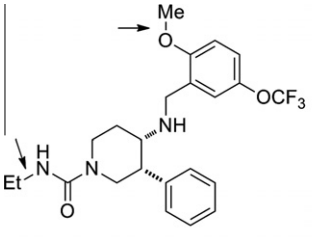
R^1	R^2 = trifluoromethoxy		R^2 = 5-(trifluoromethyl)-1*H*-tetrazol-1-yl	
	Compound[a] no.	Binding affinity[b] IC_{50} (nM)	Compound[a] no.	Binding affinity[b] IC_{50} (nM)
H	**12**	8.4	**32**	1.4
Et	**14**	4.8	**33**	1.9
$MeSo_2$	**15**	1.2	**34**	0.075
MeOCO	**16**	0.31	**35**	0.054
Ac	**17**	0.16	**36**	0.049
EtNHCO	**18**	0.17	**43**	0.039
EtNHCO	**19**	3.7	—[c]	
	55 (CP-122,721)[d]	0.012[e]	**56** (GR205171)	pK_i = 10.6[f]

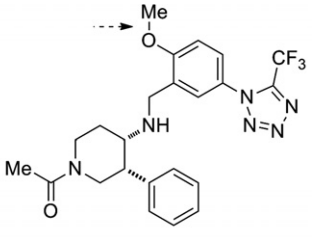
(b)

Compound[a] no.	PD assay[g], po[h] %inh. (1 mg/kg) or ID_{50} (mg/kg)	Metabolic stability in human liver microsomes[i] (μL/mg/min)
34	18%	144
35	65%	199
36	82%, ID_{50} = 0.20	111
43	70%, ID_{50} = 0.37	159

[a] All of the compounds are racemic.
[b] Inhibition of [^{125}I]Bolton–Humter-SP binding in humanIM-9 cells.
[c] Not synthesized.
[d] CP-122,721 is assessed as a single enantiomer.
[e] In-house data.
[f] Reported data.[7]
[g] Inhibition of GR73632-induced increase in locomotor activity in guinea pigs, The values of %inh. are averages of 5–10 independent experiments. ID_{50} values are compound doses causing 50% inhibition in locomotor activity.
[h] After 0.75 h of oral administration of the compounds.
[i] Measured at 1 μM.



18



36

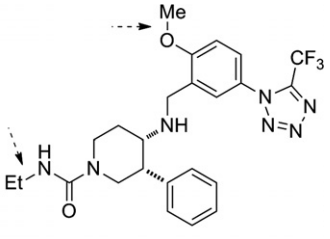


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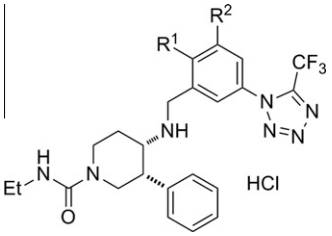
Figure 4. Metabolic sites of **18** (plain arrows) and presumed metabolic sites (dashed arrows) of **36** and **43**.

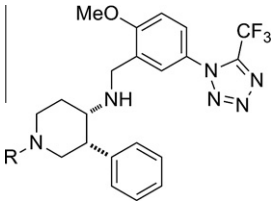
Table 3
Modification at the methoxy group

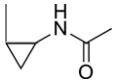
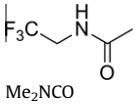
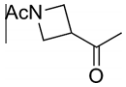
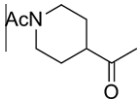
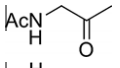
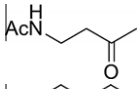
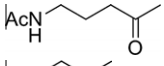
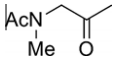


Compound[a]	R^1	R^2	binding affinity[b] IC_{50} (nM)	PD assay[g], po[h] %inh. (1 mg/kg)	metabolic stability in human liver microsomes[i] (μL/mg/min)
43	MeO	H	0.039	70%	160
48	EtO	H	0.086	35%	218
49	^{c}PrO	H	0.044	94%	217
50	$-OCH_2CH_2-$		0.093	57%	160

[a,b,g,h,i] See the corresponding footnotes in Table 2

Table 4
Effects on 1-position of {2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]}benzylamine series



Compound[a]	R	Binding affinity[b] IC_{50} (nM)	PD assay[g], po[h] %inh. (1 mg/kg) or ID_{50} (mg/kg, po)	Metabolic stability in human liver microsomes[i] (μL/mg/min)	Log *D*[j]
43	EtNHCO	0.039	70%, ID_{50} = 0.37	159	2.79
44	^{i}PrNHCO	0.081	66%	167	3.09
45		0.056	81%	107	2.86
46		0.057	65%	155	3.03
47	Me_2NCO	0.13	90%	213	3.18
36	Ac	0.049	82%, ID_{50} = 0.20	111	2.69
37		0.057	32%	27	2.31
38		0.10	85%, ID_{50} = 0.33	76	2.51
39		0.073	99%, ID_{50} = 0.26	32	2.28
40		0.054	79%, ID_{50} = 0.34	95	2.35
41		0.025	N.T.[k]	146	2.43
42		0.084	74%	73	2.31

[a,b,g,h,i] See the corresponding footnotes in Table 2
[j] At pH = 7.4
[k] Not tested

compared with the corresponding N-unsubstituted compounds. Comparison of the affinities between the two series revealed that the 5-(trifluoromethyl)-1*H*-tetrazol-1-yl series were more potent than the trifluoromethoxy series. It is noteworthy that the affinity of the compounds was influenced after reducing the basicity of the piperidine nitrogen in the both series.

The effect of stereochemistry was evaluated based on SAR comparison of compounds **18** and **19**. The results indicated that both diastereoisomers exhibited strong binding affinities from the nanomolar to subnanomolar level. Since the *cis* configuration exhibited greater affinity than the *trans* one, further evaluations were conducted using compounds with *cis* configuration.

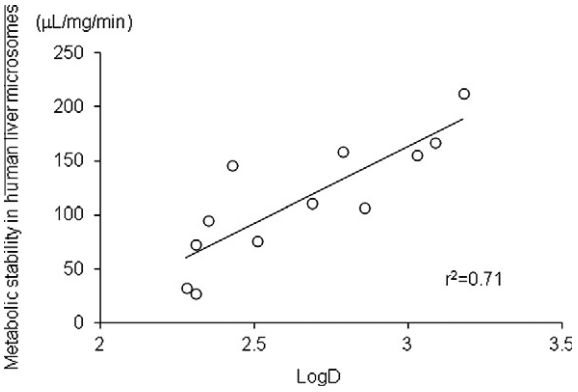


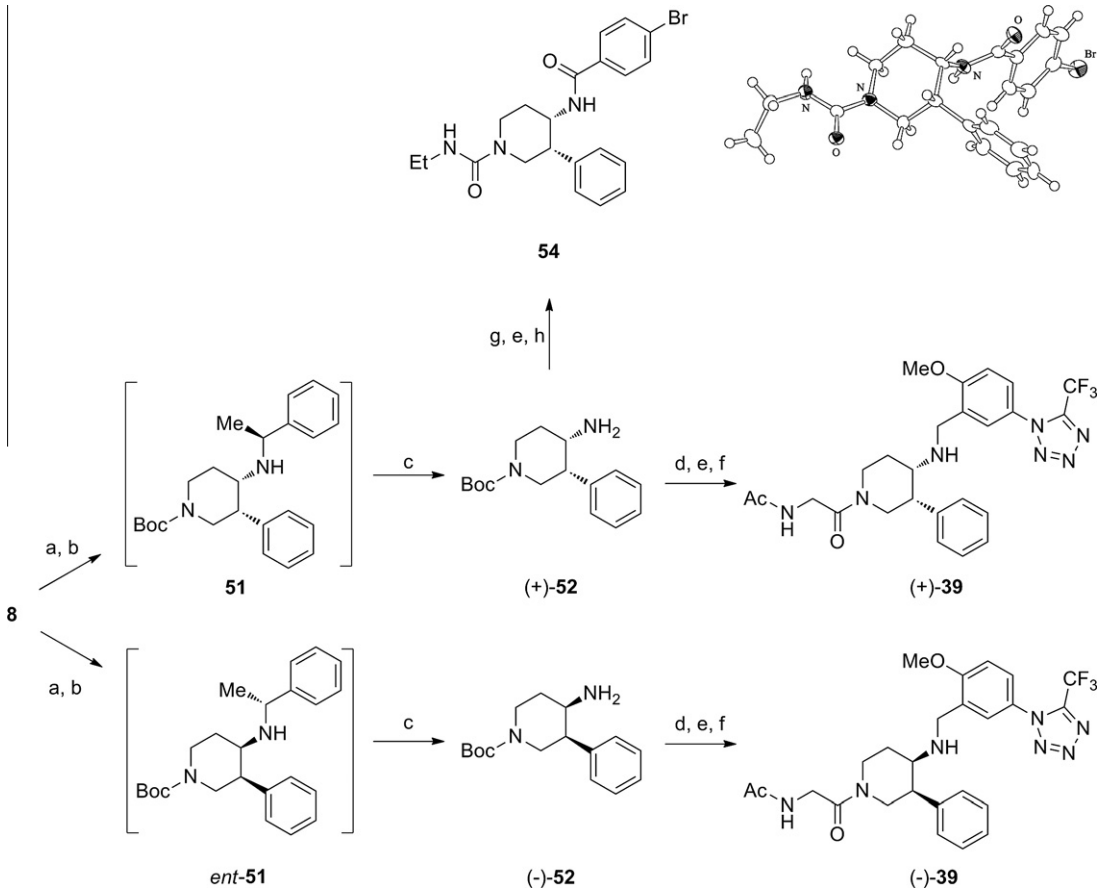
Figure 5. Correlation between metabolic stability in human liver microsomes and lipophilicity (Log*D*) of compounds in Table 4.

The compounds shown in Table 2a with an IC_{50} value less than 0.1 nM were examined for their in vivo activity in Table 2b. Compounds **35**, **36**, and **43** exhibited strong activity in the CNS with an ID_{50} value less than 1 mg/kg (po), except for **34**. However, the four compounds tested had a high rate of metabolism in human liver microsomes (>100 μL/mg/min).

To identify an appropriate site for incorporation of a metabolically stable functional group, we initially performed the metabolite characterization of **18** and presumed the metabolic sites in **36** and **43**. Cleavage of the carbon–oxygen bond at the methoxy group and carbon-nitrogen bond at the *N*-ethyl urea moiety were observed in the metabolite analysis of **18**. These results indicated that **36** and **43** might be metabolized at the methoxyl group and/or the urea moiety (Fig. 4).

The first strategy was an attempt to modify the most likely metabolism site. The methoxy group was replaced with the more bulky or benzene-fused alkoxy groups (Table 3). Introduction of an ethoxy group (**48**) slightly decreased the affinity; however, conversion to a cyclopropyloxy group (**49**) maintained the affinity and increased the in vivo activity. Dihydrobenzofuran analog **50** exhibited slightly decreased activities both in vitro and in vivo. However, no improvement in metabolic stability was observed after modification of the methoxy group, implying that blockade of another metabolic site would be effective for metabolic stabilization.

The second strategy involved substitution of ethyl urea with branched, cyclic, fluorinated, and disubstituted alkyl ureas

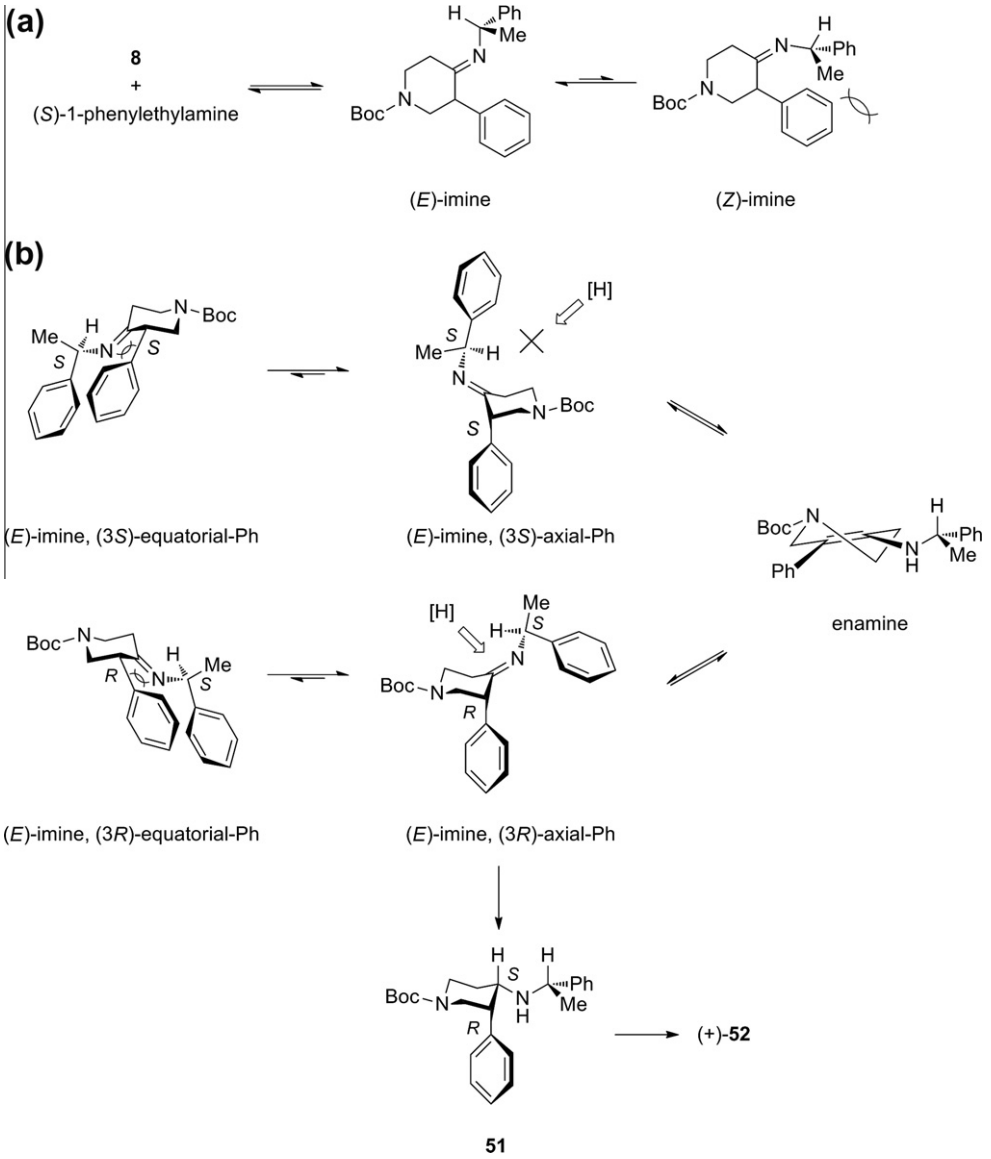


Scheme 6. Asymmetric synthesis of optically active compounds (+)- and (−)-**39**. Reagents and conditions: (a) (*S*)-1-Phenylethylamine (for (+)-**52**) or (*R*)-1-phenylethylamine (for (−)-**52**), $AlCl_3$, toluene; (b) Raney Ni, H_2, EtOH; (c) Pd-C, H_2, EtOH; (d) benzaldehyde **29**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (e) 4 N HCl–AcOEt, MeOH, 50 °C; (f) *N*-acetylglycine, HOBt, WSC, Et_3N, DMF; (g) 4-bromobenzoic acid, WSC, HOBt, DMF; (h) ethyl isocyanate, Et_3N, CH_3CN.

(Table 4). No significant effects on in vivo antagonistic activity and metabolic stability in human liver microsomes were observed in isopropyl urea **44**, whereas slight enhancement of both in vivo activity and metabolic stability was observed in cyclopropyl urea **45**. The fluorinated analog **46** showed slightly reduced in vivo activity, while its metabolic stability in human liver microsomes was almost unchanged, indicating that a CF_3 group had little effect on metabolic stability. The affinity of dimethyl urea **47** was reduced and it showed more potent in vivo activity than **43**; however, the metabolic stability of **47** was not improved. It is considered that, in the urea series, blocking the metabolically labile site increased the lipophilicity and reduced the metabolic stability.

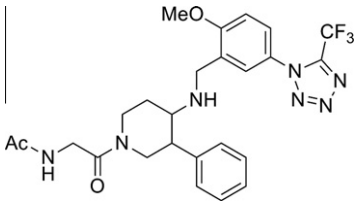
Since the urea series synthesized were not expected to exhibit strong activity with high metabolic stability, further investigations were performed to obtain acyl compounds with lower lipophilicity. Replacement of the acetyl group in **36** with an acetylazetidinecarbonyl (**37**) or acetylpiperidinecarbonyl (**38**) group remarkably improved the metabolic stability. Compound **37** exhibited less potent in vivo activity; however, the in vivo efficacy of **38** was comparable to that of **36**. We further investigated the activities of ring-opening *N*-acetyl derivatives **39**–**41**. These compounds exhibited strong in vivo activities (except for **41**) and the length of the alkyl chain was inversely proportional to the metabolic stability as illustrated in Figure 5. Among them, **39** presented the best results for in vivo activity and metabolic stability. Compound **42**, an *N*-methyl analog of **39**, showed both decreased activity and metabolic stability as compared with **39**.

Since compound **39** showed a good balance between in vivo efficacy and metabolic stability, we attempted to establish asymmetric synthesis to obtain chiral compounds for **39** efficiently. Optically active key intermediates (+)- and (−)-**52** were prepared from a racemic 3-phenylpiperidone **8** by utilizing (*S*)- and (*R*)-1-phenylethylamine as chiral auxiliaries[17] as shown in Scheme 6. Treatment with the chiral auxiliaries in the presence of a catalytic amount of $AlCl_3$ generated an unstable imine, which was successively converted to **51** or *ent*-**51** under hydrogenation conditions using Raney Ni catalyst. Removal of the chiral auxiliary afforded the intermediate (+)- and (−)-**52** in excellent chemical yield and enantiomeric excess (86% yield, 98% *ee* for (+)-**52**; 59% yield, 97% *ee* for (−)-**52**). It is noteworthy that each product in this reaction was obtained as a single stereoisomer out of the four possible stereoisomers. The stereochemistry of (+)-**52** was determined as



Scheme 7. Plausible mechanism for dynamic kinetic resolution to produce (+)-**52**; (a) preferable (*E*)-imine formation; (b) eclipsing interaction of the 3-equatorial phenyl group with the imine and hydrogenation at the less hinder face.

Table 5
Stereochemical effects



Compound	Stereochemistry	Additives	Binding affinity[b] IC_{50} (nM)	PD assay[g], po[h] ID_{50} (mg/kg, po)	Metabolic stability in human liver microsomes[i](μL/mg/min)
(+)-**39**	(3*R*,4*S*)	free	0.017	0.066	8
(−)-**39**	(3*S*,4*R*)	free	>100	N.T.[k]	N.T.[k]
53	3,4-*trans*, Racemic	HCl	0.20	N.T.[k]	34

[b,g,h,i,k] See the corresponding footnotes in Tables 2 and 4

(3*R*,4*S*)-configuration by single-crystal X-ray analysis of a 4-bromobenzoyl derivative **54** that was derived in three steps from (+)-**52**. The compounds (+)- and (−)-**52** were used to synthesize (+)- and (−)-**39**, respectively, without loss of optical purity.

Scheme 7 illustrates a plausible mechanism for the dynamic kinetic resolution by amination of racemic **8** using (*S*)-1-phenylethylamine. First, under condensation condition, **8** and (*S*)-1-phenylethylamine would preferably generate (*E*)-imine due to steric repulsion between 3- and 4-substituents. Then, 3-phenyl group would move to the axial position because the equatorial position experiences an eclipsing interaction with the imine group due to 1,3-allylic strain. An equilibrium between (3*S*)-axial-phenyl and (3*R*)-axial-phenyl (*E*)-imines would be established through an enamine. Finally, hydrogenation at the face with less hindrance would occur to produce **51** as a (3*R*,4*S*) product.

After obtaining the stereoisomers, we examined the effects of the stereochemistry of compound **39** on the binding affinity, in vivo efficacy, and metabolic stability (Table 5). Taking into account of the affinity of **53** (IC_{50} = 0.20 nM), neither isomer of *trans* is suggested to be more potent than (+)-**39** (IC_{50} = 0.017). Therefore, compound (+)-**39**, assigned as a (3*R*,4*S*)-isomer, displayed the most potent activity with high metabolic stability among the four stereoisomers.

4. Conclusion

In this study, we discovered a series of novel 3-phenyl-4-benzylaminopiperidine derivatives with potent tachykinin NK_1 receptor antagonistic activity by structural modification of a 3-benzhydrylpiperidone that was obtained from HTS. Through the SAR studies, we found that introduction of a hydrogen bonding acceptor around the piperidine nitrogen enhanced the efficacy. Compound (+)-**39** was found to be one of the most potent tachykinin NK_1 receptor antagonists with high metabolic stability. Furthermore, highly efficient and stereoselective synthesis of (+)-**39** were achieved via dynamic kinetic resolution by using (*S*)-1-phenylethylamine as a chiral auxiliary agent.

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% to 95% acetonitrile/water containing 0.1% TFA was used to elute samples. The detector wavelength was set at 220 nm. Compounds **3** and **20**, and solvents were commercially available and used as received. Compounds **4**–**8**[9], **21**–**22**[10], **26**[11], **28**[12], and **29**[13] 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 phenylacrylate (4)[9]

To a cooled suspension of sodium hydride (60% in oil dispersion, 33.6 g, 0.84 mol) in xylene (670 mL) was slowly added EtOH (85 mL) at 0 °C. Diethyl oxalate (183.0 g, 1.25 mol) was slowly added thereto and then **3** (205.3 g, 1.25 mol) was added. After stirring at room temperature for 14 h, the precipitated white powder (320.0 g) was collected by filtration with IPE. The obtained powder was dissolved in H_2O (405 mL), and then 37% aqueous HCHO (226 mL) was added. After the mixture was stirred for 1 h, K_2CO_3 (153 g, 1.11 mmol) was added thereto, and the stirring was continued for 14 h. The reaction mixture was poured into a mixture of H_2O (1000 mL) and Et_2O (500 mL). The organic layer was washed with H_2O and brine, dried and concentrated to provide **4** (220 g, quantitative) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.1 Hz), 4.29 (2H, q, J = 7.1 Hz), 5.88 (1H, d, J = 1.2 Hz), 6.34 (1H, d, J = 1.2 Hz), 7.20–7.43 (5H, m).

5.1.2. Ethyl 3-[(2-ethoxycarbonyl)ethylamino]-2-phenylpropionate (5)[9]

To a mixture of **4** (110 g, 0.628 mol) in Et_3N (96 mL, 0.690 mol) was added β-alanine·HCl (106 g, 0.690 mol), and then the mixture was stirred at room temperature for 18 h. The resulting white slurry was dispersed between Et_2O and H_2O, and then the organic layer was washed with brine. The product was back-extracted with 3 N

HCl 200 mL × 2). The aqueous phase was washed with Et_2O and made basic with 12 N NaOH (100 mL) and extracted with Et_2O (×2). The organic layer was dried and concentrated to provide **5** (101.0 g, 55%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.17–1.29 (6H, m), 2.46 (2H, t, J = 6.7 Hz), 2.90 (2H, t, J = 6.4 Hz), 2.91 (1H, dd, J = 12.2, 6.2 Hz), 3.27 (1H, dd, J = 12.2, 8.8 Hz), 3.78 (1H, dd, J = 8.4, 6.2 Hz), 4.05–4.20 (4H, m), 7.20–7.40 (5H, m). The amino NH signal was not observed.

5.1.3. 1-Benzyl-3-phenyl-4-piperidone hydrochloride (6)[9]

To a mixture of **5** (122.7 g, 0.418 mol) and sodium bicarbonate (88.6 g, 0.836 mol) in CH_3CN (328 mL) was added benzyl bromide (78.7 g, 0.459 mol) at room temperature, and then the mixture was stirred at 70 °C for 2 h. The insoluble material was filtered off, and then the filtrate was concentrated. The residue was dissolved in EtOAc (500 mL), and the solution was washed with aqueous NH_4Cl and brine, dried and concentrated to give an oil (166.5 g). The oil was dissolved in EtOH (180 mL). The solution was slowly added to a cooled suspension of sodium hydride (60% in oil dispersion, 34.7 g, 0.867 mol) in toluene (281 mL) at −5 °C, and then the mixture was heated at 75 °C for 2 h. The reaction mixture was poured into H_2O, and then the mixture was extracted with EtOAc (500 mL × 2). The extract was washed with aqueous NH_4Cl and brine, dried and concentrated to give a brown oil (151.8 g). The obtained oil was dissolved in AcOH (250 mL) and concentrated HCl (250 mL), and then the mixture was heated at 125 °C for 3 h. More AcOH (150 mL) and concentrated HCl (150 mL) were added thereto, and the stirring was continued at 125 °C for 2 h. The solvents were evaporated, and then the residue was dissolved in EtOAc (500 mL). The solution was made basic with 12 N NaOH, and extracted with EtOAc (×2). The extract was washed with aqueous NH_4Cl and brine, dried and concentrated to give an oil, which was treated with 4 N HCl–EtOAc (100 mL) and the resulting precipitate was collected by Et_2O to provide **6** (104.9 g, 83%) as white powder. ^{1}H NMR ($CDCl_3$) δ: 2.60–2.70 (1H, m), 3.15–3.40 (2H, m), 3.60–3.90 (3H, m), 4.03 (2H, s), 4.86 (1H, dd, J = 12.4, 5.4 Hz), 7.11–7.15 (2H, m), 7.30–7.40 (3H, m), 7.40–7.55 (3H, m), 3.60–7.70 (2H, m). The NMR spectrum was measured as a free base.

5.1.4. 4-Oxo-3-phenylpiperidine hydrochloride (7)[9]

A solution of **6** (30.0 g, 99.4 mmol) and 10% Pd-C (4.5 g) in MeOH (300 mL) was stirred at 55 °C under an atmosphere of H_2 (5 atm) for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residual oil was triturated with Et_2O and IPE to provide the crude **7** (20.7 g, 98%) as white powder, which was used in the next step without further purification.

5.1.5. *tert*-Butyl 4-oxo-3-phenylpiperidine-1-carboxylate (8)[9]

The obtained powder of crude **7** (5.00 g, 23.6 mmol) was dissolved in a mixture of CH_3CN (100 mL) and Et_3N (4.94 mL, 35.4 mmol) and then di-*tert*-butoxycarbonate (7.73 g, 35.4 mmol) was slowly added thereto. After stirring for 14 h, the reaction mixture was concentrated in vacuo, and then the residue was dissolved in EtOAc and H_2O. The organic layer was washed with aqueous NH_4Cl and brine, dried and concentrated to provide **8** (5.46 g, 84%) as white solids. ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.57 (2H, t, J = 6.1 Hz), 3.45–3.74 (3H, m), 4.10–4.40 (2H, m), 7.15–7.21 (2H, m), 7.28–7.41 (3H, m).

5.1.6. *tert*-Butyl 4-amino-3-phenyl-piperidine-1-carboxylate (9)

To a solution of **8** (15.0 g, 54.4 mmol), concentrated HCl (13.8 mL) and sodium acetate (13.4 g, 163 mmol) in H_2O (100 mL) and EtOH (150 mL) was added 50% aqueous hydroxylamine (9.70 mL, 163 mmol), and then the mixture was heated at 75 °C for 2 h. The reaction mixture was concentrated in vacuo, and then the mixture was extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated to give white paste (20.8 g). The obtained product was dissolved in EtOH (150 mL) and THF (150 mL), and then Raney Ni (washed with H_2O and EtOH, ca. 15 g) was added. The mixture was stirred under an atmosphere of H_2 (5 atm) at 75 °C for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to provide **9** as a mixture of *cis* and *trans* isomers (7.18 g, 72%) as white powder, which was used in the next step without further purification.

5.1.7. *tert*-Butyl *cis*-4-{[2-methoxy-5-(trifluoromethoxy) benzyl]amino}-3-phenyl-piperidine-1-carboxylate (10)

A mixture of **9** (4.17 g, 15.1 mmol) and 2-methoxy-5-(trifluoromethoxy)benzaldehyde (3.32 g, 15.1 mmol) in CH_2Cl_2 (45 mL) and AcOH (0.5 mL) was added $NaBH(OAc)_3$ (4.80 g, 22.6 mmol). After stirring at room temperature for 14 h, the reaction mixture was poured into aqueous $NaHCO_3$, and then extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient eluent of 10–20% EtOAc/hexane. Evaporation of the less polar fractions provided **10** (5.51 g, 76%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.60–1.75 (1H, m), 1.85–2.00 (1H, m), 2.92–3.00 (2H, m), 3.30–3.40 (1H, m), 3.46 (1H, d, J = 14.1 Hz), 3.47 (3H, s), 3.72 (1H, d, J = 14.1 Hz), 3.50–4.20 (4H, m), 6.63 (1H, d, J = 8.7 Hz), 6.89 (1H, d, J = 2.7 Hz), 6.98–7.04 (1H, m), 7.07–7.15 (2H, m), 7.20–7.35 (3H, m); ESI-MS m/z 481 [$(M+H)^+$], 425 [$(M-{}^t\mathrm{Bu}+2H)^+$]; chemical purity: 100.0%.

5.1.8. *tert*-Butyl *trans*-4-{[2-methoxy-5-(trifluoromethoxy) benzyl]amino}-3-phenylpiperidine-1-carboxylate (11)

Evaporation of the more polar fractions described for the purification of **10** provided **11** (0.89 g, 12%) as white powder. ^{1}H NMR ($CDCl_3$) δ: 1.40–1.60 (10H, m), 2.09–2.14 (1H, m), 2.57–2.85 (4H, m), 3.04 (3H, s), 3.56 (1H, d, J = 13.5 Hz), 3.78 (1H, d, J = 13.5 Hz), 4.00–4.40 (2H, m), 6.67 (1H, d, J = 8.7 Hz), 6.92 (1H, d, J = 2.4 Hz), 7.02–7.12 (3H, m), 7.20–7.34 (3H, m). The amino NH signal was not observed; ESI-MS m/z 481 [$(M+H)^+$], 425 [$(M-{}^t\mathrm{Bu}+2H)^+$]; chemical purity: 94.2%.

5.1.9. *cis*-*N*-[2-Methoxy-5-(trifluoromethoxy)benzyl]-3-phenyl-4-piperidinamine dihydrochloride (12)

To a cooled mixture of **10** (5.40 g, 11.2 mmol) in THF (30 mL) was added TFA (10 mL) at 0 °C. After stirring for 10 min, the volatiles were evaporated, and the residue was poured into aqueous NaOH and EtOAc. The organic layer was washed with brine, dried and concentrated to give an oil. The obtained oil was treated with 2 equiv of 4 N HCl–EtOAc (5.5 mL) and triturated with Et_2O to provide **12** (3.95 g, 78%) as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.60–2.00 (2H, m), 2.83 (1H, dt, J = 12.0, 3.9 Hz), 2.95–3.08 (3H, m), 3.16 (1H, dt, J = 12.3, 3.0 Hz), 3.41–3.51 (1H, m), 3.47 (3H, s), 3.70 (1H, d, J = 14.7 Hz), 3.89 (1H, dd, J = 7.8, 3.6 Hz), 6.63 (1H, d, J = 8.7 Hz), 6.90 (1H, d, J = 2.7 Hz), 7.00 (1H, dd, J = 9.3, 2.4 Hz), 7.12–7.18 (2H, m), 7.18–7.34 (3H, m). The NMR spectrum was measured as a free base, and the amino NH signals were not observed; ESI-MS m/z 381 [$(M-2HCl+H)^+$]; Anal. Calcd for $C_{20}H_{25}Cl_2N_2O_2F_3\cdot 0.5H_2O$: C, 51.96; H, 5.67; N, 6.06. Found: C, 51.81; H, 5.62; N, 6.07.

5.1.10. *trans*-*N*-[2-Methoxy-5-(trifluoromethoxy)benzyl]-3-phenyl-4-piperidinamine hydrochloride (13)

To a cooled mixture of **11** (0.86 g, 1.79 mmol) in THF (5 mL) was added TFA (2 mL) at 0 °C. After stirring for 10 min, the solvents were evaporated, and the residue was poured into aqueous NaOH and EtOAc. The organic layer was washed with brine, dried and concentrated to give an oil. The obtained oil was treated with 1 equiv of 4 N

HCl–EtOAc (0.45 mL) and triturated with Et_2O to provide **13** (0.74 g, 99%) as white powder. Mp 200–202 °C; ^{1}H NMR ($CDCl_3$) δ: 1.77–1.95 (1H, m), 2.24–2.36 (1H, m), 2.71 (1H, dt, J = 10.6, 3.6 Hz), 2.82–3.10 (3H, m), 3.34–3.60 (6H, m), 3.72 (1H, d, J = 13.8 Hz), 5.00–6.60 (2H, br), 6.87 (1H, d, J = 2.4 Hz), 7.02–7.10 (3H, m), 7.24–7.34 (3H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS m/z 381 [(M−HCl+H)$^+$]; Anal. Calcd for $C_{20}H_{24}ClN_2O_2F_3$: C, 57.62; H, 5.80; N, 6.72. Found: C, 57.38; H, 5.82; N, 6.63.

5.1.11. *cis*-1-Ethyl-*N*-[2-methoxy-5-(trifluoromethoxy)benzyl]-3-phenyl-4-piperidinamine hydrochloride (14)

A solution of **12** (0.23 g, 0.50 mmol) ethyl iodide (0.083 g, 0.53 mmol) and Et_3N (0.10 g, 1.0 mmol) in THF (5 mL) and DMF (1 mL) was stirred at room temperature for 3 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil. The oil was treated with 1 equiv of 4 N HCl–EtOAc (0.085 mL) to provide **14** (0.15 g, 67%) as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, J = 7.2 Hz), 1.71–2.04 (2H, m), 2.55 (2H, q, J = 7.2 Hz), 2.69-3.18 (5H, m), 3.41-3.51 (6H, m), 3.68-3.72 (1H, m), 6.64 (1H, d, J = 9.0 Hz), 6.90 (1H, s like), 6.99-7.04 (1H, m), 7.11-7.31 (5H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS m/z 409 [(M−HCl+H)$^+$]; chemical purity: 97.9%.

5.1.12. *cis*-*N*-[2-Methoxy-5-(trifluoromethoxy)benzyl]-1-(methyl sulfonyl)-3-phenyl-4-piperidin-4-amine hydrochloride (15)

To a cooled mixture of **12** (0.23 g, 0.50 mmol) and Et_3N (0.14 mL, 1.00 mmol) in THF (1 mL) and DMF (0.2 mL) was added methanesulfonyl chloride (0.060 g, 0.52 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was added to aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc (0.10 mL) to give **15** (0.19 g, 83%) as white powder. Mp 175–179 °C; ^{1}H NMR ($CDCl_3$) δ: 1.80–1.93 (1H, m), 2.00–2.10 (1H, m), 2.85 (3H, s), 2.95–3.00 (1H, m), 3.14–3.28 (3H, m), 3.40–3.60 (3H, m), 3.45 (3H, s), 3.65-3.76 (2H, m), 6.64 (1H, d, J = 9.0 Hz), 6.89 (1H, s like), 6.99–7.15 (3H, m), 7.20–7.35 (3H, m); Anal. Calcd for $C_{21}H_{26}ClN_2O_4F_3S{\cdot}0.5H_2O$: C, 50.05; H, 5.40; N, 5.56. Found: C, 49.71; H, 5.40; N, 5.46; ESI-MS m/z 459 [(M–HCl+H)$^+$].

5.1.13. Methyl *cis*-4-{[2-methoxy-5-(trifluoromethoxy)benzyl]amino}-3-phenylpiperidine-1-carboxylate hydrochloride (16)

To a cooled mixture of **12** (0.23 g, 0.50 mmol) and Et_3N (0.14 mL, 1.00 mmol) in THF (1 mL) and DMF (0.2 mL) was added methyl chloroformate (0.050 g, 0.53 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was added aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc (0.10 mL) to provide **16** (0.19 g, 87%) as white powder. Mp 166–170 °C; ^{1}H NMR ($CDCl_3$) δ: 1.80–1.93 (1H, m), 2.00–2.10 (1H, m), 2.91-3.00 (3H, m), 3.10–4.15 (12H, m), 6.64 (1H, d, J = 9.0 Hz), 6.89 (1H, s like), 6.99–7.15 (3H, m), 7.20–7.35 (3H, m). The NMR spectrum was measured as a free base; Anal. Calcd for $C_{22}H_{25}N_2O_4F_3{\cdot}HCl{\cdot}0.5H_2O$: C, 54.61; H, 5.62; N, 5.79. Found: C, 54.39; H, 5.78; N, 5.65; ESI-MS m/z 439 [(M–HCl+H)$^+$].

5.1.14. *cis*-1-Acetyl-*N*-(2-methoxy-5-(trifluoromethoxy)benzyl)-3-phenyl-4-piperidinamine hydrochloride (17)

To a cooled mixture of **12** (0.20 g, 0.44 mmol) and Et_3N (0.19 mL) in THF (10 mL) was added acetyl chloride (0.032 mL, 0.44 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was poured into H_2O, and then the mixture was extracted with EtOAc. The extract was washed with aqueous NH_4Cl and brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc (0.10 mL) to provide **17** as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.80 (1H, m), 1.90–2.05 (1H, m), 2.05–2.14 (3H, m), 2.90–3.05 (2H, m), 3.25–3.98 (9H, m), 4.20–4.59 (1H, m), 6.64 (1H, dd, J = 8.7, 5.1 Hz), 6.89 (1H, s like), 6.98–7.15 (3H, m), 7.20–7.35 (3H, m). The NMR spectrum was measured as a free base; ESI-MS m/z 423 [(M−HCl+H)$^+$]; chemical purity: 96.5%.

5.1.15. *cis*-*N*-Ethyl-4-{[2-methoxy-5-(trifluoromethoxy)benzyl]amino}-3-phenylpiperidine-1-carboxamide (18)

To a cooled solution of **12** (0.60 g, 1.3 mmol) and Et_3N (0.37 mL, 2.6 mmol) in CH_3CN (10 mL) was added ethyl isocyanate (0.11 mL, 1.3 mmol) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous NH_4Cl and brine, dried and concentrated. The residue was purified by preparative HPLC to provide **18** (0.47 g, 78%) as white powder. Mp 68–70 °C; ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, J = 6.6 Hz), 1.60–1.80 (1H, m), 1.85–1.95 (1H, m), 2.95–3.06 (2H, m), 3.22–3.34 (2H, m), 3.37–3.35 (5H, m), 3.60–3.84 (5H, m), 4.38–4.42 (1H, m), 6.65 (1H, d, J = 9.0 Hz), 6.91 (1H, d, J = 2.4 Hz), 7.02 (1H, dd, J = 8.7, 3.0 Hz), 7.12–7.17 (2H, m), 7.20–7.34 (3H, m). The *cis* stereochemistry was assigned on the basis of an NOE compared with that of **19**; ESI-MS m/z 452 [(M+H)$^+$]; Anal. Calcd for $C_{23}H_{28}N_3O_3F_3$: C, 61.19; H, 6.25; N, 9.31. Found: C, 61.02; H, 6.24; N, 9.03.

5.1.16. *trans*-*N*-Ethyl-4-{[2-methoxy-5-(trifluoromethoxy)benzyl]amino}-3-phenylpiperidine-1-carboxamide (19)

Compound **19** was synthesized by the similar procedure described for **18** by using **13** and ethyl isocyanate in 42% yield as white powder. Mp 94–96 °C; ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, J = 7.2 Hz), 1.35–1.53 (1H, m), 2.10–2.18 (1H, m), 2.55–2.88 (4H, m), 3.21–3.31 (2H, m), 3.47 (3H, s), 3.56 (1H, d, J = 13.8 Hz), 3.74 (1H, d, J = 13.8 Hz), 3.84–3.93 (1H, m), 4.05–4.14 (1H, m), 4.37–4.44 (1H, t like), 3.80–4.80 (1H, br), 6.68 (1H, d, J = 9.0 Hz), 6.92 (1H, d, J = 2.4 Hz), 7.03–7.12 (3H, m), 7.20–7.34 (3H, m). The *trans* stereochemistry was assigned on the basis of an NOE between the protons at C5 and benzyl carbon; ESI-MS m/z 452 [(M+H)$^+$]; Anal. Calcd for $C_{23}H_{28}N_3O_3F_3{\cdot}0.1H_2O$: C, 60.94; H, 6.27; N, 9.27. Found: C, 60.88; H, 6.23; N, 9.34.

5.1.17. 5-Nitro-2,3-dihydro-1-benzofuran (21)[10]

To a solution of **20** (27.1 g, 226 mmol) in acetic acid (100 mL) was slowly added nitric acid (11.2 mL, 249 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was poured into a mixture of ice and 12 N NaOH (100 mL). The mixture was extracted with EtOAc. The extract was washed with aqueous NH_4Cl and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with an eluent of 20% EtOAc/hexane to provide **21** (6.4 g, 17%) as a pale brown powder; ^{1}H NMR ($CDCl_3$) δ: 3.30 (2H, t, J = 8.7 Hz), 4.74 (2H, t, J = 8.7 Hz), 6.81 (1H, d, J = 9.6 Hz), 8.07–8.12 (2H, m); Anal. Calcd for C_8H_7NO: C, 58.18; H, 4.27; N, 8.48. Found: C, 57.93; H, 4.19; N, 8.27.

5.1.18. 2,3-Dihydro-1-benzofuran-5-ylamine (22)[10]

A mixture of **21** (6.3 g, 38.1 mmol) and 10% palladium on carbon (1.0 g) in EtOH (50 mL) and THF (50 mL) was stirred under an atmosphere of H_2 (1 atm) at room temperature for 13 h. The catalyst was removed by filtration, and then the filtrate was concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient eluent of 0–10% MeOH/EtOAc to provide **22** (4.63 g, 90%) as a pale brown powder; ^{1}H NMR ($CDCl_3$) δ: 3.12 (2H, t, J = 8.6 Hz), 3.37 (2H, s), 4.49 (2H, t, J = 8.6 Hz), 6.43–6.47

(1H, m) 6.57–6.61 (2H, m); Anal. Calcd for C_8H_9NO: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.92; H, 6.60; N, 10.09.

5.1.19. *N*-2,3-Dihydro-1-benzofuran-5-yl-2,2,2-trifluoroacetamide (23)

To a cooled mixture of **22** (4.51 g, 33.4 mmol) and Et_3N (6.1 mL, 43.4 mmol) in CH_2Cl_2 (40 mL) was added trifluoroacetic anhydride (6.2 mL, 43.4 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was added into aqueous NH_4Cl and extracted with EtOAc. The extract was washed with brine, dried and concentrated to provide **23** (7.1 g, 98%) as white powder; ^{1}H NMR ($CDCl_3$) δ: 3.23 (2H, t, J = 8.7 Hz), 4.60 (2H, t, J = 8.7 Hz), 6.76 (1H, d, J = 8.1 Hz), 7.12 (1H, dd, J = 8.7, 2.4 Hz), 7.51 (1H, m), 7.65–7.80 (1H, m); Anal. Calcd for $C_{10}H_8NO_2F$: C, 51.96; H, 3.49; N, 6.06. Found: C, 52.13; H, 3.39; N, 6.38.

5.1.20. 1-(2,3-Dihydro-1-benzofuran-5-yl)-5-(trifluoromethyl)-1*H*-tetrazole (24)

To a suspension of **23** (6.91 g, 32.1 mmol) in CCl_4 (90 mL) was added triphenylphosphine (12.6 g, 48.2 mmol), and the mixture was stirred at 95 °C for 24 h. After the evaporation of the solvent, the residue was dissolved in DMF (120 mL), and then sodium azide (3.34 g, 51.4 mmol) was added. After stirring at room temperature for 30 min, the reaction mixture was poured into H_2O, and then the mixture was extracted with EtOAc. The extract was washed with H_2O and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with an eluent of 10% EtOAc/hexane to provide **24** (7.12 g, 87%) as white powder. ^{1}H NMR ($CDCl_3$) δ: 3.33 (2H, t, J = 9.0 Hz), 4.73 (2H, t, J = 9.0 Hz), 6.92 (1H, dd, J = 8.7, 0.6 Hz), 7.17–7.21 (1H, m), 7.24–7.28 (1H, m); Anal. Calcd for $C_{10}H_7N_4OF_3$: C, 46.88; H, 2.75; N, 21.87. Found: C, 47.05; H, 2.79; N, 21.84.

5.1.21. 5-[5-(Trifluoromethyl)-1*H*-tetrazol-1-yl]-2,3-dihydro-1-benzofuran-7-carbaldehyde (25)

A mixture of **24** (1.8 g, 7.0 mmol) and hexamethylenetetramine (4.0 g, 28.2 mmol) in methanesulfonic acid (50 mL) was stirred at 90 °C for 2 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with an eluent of 33% EtOAc/hexane to provide to provide **25** (1.4 g, 71%) as white powder. Mp 144–146 °C; ^{1}H NMR ($CDCl_3$) δ: 3.41 (2H, t, J = 8.7 Hz), 4.93 (2H, t, J = 8.7 Hz), 7.46–7.47 (1H, m), 7.71–7.72 (1H, m), 10.24 (1H, s); Anal. Calcd for $C_{11}H_7N_4O_2F_3$: C, 46.49; H, 2.48; N, 19.71. Found: C, 46.47; H, 2.52; N, 19.62.

5.1.22. 2-Ethoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzaldehyde (27)

A mixture of 2-hydroxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzaldehyde **26**[11] (1.1 g, 4.1 mmol), ethyl iodide (0.66 mL, 8.9 mL) and K_2CO_3 (1.3 g, 9.3 mmol) in DMF (20 mL) was heated at 85 °C for 8 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous NH_4Cl and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient eluent of 10–33% EtOAc/hexane to provide **27** (1.06 g, 86%) as white powder. Mp 90–95 °C; ^{1}H NMR ($CDCl_3$) δ: 1.57 (3H, t, J = 7.1 Hz), 4.29 (2H, q, J = 7.1 Hz), 7.20 (1H, d, J = 9.0 Hz), 7.63 (1H, dd, J = 9.0, 3.0 Hz), 7.95 (1H, d, J = 3.0 Hz), 10.51 (1H, s); Anal. Calcd for $C_{11}H_9N_4O_2F_3$: C, 46.16; H, 3.17; N, 19.58. Found: C, 46.25; H, 3.17; N, 19.73.

5.1.23. *tert*-Butyl *cis*-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidine-1-carboxylate (30)

See Section 5.1.24.

5.1.24. *tert*-Butyl *trans*-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidine-1-carboxylate (31)

Compounds **30** and **31** were synthesized by the similar procedure described for **10** and **11** by using **9** and 2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzaldehyde **29** as starting materials. Compound **30**: ^{1}H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.60–1.80 (1H, m), 1.85–1.95 (1H, m), 2.94–3.00 (2H, m), 3.27–3.36 (1H, m), 3.50–3.68 (5H, m), 3.76–3.86 (3H, m), 6.83 (1H, d, J = 8.7 Hz), 7.02 (1H, d, J = 2.7 Hz), 7.07–7.28 (6H, m). The NH signal was not observed.; ESI-MS m/z 533 [$(M+H)^+$]; chemical purity: 91.3%.

Compound **31**: ^{1}H NMR ($CDCl_3$) δ: 1.40–1.55 (10H, m), 2.05–2.18 (1H, m), 2.50–2.90 (3H, m), 3.60 (3H, s), 3.63 (1H, d, J = 15.0 Hz), 3.82 (1H, d, J = 15.0 Hz), 4.00–4.35 (3H, br), 6.86 (1H, d, J = 9.0 Hz), 7.06–7.15 (2H, m), 7.18–7.35 (5H, m). The NH signal was not observed; ESI-MS m/z 533 [$(M+H)^+$]; chemical purity: 95.4%.

5.1.25. *cis*-*N*-{2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}-3-phenylpiperidin-4-amine dihydrochloride (32)

Compound **32** was synthesized by the similar procedure described for **12** by using **30** as starting material in 95% yield as white powder: ^{1}H NMR ($CDCl_3$) δ: 1.60–2.00 (2H, m), 2.76–2.87 (1H, m), 2.96–3.19 (4H, m), 3.41 (1H, t, J = 12.2 Hz), 3.57 (1H, d, J = 15.4 Hz), 3.65 (3H, s), 3.80 (1H, d, J = 15.4 Hz), 6.83 (1H, d, J = 8.8 Hz), 7.00–7.02 (1H, m), 7.07–7.27 (6H, m). The NMR spectrum was measured as a free base, and the amino NH signals were not observed; Anal. Calcd for $C_{21}H_{23}N_6OF_3{\cdot}2HCl{\cdot}0.75H_2O$: C, 48.61; H, 5.15; N, 16.20. Found: C, 48.55; H, 5.45; N, 15.94; ESI-MS m/z 433 [$(M-2HCl+H)^+$].

5.1.26. *cis*-1-Ethyl-*N*-{2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]}benzyl)-3-phenyl-4-piperidinamine hydrochloride (33)

This compound was synthesized by the similar procedure described for **14** by using **32** as starting material in 72% yield. White amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.13 (3H, t, J = 7.2 Hz), 1.80–1.99 (3H, m), 2.46–2.58 (3H, m), 2.76–2.88 (4H, m), 3.14–3.20 (1H, m), 3.57 (1H, d, J = 15.6 Hz), 3.65 (3H, S), 3.78 (1H, d, J = 15.6 Hz), 6.82 (1H, d, J = 11.0 Hz), 7.00–7.27 (6H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS m/z 461 [$(M-HCl+H)^+$]; chemical purity: 95.4%.

5.1.27. *cis*-*N*-{2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}-1-(methylsulfonyl)-3-phenyl-4-piperidinamine (34)

To a mixture of **32** (152 mg, 0.30 mmol), Et_3N (61 mg, 0.60 mmol) in a mixture of THF and DMF (5/1, v/v, 3 mL) was added methanesulfonyl chloride (72 mg, 0.63 mmol). After stirring at room temperature for 18 h, the reaction mixture was poured into H_2O and extracted with EtOAc (×2). The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated in vacuo. The residue was purified by preparative HPLC to give **34** (50 mg, 33%) as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.80–1.95 (1H, m), 2.00–2.11 (1H, m), 2.85 (3H, s), 2.95–2.99 (1H, m), 3.18–3.27 (2H, m), 3.47–3.60 (4H, m), 3.60 (3H, s), 3.71–3.83 (2H, m), 6.85 (1H, d, J = 8.7 Hz), 7.04–7.32 (7H, m); ESI-MS m/z 511 [$(M+H)^+$]; chemical purity: 92.6%.

5.1.28. Methyl *cis*-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinecarboxylate (35)

To a mixture of **32** (152 mg, 0.30 mmol), Et_3N (61 mg, 0.60 mmol) in a mixture of THF and DMF (5/1, v/v, 3 mL) was added methyl chloroformate (30 mg, 0.32 mmol). After stirring at room temperature for 12 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with

aqueous $NaHCO_3$ and brine, dried and concentrated in vacuo. The residue was purified by preparative HPLC to give **35** (128 mg, 87%) as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.62–2.00 (2H, m), 2.95–3.05 (2H, m), 3.31–3.39 (1H, m), 3.54–3.89 (9H, m), 3.62 (3H, s), 6.82–6.86 (1H, m), 7.00–7.29 (7H, m); ESI-MS *m/z* 491 [(M+H)$^+$]; chemical purity: 99.1%.

5.1.29. 1-Acetyl-*N*-{2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}-3-phenyl-4-piperidinamine hydrochloride (36)

To a mixture of **32** (0.15 g, 0.30 mmol), acetic acid (0.022 g, 0.36 mmol), HOBt (0.055 g, 0.36 mmol) and Et_3N (0.061 g, 0.60 mmol) in DMF (3 mL) was added WSC (0.069 g, 0.36 mmol). After stirring at room temperature for 14 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc to provide **36** (0.12 g, 78%) as a white amorphous. ^{1}H NMR($CDCl_3$) δ: 1.68–1.72 (1H, m), 1.90–2.03 (1H, m), 2.15 (1.5H, s), 2.17 (1.5H, s), 2.91–3.04 (2H, m), 3.25–3.42 (1H, m), 3.52–3.96 (5H, m), 3.60 (1.5H, s), 3.66 (1.5H, s), 4.22–4.27 (0.5H, m), 4.57–4.62 (0.5H, m), 6.82–6.87 (1H, m), 7.03–7.29 (7H, m); ESI-MS *m/z* 475 [(M−HCl+H)$^+$]; chemical purity: 87.4%.

The following compounds from **37** to **42** were prepared in a manner similar to that described for the synthesis of **36**.

5.1.30. *cis*-1-[(1-Acetyl-3-azetidinyl)carbonyl]-*N*-{2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}-3-phenyl-4-piperidinamine hydrochloride (37)

Yield 40%. White amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.83–1.93 (1H, m), 2.01–2.06 (2H, m), 2.40 (3H, s), 3.04–3.05 (1H, m), 3.05–3.18 (1H, m), 3.39–4.00 (10H, m), 3.64 (3H, s), 6.85–6.88 (1H, m), 7.05–7.30 (7H, m); ESI-MS *m/z* 558 [(M−HCl+H)$^+$]; chemical purity: 98.0%.

5.1.31. *cis*-1-[(1-Acetyl-4-piperidinyl)carbonyl]-*N*-{2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}-3-phenyl-4-piperidinamine hydrochloride (38)

Yield 99%. White amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.50–2.15 (7H, m), 2.50–4.00 (16H, m), 4.20–4.65 (2H, m), 6.82–7.34 (8H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 586 [(M–HCl+H)$^+$]; chemical purity: 97.6%.

5.1.32. *N*-{2-[*cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}acetamide (39)

Yield 88%. White powder. Mp 165–168 °C; ^{1}H NMR ($CDCl_3$) δ: 1.60–1.75 (1H, m), 1.95–1.99 (1H, m), 2.03 (1.5H, s), 2.05 (1.5H, s), 2.95–3.10 (2H, m), 3.25–4.19 (11H, m), 4.25–4.40 (0.5H, m), 4.53–4.58 (0.5H, m), 6.60–6.70 (1H, m), 6.84 (1H, dd, *J* = 9.0, 3.0 Hz), 7.00–7.08 (3H, m), 7.18–7.28 (4H, m); ESI-MS *m/z* 532 [(M+H)$^+$]; chemical purity: 98.5%.

5.1.33. *N*-{3-[*cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-3-oxopropyl}acetamide hydrochloride (40)

Yield 92%. White amorphous; ^{1}H NMR ($CDCl_3$) δ: 1.60–1.75 (1H, m), 1.95–1.99 (1H, m), 2.03 (1.5H, s), 2.05 (1.5H, s), 2.95–3.10 (2H, m), 3.25–4.19 (11H, m), 4.25–4.40 (0.5H, m), 4.53–4.58 (0.5H, m), 6.60–6.70 (1H, m), 6.84 (1H, dd, *J* = 9.0, 3.0 Hz), 7.00–7.08 (3H, m), 7.18–7.28 (4H, m); ESI-MS *m/z* 546 [(M−HCl+H)$^+$]; chemical purity: 98.9%.

5.1.34. *N*-{4-[*cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidin-1-yl]-4-oxobutyl}acetamide hydrochloride (41)

Yield 95%. White amorphous; ^{1}H NMR ($CDCl_3$) δ: 1.63–2.03 (7H, m), 2.36 (1H, t, *J* = 6.0 Hz), 2.47 (1H, t, *J* = 6.0 Hz), 2.90–3.02 (2H, m), 3.21–3.92 (11H, m), 4.20–4.31 (0.5H, m), 4.54–4.62 (0.5H, m), 6.30–6.45 (1H, m), 6.86 (1H, dd, *J* = 9.0, 3.0 Hz), 7.06–7.35 (7H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 560 [(M−HCl+H)$^+$].

5.1.35. *N*-{2-[*cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}-*N*-methylacetamide hydrochloride (42)

Yield 27%. White amorphous; ^{1}H NMR ($CDCl_3$) δ: 1.65–1.82 (1H, m), 1.91–2.03 (1H, m), 2.11 (1.5H, s), 2.16 (1.5H, s), 2.91–3.10 (2H, m), 3.07 (1.5H, s), 3.10 (1.5H, s), 3.32–4.42 (8.5H, m), 3.61 (1.5H, s), 3.65 (1.5H, s), 4.52–4.56 (0.5H, m), 6.84 (1H, m), 7.01–7.29 (7H, m). The NMR spectrum was measured as a free base; ESI-MS *m/z* 546 [(M−HCl+H)$^+$]; chemical purity: 99.8%.

5.1.36. *cis*-*N*-Ethyl-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidine-1-carbox! amide hydrochloride (43)

Compound **43** was synthesized by the similar procedure described for **18** by using **32** and ethyl isocyanate as starting materials in 59% yield as white powder. Mp 143–145 °C: ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, *J* = 7.2 Hz), 1.65–1.80 (1H, m), 1.85–1.95 (1H, m), 2.95–3.06 (2H, m), 3.22–3.32 (2H, m), 3.36–3.46 (1H, m), 3.55–3.70 (6H, m), 3.76–3.84 (2H, m), 4.36–4.44 (1H, m), 6.84 (1H, d, *J* = 8.7 Hz), 7.04 (1H, d, *J* = 2.7 Hz), 7.12–7.30 (6H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; Anal. Calcd for $C_{24}H_{28}N_7O_2F_3{\cdot}HCl{\cdot}0.25H_2O$: C, 51.25; H, 5.64; N, 17.43. Found: C, 51.51; H, 5.41; N, 16.89; ESI-MS *m/z* 504 [(M−HCl+H)$^+$].

5.1.37. *cis*-*N*-Isopropyl-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinecarboxamide hydrochloride (44)

To a cooled solution of **32** (0.30 g, 0.59 mmol) and Et_3N (0.17 mL, 1.19 mmol) in CH_3CN (10 mL) was added isopropyl isocyanate (0.059 mL, 0.59 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous NH_4Cl and brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc and triturated with IPE to provide **44** (0.19 g, 57%) as a white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.13 (6H, d, *J* = 6.6 Hz), 1.65–1.80 (1H, m), 1.85–1.95 (1H, m), 2.94–3.06 (2H, m), 3.34–3.46 (1H, m), 3.54–3.70 (6H, m), 3.72–3.84 (2H, m), 3.98 (1H, septet, *J* = 6.6 Hz), 4.21 (1H, d, *J* = 7.5 Hz), 6.85 (1H, d, *J* = 8.7 Hz), 7.04 (1H, d, *J* = 2.4 Hz), 7.12–7.30 (6H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 518 [(M−HCl+H)$^+$]; chemical purity: 85.0%.

5.1.38. *cis*-*N*-Cyclopropyl-4-({2-methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinecarboxamide hydrochloride (45)

To a mixture of **32** (0.20 g, 0.40 mmol), Et_3N (0.11 mL, 0.80 mmol) in DMF (3 mL) was added 4-nitrophenyl cyclopropylcarbamate (0.11 g, 0.48 mmol). After stirring at room temperature for 8 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by preparative HPLC to give an oil, which was treated with 1 equiv of

4 N HCl–EtOAc and triturated with IPE to provide **45** (0.15 g, 68%) as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 0.43–0.48 (2H, m), 0.67–0.73 (2H, m), 1.67–1.75 (1H, m), 1.88–1.94 (1H, m), 2.62–2.67(1H, m), 2.97–3.01 (2H, m), 3.36–3.45 (1H, m), 3.54–3.63 (3H, m), 3.63 (3H, s), 3.77–3.82 (2H, m), 4.89 (1H, s), 6.83–6.86 (1H, m), 7.02–7.28 (7H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 516 [(M−HCl+H)$^+$]; chemical purity: 86.9%.

5.1.39. *cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-*N*-(2,2,2-trifluoroethyl)-1-piperidinecarboxamide hydrochloride (46)

This compound was synthesized by the similar procedure described for **45** by using **32** and 4-nitrophenyl (2,2,2-trifluoroethyl)carbamate as starting materials in 33% yield. White amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.67–1.75 (1H, m), 1.88–1.94 (1H, m), 2.97–3.01 (2H, m), 3.36–3.60 (3H, m), 3.54–3.63 (3H, m), 3.63 (3H, s), 3.77–3.82 (2H, m), 4.89 (1H, s), 6.83–6.86(1H, m), 7.02–7.28 (7H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 558 [(M−HCl+H)$^+$]; chemical purity: 96.3%.

5.1.40. *cis*-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-*N*,*N*-dimethyl-3-phenyl-1-piperidinecarboxamide hydrochloride (47)

To a cooled mixture of **32** (0.30 g, 0.59 mmol), Et_3N (0.17 mL, 1.20 mmol) in CH_3CN (10 mL) was added dimethylcarbamyl chloride (0.10 mL, 1.13 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with an eluent of EtOAc/hexane/MeOH (40:10/1, v/v) to give an oil, which was treated with 1 equiv of 4 N HCl–EtOAc and triturated with IPE to provide **47** (0.29 g, 97%) as a white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.70–2.00 (2H, m), 2.77 (6H, s), 2.95–3.02 (1H, m), 3.04–3.12 (1H, m), 3.32 (1H, dt, *J* = 10.2, 3.0 Hz), 3.40–3.50 (1H, m), 3.52–3.68 (6H, m), 3.79 (1H, d, *J* = 15.3 Hz), 6.54 (1H, d, *J* = 11.7 Hz), 7.00 (1H, d, *J* = 2.7 Hz), 7.08–7.29 (6H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 504 [(M−HCl+H)$^+$]; chemical purity: 97.0%.

5.1.41. *cis*-*N*-Ethyl-4-({2-ethoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidine-1-carboxamide hydrochloride (48)

This compound was synthesized by the similar procedure described for **43** by using **9** and **27** as starting materials in 46% yield as a white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.34 (6H, m), 1.66–1.80 (1H, m), 1.86–1.96 (1H, m), 2.97–3.06 (2H, m), 3.22–3.34 (2H, m), 3.35–3.46 (1H, m), 3.56 (1H, d, *J* = 15.3 Hz), 3.60–3.70 (2H, m), 3.82 (1H, d, *J* = 15.3 Hz), 3.77–4.00 (3H, m), 4.38–4.46 (1H, m), 6.83 (1H, d, *J* = 8.7 Hz), 7.03 (1H, d, *J* = 2.4 Hz), 7.08–7.14 (2H, m), 7.15–7.27 (4H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 518 [(M−HCl+H)$^+$]; chemical purity: 97.7%.

5.1.42. *cis*-*N*-Ethyl-4-({2-(cyclopropyloxy)-5-[5-(trifluoromethyl) -1*H*-tetrazol-1-yl]benzyl}amino)-3-phenylpiperidine-1-carboxamide hydrochloride (49)

This compound was synthesized by the similar procedure described for **43** by using **9** and 2-(cyclopropyloxy)-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzaldehyde **28** as starting materials in 36% yield as a white amorphous. ^{1}H NMR ($CDCl_3$) δ: 0.57–0.65 (2H, m), 0.74–0.82 (2H, m), 1.12 (3H, t, *J* = 7.2 Hz), 1.65–1.80 (1H, m), 1.84–1.93 (1H, m), 2.95–3.05 (2H, m), 3.21–3.32 (2H, m), 3.33–3.44 (1H, m), 3.53 (1H, d, *J* = 15.3 Hz), 3.72 (1H, d, *J* = 15.3 Hz), 3.55–3.80 (4H, m), 4.35–4.44 (1H, m), 7.02 (1H, s), 7.10–7.25 (7H, m). The NMR spectrum was measured as a free base, and the amino NH signal was not observed; ESI-MS *m/z* 530 [(M−HCl+H)$^+$]; chemical purity: 98.7%.

5.1.43. *cis*-*N*-Ethyl-3-phenyl-4-[({5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]-2,3-dihydro-1-benzofuran-7-yl}methyl)amino] piperidine-1-carboxamide (50)

This compound was synthesized by the similar procedure described for **43** by using **9** and **25** as starting materials in 68% yield as white powder. Mp 157–159 °C: ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, *J* = 7.2 Hz), 1.65–1.80 (1H, m), 1.84–1.94 (1H, m), 2.95–3.06 (2H, m), 3.18–3.33 (4H, m), 3.36–3.46 (1H m), 3.55 (1H, d, *J* = 14.7 Hz), 3.60–3.70 (2H, m), 3.75 (1H, d, *J* = 14.7 Hz), 3.75–3.82 (1H, m), 4.38–4.60 (3H, m), 6.79 (1H, s), 7.09 (1H, s), 7.12–7.20 (3H, m), 7.22–7.25 (2H, m). The amino NH signal was not observed; Anal. Calcd for $C_{25}H_{28}N_7O_2F_3$: C, 58.24; H, 5.47; N, 19.02. Found: C, 58.19; H, 5.47; N, 18.85; ESI-MS *m/z* 516 [(M+H)$^+$].

5.1.44. *tert*-Butyl (3*R*, 4*S*)-4-amino-3-phenylpiperidine-1-carboxylate ((+)-52)

The mixture of **8** (60.6 g, 220 mmol), (*S*)-1-phenylethylamine (40 g, 330 mmol), $AlCl_3$ (1.5 g, 11 mmol) and toluene (750 mL) was stirred under reflux temperature for 8 h, and then concentrated. The mixture of the residue, Raney nickel (110 g) and ethanol (500 mL) was stirred under a hydrogen pressure of 0.5 MPa at room temperature for 62 h. Insoluble materials were filtered off and the filtrate was concentrated. The residue was purified by flash chromatography on SiO_2 with an eluent of 25% EtOAc/hexane to give a hydrogenated compound **51** as an oil. The mixture of the compound, Pd-C (11.1 g), and ethanol (500 mL) was stirred at a hydrogen pressure of 0.5 MPa at 45 °C for 13 h. Insoluble materials were filtered off and the filtrate was concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient eluent from 33% EtOAc/hexane to 33% MeOH/EtOAc to give (+)-**52** (52.0 g, 86%, 98% ee) as white powder: Mp 100–103 °C; ^{1}H NMR ($CDCl_3$) δ: 0.87 (1H, br s), 1.20 (1H, br s), 1.46 (9H, s), 1.61 (1H, m), 1.88 (1H, m), 2.93 (1H, m), 3.30–4.11 (5H, m), 7.00–7.52 (5H, m); $[\alpha]_D^{25}$ = + 103 (c 1.0, $CHCl_3$).

5.1.45. *tert*-Butyl (3*S*, 4*R*)-4-amino-3-phenylpiperidine-1-carboxylate ((−)-52)

This compound was synthesized by the similar procedure described for (+)-**52** by using **8** and (*R*)-1-phenylethylamine as starting materials in 59% yield as white powder. Optical purity 97% ee; The NMR spectrum was the same as that of (+)-**52**: Mp 100–103 °C; $[\alpha]_D^{25}$ = −91.0 (*c* 1.0, MeOH).

5.1.46. *N*-{2-[(3*R*,4*S*)-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}acetamide ((+)-39)

This compound was synthesized by the similar procedure described for **39** by using (+)-**52** as starting material in 78% yield as white powder. Optical purity >98% ee: Mp 117–118 °C; NMR and MS spectrum were the same as those of **39**; Anal. Calcd for $C_{25}H_{28}F_3N_7O_3$: C, 56.49; H, 5.31; N, 18.45. Found: C, 56.46; H, 5.48; N, 18.51. $[\alpha]_D^{25}$ = +70.6 (*c* 1.0, MeOH).

5.1.47. *N*-{2-[(3*S*,4*R*)-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}acetamide ((−)-39)

This compound was synthesized by the similar procedure described for **39** by using (−)-**52** as starting material in 94% yield as white powder. Optical purity >98% ee: Mp 116–117 °C; NMR

and MS spectrum were the same as those of **39**; Anal. Calcd for $C_{25}H_{28}F_3N_7O_3$: C, 56.49; H, 5.31; N, 18.45. Found: C, 56.23; H, 5.10; N, 18.48. $[\alpha]_D^{25} = -73.3$ (*c* 1.0, MeOH)

5.1.48. *trans*-*N*-{2-[-4-({2-Methoxy-5-[5-(trifluoromethyl)-1*H*-tetrazol-1-yl]benzyl}amino)-3-phenyl-1-piperidinyl]-2-oxoethyl}acetamide (53)

This compound was synthesized by the similar procedure described for **39** by using **31** as starting material in 75% yield as white amorphous. ^{1}H NMR ($CDCl_3$) δ: 1.29–1.50 (1H, m), 2.04–2.05 (3H, m), 2.17–2.25 (1H, m), 2.48–2.65 (3H, m), 3.02–3.16 (1H, m), 3.61 (3H, s), 3.61–4.17 (6H, m), 4.61–4.77 (1H, m), 6.60 (1H, br s), 6.88 (1H, d, *J* = 6.0 Hz), 7.06–7.15 (3H, m), 7.20–7.35 (4H, m); ESI-MS *m/z* 532 [(M+H)$^+$]; chemical purity: 100.0%.

5.1.49. (3*R*,4*S*)-4-[(4-Bromobenzoyl)amino]-*N*-ethyl-3-phenyl-1-piperidinecarboxamide (54)

To a mixture of (+)-**52** (1.00 g, 3.62 mmol, 98% ee), 4-bromobenzoic acid (1.09 g, 5.43 mmol) and HOBt (0.83 g, 5.43 mmol) in DMF (20 mL) was added WSC (1.04 g, 5.43 mmol) at rt. After stirring for 4 h, the reaction mixture was poured into H_2O, and extracted with EtOAc. The extract was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was purified by flash chromatography on SiO_2 with a gradient eluent of 10–40% EtOAc/hexane to give a white powder (1.28 g). The obtained powder (1.28 g) was dissolved in MeOH (10 mL), and then 4 N HCl–EtOAc (2.8 mL) was added. After stirring at 50 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in aqueous NaOH, and the mixture was extracted with EtOAc. The extract was washed with and brine, dried and concentrated to provide a white powder (0.70 g, 1.95 mmol, 36%); ^{1}H NMR ($CDCl_3$) δ: 1.80–2.00 (2H, m), 2.92–3.02 (1H, m), 3.04–3.14 (1H, m), 3.16–3.32 (3H, m), 4.48–4.57 (1H, m), 5.78–5.85 (1H, m), 7.23–7.41 (6H, m), 7.47–7.56 (3H, m); $[\alpha]_D^{25} = +117.3$ (*c* 1.0, MeOH); Anal. Calcd for $C_{18}H_{19}N_2OBr{\cdot}0.25H_2O$: C, 59.43; H, 5.40; N, 7.70. Found: C, 59.59; H, 5.28; N, 7.72.

To a cooled solution of the powder (1.00 g, 2.53 mmol) and Et_3N (0.71 mL, 5.08 mmol) in CH_3CN (15 mL) was added ethyl isocyanate (0.71 mL, 3.76 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was poured into H_2O, and extracted with EtOAc. The extract was washed with 0.1 N HCl, aqueous $NaHCO_3$ and brine, dried and concentrated to provide **54** (0.93 g, 2.16 mmol, 86%) as a white powder; ^{1}H NMR ($CDCl_3$) δ: 1.13 (3H, t, *J* = 7.2 Hz), 1.80–2.00 (2H, m), 3.10–3.24 (1H, m), 3.24–3.35 (3H, m), 3.64 (1H, dd, *J* = 13.5, 3.9 Hz), 3.88 (1H, dd, *J* = 13.5, 5.1 Hz), 4.05–4.15 (1H, m), 4.45–4.60 (2H, m), 5.69 (1H, d, *J* = 9.0 Hz), 7.26–7.35 (5H, m), 7.39 (2H, d, *J* = 8.4 Hz), 7.51 (2H, d, *J* = 8.4 Hz); $[\alpha]_D^{25} = +25.0$ (*c* 1.0, MeOH); Anal. Calcd for $C_{21}H_{24}N_3O_2Br$: C, 58.61; H, 5.62; N, 9.76. Found: C, 58.53; H, 5.62; N, 9.79.

5.2. Single-crystal X-ray analysis of 54

A crystal of **54** was obtained by recrystallization from acetone and Et_2O. 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. The absolute configuration was determined by the Flack parameter of −0.02(1).

Table 6
Crystal data and summary of data collection

Empirical formula	$C_{21}H_{24}BrN_3O_2$
Formula weight	430.34
Crystal color, habit	colorless, needle
Crystal dimensions	0.80 × 0.070 × 0.01 mm
Crystal system	monoclinic
Lattice type	C-centered
Lattice parameters	a = 74.60(3) Å b = 5.670(2) Å c = 18.899(7) Å β = 96.24(3)° V = 7946(4) Å^3
Space group	C2 (#5)
Z value	16
D_{calc}	1.439 g/cm^3
F_{000}	3552.00
Diffractometer	Rigaku RAXIS-RAPID Imaging Plate
Radiation	MoKα (λ = 0.71075 Å^3) graphite monochromated
Temperature	−150 °C
No. of reflections measured	Total: 38315 Unique: 16316 (R_{int} = 0.150)
Corrections	Lorentz-polarization Absorption (*trans*. factors: 0.2080–0.9793)
Structure solution	Direct Methods (SHELXS-97)
Refinement	Full-matrix least-squares (SHELXL-97)
Function minimized	$\Sigma\omega(Fo^2-Fc^2)^2$
Least squares weights	$\omega = [\sigma^2(Fo^2) + (0.0010P)^2 + 0.0000P]^{-1}$ where $P = (Fo^2 + 2Fc^2)/3$
No. of reflections	10040
No. variables	978
Reflection/parameter ratio	10.27
Residuals: R; Rw	0.069; 0.169
Goodness of fit indicator	0.81
Max Shift/error in final Cycle	0.00
Maximum peak in final diff. map	0.99 e^-/Å^3
Minimum peak in final diff. map	−0.74 e^-/Å^3
Flack parameter	−0.02(1)

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 in the literature with minor modification.[14] IM-9 cells (2 × l0^5 cells/mL) were inoculated and incubated for three 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 a tachykinin NK_1 receptor agonist, GR73637, in guinea pigs

Experiments were performed according to the methods reported by Rupniak et al. with a minor modification.[15] Male Hartley guinea pigs weighing 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 tachykinin 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 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 ambulatory activity was started. Forty-five minutes before the measurement, 0.5% methylcellulose solution or test compounds suspended in 0.5% methylcellulose solution were orally administered in a volume of 2 mL/kg. An animal infused with saline 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, 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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References and notes

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