Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 4645–4665
Bioorganic & Medicinal Chemistry

Highly potent PDE4 inhibitors with therapeutic potential

Hiroshi Ochiai,[a] Tazumi Ohtani,[a] Akiharu Ishida,[a] Kensuke Kusumi,[a] Masashi Kato,[a] Hiroshi Kohno,[a] Yoshihiko Odagaki,[a] Katuya Kishikawa,[b] Susumu Yamamoto,[a] Hiroshi Takeda,[a] Takaaki Obata,[a] Hisao Nakai[a,*] and Masaaki Toda[a]

[a]*Minase Research Institute, Ono Pharmaceutical Co., Ltd, 3-1-1 Sakurai, Shimamoto, Osaka, Mishima 618-8585, Japan*
[b]*Development Planning, Ono Pharmaceutical Co., Ltd, 2-1-5 Doshomachi, Osaka, Chuo-ku 541-8526, Japan*

Received 29 May 2004; revised 23 June 2004; accepted 23 June 2004
Available online 20 July 2004

Abstract—The hypothesis that the dose-limiting side effects of PDE4 inhibitors could be mediated via the central nervous system prompted us to design and synthesize a hydrophilic piperidine analog to improve the side effect profile of Ariflo™ **1**, which is an orally active second-generation PDE4 inhibitor. During evaluation of various water-soluble piperidine analogs, **2a–b**, **11b–14b**, and **17a** showed therapeutic potential in cross-species comparison studies. The following three findings were obtained: (1) The hydroxamic acid group, a well known metal chelator, caused a marked increase of inhibitory activity. (2) Water-soluble piperidine analogs lacked the configurational isomerism of Ariflo **1** without loss of inhibitory activity. (3) Replacement of the 4-methoxy residue with a difluoromethoxy residue led to an increase of in vivo potency. Structure–activity relationships are presented. Single-dose rat pharmacokinetic data for **11b**, **12b**, and **17a** are also presented.

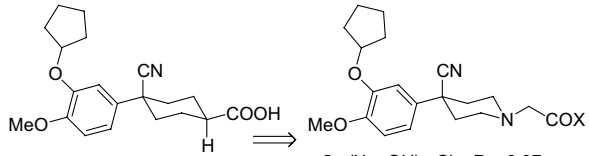
1. Introduction

The cyclic nucleotide phosphodiesterases (PDEs)[1–4] are enzymes that regulate the cellular levels of cyclic adenosine monophosphate (*c*AMP) and cyclic guanosine monophosphate (*c*GMP) through hydrolysis of these second messengers. Phosphodiesterase type 4 (PDE4) is a *c*AMP-specific PDE expressed by immune and inflammatory cells, including neutrophils, T-lymphocytes, macrophages, and eosinophils. Inhibition of PDE4 in inflammatory cells influences various specific responses, such as the production and/or release of pro-inflammatory mediators, cytokines, and active oxygen species.[5] The potential of PDE4 inhibitors as anti-inflammatory agents for the treatment of asthma and rheumatoid arthritis has received considerable attention.[5,6] However, no selective PDE4 inhibitor has been released yet because the pioneer compounds, typified by rolipram, had dose-limiting side effects (including nausea and emesis) that severely restricted their therapeutic utility.

Ariflo™ **1**[7] (Fig. 1) has a markedly improved side effect profile relative to that of the classical PDE4 inhibitor rolipram. The improved therapeutic index of Ariflo has been attributed to more selective inhibition of PDE4 catalytic activity (LPDE4) versus its competition for the high-affinity[^{3}H]rolipram-binding site (HPDE4).[8,9]

A second strategy to decrease side effects is related to the design of PDE4 subtype selective inhibitors.[10–13] Recently, naphthyridine-related PDE4 inhibitors have been reported to be PDE4D-selective.[14] Ariflo is an orally active, second-generation PDE4 inhibitor that is also reported to be PDE4D-selective.

The dose-limiting side effects of PDE4 inhibitors, such as nausea and emesis, are thought to be mediated via



1 (Ariflo) ; ClogP = 3.20
2a (X = OH) ; ClogP = 0.07
2b (X = NHOH) ; ClogP = 1.30

Figure 1. Molecular design of hydrophilic molecules.

Keywords: PDE4 inhibitor; Piperidine analog; Hydroxamic acid; Difluoromethoxy; Pharmacokinetic data.
* Corresponding author. Tel.: +81-75-961-1151; fax: +81-75-962-9314; e-mail: hi.nakai@ono.co.jp

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.06.032

the central nervous system (CNS).[15,16] Accordingly, the negative charge of Ariflo **1** at a physiological pH might decrease the occurrence of side effects because of its limited penetration into the CNS. Based on this hypothesis, the design of a highly hydrophilic PDE4 inhibitor was thought to be another possible approach to controlling side effects because compounds that show good penetration into the CNS have a relatively high lipophilicity.[17,18]

As shown in Figure 1, focus was placed on the design and synthesis of a less lipophilic and/or zwitter-ionic piperidine analog, which was structurally related to Ariflo **1** and was expected to show more limited CNS penetration.[19] A structure–activity relationship (SAR) study was started with the expectation that this approach would provide compounds with an improved therapeutic index. As a result, many analogs were found that showed increased potency in both in vitro and in vivo tests. Several of these analogs were estimated to be unlikely to cause emesis at therapeutic doses based on the difference between the dose producing the desired effects and side effects in cross-species and same-species comparisons (Table 6).

This report covers the process of identifying a number of highly potent PDE4 inhibitors with an improved therapeutic potential. SAR data for the piperidine analogs and rat pharmacokinetic data for selected compounds are also reported.

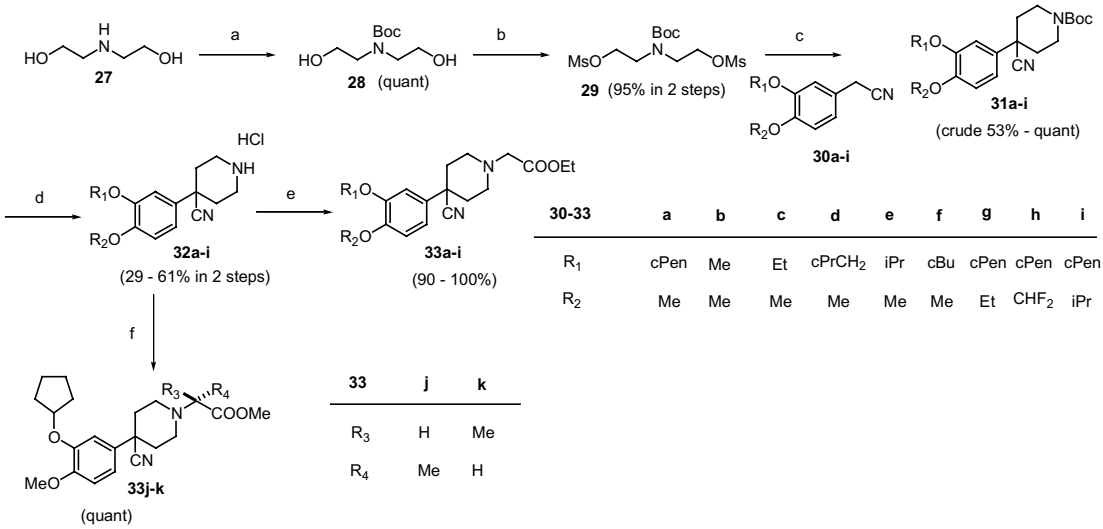
2. Chemistry

The synthesis of the compounds **2**–**26** listed in Tables 1–5 is described in Schemes 1–3. As outlined in Scheme 1, intermediates **32a**–**i** were prepared from a benzyl cyanide **30**.[7] Dialkylation of an appropriately substituted benzyl cyanide **30a**–**i** with a methane sulfonate **29**, which was prepared from **27** in two steps, afforded **31a**–**i**, respectively, while acidic deprotection produced **32a**–**i**, respectively. *N*-Alkylation of **32a**–**i** with ethyl bromoacetate gave **33a**–**i**, respectively. *N*-Alkylation of **32a** with triflates, which was derived from methyl (*S*)-(−)-lactate and methyl (*R*)-(+)-lactate, respectively, resulted in **33j** and **33k**, respectively.[20] The synthesis of **33l**–**n** is outlined in Scheme 2A. The key intermediates **36a**–**e** were prepared by ozonolysis of **35a**–**e**, which were obtained by dialkylation of appropriately substituted benzyl cyanides (**30a**,**c**, and **j**–**l**) with the dichloride **34**. Reductive amination[21] of **36e** and **36d** with an appropriate amino ester provided **33l**–**n**, respectively.

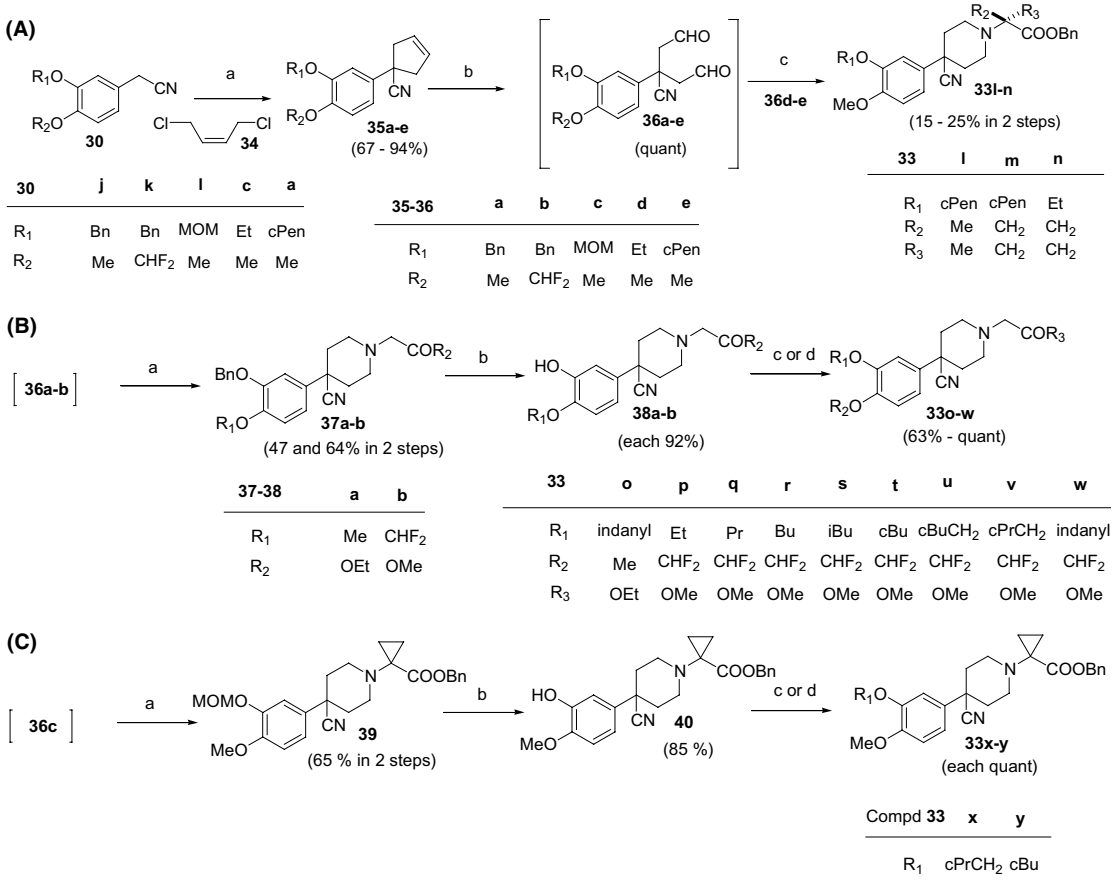
As outlined in Scheme 2B, reductive amination of **36a**–**b** with an appropriate amino ester afforded **37a**–**b**, respectively. Catalytic hydrogenolysis of **37a**–**b** gave **38a**–**b**, respectively, while *O*-alkylation of **38a**–**b** by the appropriate method resulted in **33o**–**w**, respectively.

As described in Scheme 2C, reductive amination of **36c** with an appropriate amino ester afforded **39**, acidic deprotection of which provided **40**. Then *O*-alkylation of **40** under appropriate reaction condition, gave **33x**–**y**, respectively.

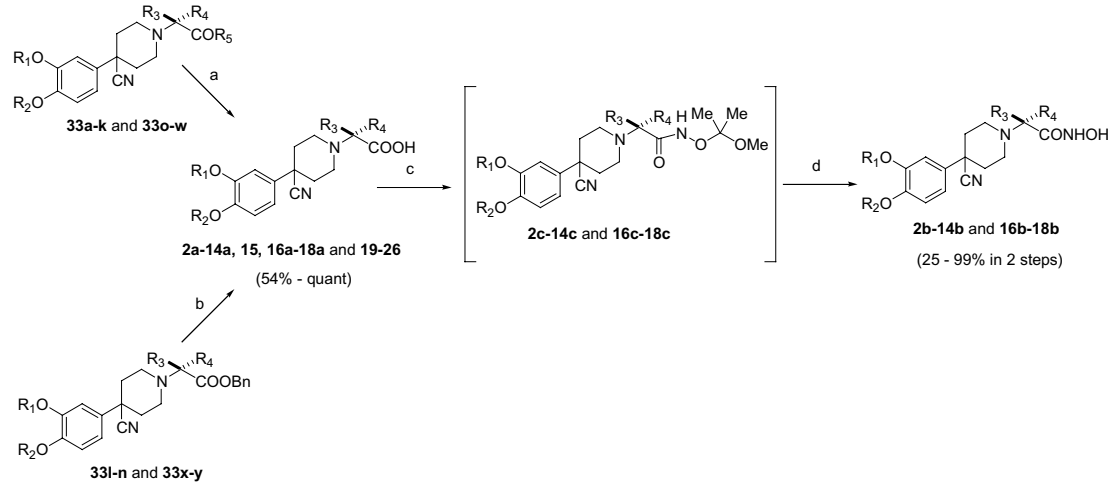
The synthesis of **2b**–**14b** and **16b**–**18b** is outlined in Scheme 3. Alkaline hydrolysis of **33a**–**k** and **33o**–**w** produced the corresponding carboxylic acids **2a**, **10a**–**14a**, **16a**–**18a**, **3a**–**4a**, and **15**, **19**–**26**, respectively. Catalytic hydrogenation of **33l**–**n** and **33x**–**y** afforded the corresponding carboxylic acids **5a**–**7a** and **8a**–**9a**, respectively. Condensation of **2a**–**14a** and **16a**–**18a** with *O*-protected hydroxylamine, followed by acidic deprotection, resulted in the corresponding hydroxamic acids **2b**–**14b** and **16b**–**18b**, respectively.



Scheme 1. Synthesis of **33a**–**k**. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, 0 °C; (b) MsCl, Et_3N, CH_2Cl_2, −78 °C; (c) **30a**–**i**, LiHMDS, THF, −78 °C; (d) TFA, $C_6H_5SCH_3$, CH_2Cl_2, then 4 N HCl/EtOAc; (e) K_2CO_3, $BrCH_2COOEt$, DMF; (f) methyl-*S*-(−)-lactate or methyl-*R*-(+)-lactate, Tf_2O, CH_2Cl_2, 2,6-lutidine, Et_3N.



Scheme 2. (A) Synthesis of **33l**–**n**. Reagents and conditions: (a) **34**, LiHMDS, THF, −78 °C; (b) O_3, CH_2Cl_2, then PPh_3, −78 °C; (c) $NaBH(OAc)_3$, AcOH, DCE, or DMF, $NH_2CR_2R_3COOBn$. (B) Synthesis of **33o**–**w**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DCE, or DMF, $NH_2CH_2COR_5$; (b) H_2, 10% Pd/C, MeOH; (c) alcohol, DEAD, PPh_3, THF; (d) alkyl halide, K_2CO_3, DMF. (C) Synthesis of **33x**–**y**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DCE, or DMF, $NH_2C(CH_2)_2COOBn$; (b) 4*N* HCl/EtOAc, CH_2Cl_2; (c) alcohol, DEAD, PPh_3, THF; (d) alkyl halide, K_2CO_3, DMF.



Scheme 3. Synthesis of **2a**–**14a**, **15**, **16a**–**18a**, **19**–**26**, **2b**–**14b**, and **16b**–**18b**. Reagents and conditions: (a) 2 N NaOHaq, EtOH; (b) H_2, 10% Pd/C, MeOH; (c) EDC, HOBt, DMF, $NH_2OC(CH_3)_2(OCH_3)$; (d) 2 N HClaq, MeOH.

3. Results and discussion

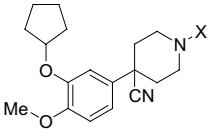
The series of piperidine analogs listed in Tables 1–5 were synthesized and biologically evaluated to assess the inhibitory effect on PDE4 obtained from U937 cells[22] (derived from human monocytes). The results of the assays were expressed as IC_{50} values, that is, the test compound concentration that caused 50% inhibition. Test

compounds were also evaluated for the inhibitory effect lipopolysaccharide (LPS)-induced production of tumor necrosis factor-α (TNF-α) in rats[23] and results were expressed as ID_{50} values (i.e., the dose that achieved 50% inhibition relative to the effect of the vehicle).

Table 1 summarized the biological activities of these piperidine analogs. Compound **2a** was designed to have less hydrophobicity than Ariflo **1** and to remove the configurational isomerism; it exhibited potent LPDE4 activity, with an oral dose of 3 mg/kg causing 74% inhibition of LPS-induced TNF-α production in rats. Based on reported information,[11] the corresponding hydroxamic acid analog **2b** was extremely potent in the in vitro LPDE4 assay and also showed an oral ID_{50} value of 0.04 mg/kg in the in vivo TNF-α production assay. Thus, the hydroxamic acid group, a well known metal chelator, was confirmed to cause a striking increase of inhibitory activity.

According to our in vivo evaluation, **2b** had no effect on LPS-induced TNF-α production at 3 h after oral administration (0.1 mg/kg), although it showed a very potent effect at 0.5 h. In the early stage of these investigations, rapid hydrolysis of the hydroxamic acid moiety of **2b** to a carboxylic acid was predicted to be one of the reasons for its short duration of action.

Table 1. Activity profile of piperidine analogs

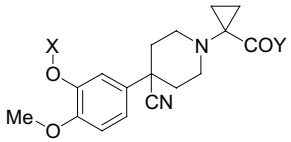


Compound	X	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[b] ID_{50} (mg/kg, po)
2a (Y = OH)		66	(74%)[c]
2b (Y = NHOH)		0.080	0.043
3a (Y = OH)		>300	NT[e]
3b (Y = NHOH)		0.91	(26%)[d]
4a (Y = OH)		>300	NT[e]
4b (Y = NHOH)		5.9	(49%)[d]
5a (Y = OH)		>1000	NT[e]
5b (Y = NHOH)		34	NT[e]
6a (Y = OH)		>1000	NT[e]
6b (Y = NHOH)		4.6	2.0

[a] Inhibition of PDE4 prepared from U937 cells (a cell line derived from human monocytes). IC_{50} represent a mean of $n = 2$.
[b] ID_{50} for inhibition of LPS-induced TNF-α production in rats ($n = 7$) 0.5 h after oral dosing of a test compound.
[c] Inhibition% at 3 mg/kg, po.
[d] Inhibition% at 1 mg/kg, po.
[e] Not tested.

To avoid the predicted rapid hydrolysis of **2b**, design and synthesis of α-substituted and α,α-disubstituted analogs **3b**–**6b** listed in Table 1 was carried out. Compounds **3a**–**6a** were also synthesized and evaluated for comparison with the corresponding hydroxamic acid analogs. Introduction of an *R*-methyl group at the α-position of **2b** gave **3b**, which was over 10-fold less potent in vitro and also showed marked loss of in vivo activity. The corresponding *S*-isomer **4b** exhibited even lower in vitro activity, but was slightly more potent in vivo. The *R*-methyl configuration was found to be superior to the *S*-methyl configuration, although introduction of an alkyl moiety failed to increase the activity of **2b**. Introduction of an α,α-dimethyl moiety into **2b** gave **6b**, which showed markedly lower in vitro activity. Introduction of an α,α-dimethylene moiety, which was predicted to be smaller than the α,α-dimethyl moiety, into **2b** gave **5b**, which also showed lower activity both in vitro and in vivo. All of the corresponding carboxylic acid analogs **3a**–**6a** were far less potent in the LPDE4 assay than their corresponding hydroxamic acid analogs **3b**–**6b**, respectively. Compounds **3a**–**4a** and **5a**–**6a** caused less than 50% inhibition at 0.3 and 1 μM, respectively. Thus, this modification caused marked reduction of the activity of the new chemical lead **2b**. The enantiomers **3b** and **4b** achieved 26% inhibition and 49% inhibition, respectively, in the LPS-induced TNF-α production assay at 0.5 h after oral administration, but showed no effect at 3 h after dosing. Accordingly, neither the inhibitory activity nor the duration of action was not improved by this modification. Further optimization of **6a**–**b**, which was expected to resist hydrolysis better than the monomethyl derivatives **3b**–**4b** and exhibit more potent LPDE4 activity than the α,α-dimethyl derivatives **5a**–**b**, was performed as described in Table 2. Replacement of the 3-cyclopentyloxy residue of **6a**–**b** with an ethoxy residue afforded **7a**–**b**, which showed more potent and nearly 1.7-fold weaker LPDE4 activity, respectively. Replacement of the cyclopentyloxy residue of **6a**–**b** with a cyclopropylmethyloxy residue gave **8a**–**b**,

Table 2. Activity profile of α,α-dimethylene analogs



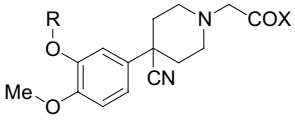
Compound	X =	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[a] ID_{50} (mg/kg, po)
7a (Y = OH)	Et-	870	NT[a]
7b (Y = NHOH)		8.0	(20%)[b]
8a (Y = OH)		>1000	NT[a]
8b (Y = NHOH)		14	0.87
9a (Y = OH)		940	NT[a]
9b (Y = NHOH)		7.6	5.6

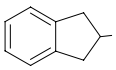
[a] See corresponding footnotes from Table 1.
[b] Inhibition% at 3 mg/kg, po.

showing no increase and nearly 3-fold weaker LPDE4 activity, respectively. Replacement of the cyclopentyloxy residue of **6a**–**b** with a cyclobutyloxy residue led to **9a**–**b**, which showed stronger and nearly 1.6-fold weaker LPDE4 activity, respectively. With regard to the effect on TNF-α production, **8b** was more than 2-fold stronger than **6b** while **7b** and **9b** showed less activity. The duration of action was also not improved by this modification.

Therefore, further attempts at optimization of the cyclopentyl moiety of **2a**–**b** were made as shown in Table 3. Replacement of the cyclopentyl moiety of **2b** with *n*-alkyl chains, such as methyl and ethyl moieties, resulted in **10b** and **11b**, respectively. Compound **10b** showed lower potency in both the in vitro and in vivo tests, while **11b** showed 6.5-fold lower in vitro activity, but retained its in vivo potency. Replacement of the cyclopentyl moiety of **2b** with a cyclopropylmethyl moiety gave **12b**, which also had reduced in vitro potency, but retained its in vivo activity. Replacement of the cyclopentyl moiety of **2b** with a branched alkyl moiety, such as an isopropyl moiety, gave **13b**, which showed a more than 10-fold reduction of in vitro potency and a nearly 2-fold reduction of in vivo potency. Ring contraction of the cyclopentyl moiety of **2b** produced a cyclobutyloxy analog **14b**, which had nearly 2-fold lower in vitro activity, but retained its in vivo potency. The corresponding carboxylic acids **10a**–**14a** demonstrated a marked loss of LPDE4 inhibitory activity compared with **2a**. Interestingly, the indan-2-yloxy analog **15**[24] demonstrated equipotent inhibition of LPDE4 relative to **2a**, but exhibited lower in vivo activity.

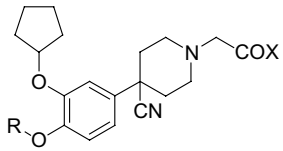
Table 3. Optimization of 3-alkoxy residue



Compound	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[a] ID_{50} (mg/kg, po)
10a (X = OH)	Me-	>100	NT[a]
10b (X = NHOH)		4.7	0.23
11a (X = OH)	Et-	>100	(0%)[b]
11b (X = NHOH)		0.52	0.04
12a (X = OH)		>100	(12%)[b]
12b (X = NHOH)		0.29	0.03
13a (X = OH)	*i*-Propyl-	>100	NT[a]
13b (X = NHOH)		1.0	0.09
14a (X = OH)	*c*-Butyl-	>100	NT[a]
14b (X = NHOH)		0.18	0.03
15 (X = OH)		67	(29%)[c]

[a] See corresponding footnotes from Table 1.
[b] Inhibition% at 1 mg/kg, po.
[c] Inhibition% at 3 mg/kg, po.

Table 4. Optimization of 4-alkoxy residue

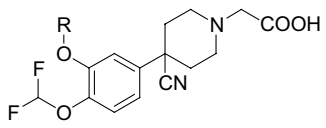


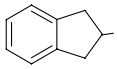
Compound	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[a] ID_{50} (mg/kg, po)
16a (X = OH)	Et-	2500	NT[a]
16b (X = NHOH)		6.2	0.7
17a (X = OH)	CHF_2-	65	1.1
17b (X = NHOH)		0.051	0.02
18a (X = OH)	*i*-Propyl-	>1000	NT[a]
18b (X = NHOH)		>30	NT[a]

[a] See corresponding footnotes from Table 1.

As shown in Table 4, further optimization of the methoxy moiety of **2a**–**b** was carried out. Replacement of the methoxy moiety of **2b** with an ethoxy moiety gave **16b**, which had 75-fold lower LPDE4 inhibitory activity and nearly 17-fold lower in vivo activity. Replacement of the methoxy moiety of **2b** with an isopropyloxy moiety resulted in **18b**, which also showed a marked decrease of LPDE4 inhibitory activity. Interestingly, replacement of the methoxy moiety of **2b** with a difluoromethoxy[25] moiety produced **17b**, which was nearly equipotent with respect to LPDE4 activity and demonstrated a 2-fold increase of in vivo activity. The corresponding carboxylic acid derivatives **16a** and **18a** showed a much lower potency both in vitro and in vivo, although **17a** had nearly the same potency as **2a** in both the LPDE4 assay and the LPS-induced TNF-α production assay. As described above, the acceptable modifications of the methoxy moiety of **2a**–**b** were found to be much more limited than those of the cyclopentyl moiety, while the difluoromethoxy moiety was identified as another optimized moiety that could be replaced by a methoxy. Among the several possible changes in the physical properties of a molecule that occur when a hydrogen atom is replaced by a fluorine atom, an increase of at least three factors (steric bulkiness, lipophilicity, and metabolic stabilization) would be expected. As shown in Table 8, these three changes of the physical properties were estimated from the calculated molal volume (Mol vol),[26] the log *D* and the duration of action in vivo, respectively. According to our calculations using the QMPRPLUS program,[27] the molal volume of CHF_2 was located between the Me and Et moieties. The log *D* values of **2a** and **17a** were 0.1 and 1.0, respectively. As a result, the lipophilicity of **17a** was found to increase remarkably with a small change of the molal volume. Additionally, a longer duration of action was obtained by this modification of **17a**, presumably because of

Table 5. Activity profile of 4-difluoromethoxy analog



Compound	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[a] ID_{50} (mg/kg, po)
19	Et-	530	1.4
20	*n*-Propyl-	490	0.68
21	*n*-Butyl-	760	1.3
22	*i*-Butyl-	290	1.4
23	*c*-Butyl-	450	1.1
24		460	2.9
25		150	0.72
26		180	NT[a]

[a] See corresponding footnotes from Table 1.

increased stability due to metabolic changes such as demethylation.[25]

Accordingly, further optimization of the 3-alkoxy residue of 4-difluoromethoxyphenyl analogs was carried out, as illustrated in Table 5. Replacement of the 3-cyclopentyloxy residue of the aromatic moiety of **17a** with appropriate alkyl residues afforded **19**–**26**. The 3-ethoxy analog **19** was nearly 8-fold weaker and slightly less potent than **17a** in the in vitro and in vivo evaluations, respectively. The 3-^{n}propyloxy analog **20** showed nearly 7-fold lower inhibition of LPDE4 activity than **17a**, while it was slightly more potent in the TNF-α production assay. The 3-^{n}butyloxy analog **21** exhibited nearly 12-fold lower inhibition of LPDE4 activity than **17a**, while it was only slightly less potent in the TNF-α production assay. When the 3-^{t}butyloxy analog **22** was tested, it showed nearly 5-fold weaker inhibition of LPDE4 activity than **17a**, but showed only slightly lower potency in the TNF-α production assay. A 3-cyclobutyloxy analog **23** exhibited 7-fold less potent LPDE4 inhibitory activity than **17a**, while it was equipotent in the TNF-α production assay. The 3-cyclobutylmethyloxy analog **24** also showed 7-fold less potent LPDE4 inhibitory activity than **17a** and was nearly 3-fold less potent in the TNF-production assay. Finally, the 3-cyclopropylmethyloxy analog **25** exhibited nearly 2-fold weaker inhibition of LPDE4 activity than **17a**, but had a slightly higher ID_{50} value in the TNF-α production assay, and the 3-isoindanyloxy analog **26** showed nearly 3-fold lower inhibition of LPDE4 activity than **17a**.

Further biological evaluation of compounds **2a**–**b**, **11b**–**14b**, and **17a**, which were selected based on the in vitro LPDE4 assay and in vivo TNF-α production assay, was carried out as shown in Table 6. Inhibition of slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction[28,29] was investigated in guinea pigs as a beneficial effect and inhibition of gastric emptying was assessed in rats[30] as an effect. The results were expressed as ID_{50} values, that is, the dose that achieved 50% inhibition relative to the vehicle. These compounds were also evaluated for inhibition of LPS-induced TNF-α production in human whole blood (HWB)[31] to estimate their clinical potential and results were expressed

Table 6. Further biological evaluation of **1**, **2a**–**b**, **11b**–**14b**, and **17a**

Compound	SRS-A-mediated bronchoconstriction[a] ID_{50} (mg/kg, po)		Inhibition of TNF-α production[b] ID_{50} (mg/kg, po)	Inhibition of gastric emptying[c] ID_{50} (mg/kg, po)	Inhibition of TNF-α production[d] in HWB IC_{50} (μM)	Ferret emesis[e] (vomiting/tested)				
	OVA challenge (mg/kg, iv)					0.1	0.3	1	3	10
						(mg/kg, po)				
	0.15	0.5								
1 (Ariflo)	4.5	10	1.7	5.7	18	NT[g]	NT[g]	NT[g]	NT[g]	NT[g]
2a	NT[g]	(0%)[h]	(74%)[i]	NT[g]	5.2	NT[g]	NT[g]	NT[g]	NT[g]	NT[g]
2b	0.3	NT[g]	0.04	0.3	0.0089	0/4	0/4	2/2	NT[g]	NT[g]
11b	0.04	NT[g]	0.04	0.7	0.0050	NT[g]	0/2	1/2	NT[g]	NT[g]
12b	0.3	1.1	0.03	0.3	0.0021	NT[g]	0/5	3/5	2/2	NT[g]
13b	(57%)[f]	NT[g]	0.09	0.8	0.012	NT[g]	0/2	1/2	NT[g]	NT[g]
14b	(77%)[f]	NT[g]	0.027	0.3	0.0027	NT[g]	0/2	0/2	NT[g]	NT[g]
17a	NT[g]	4.2	1.1	4.7	0.84	NT[g]	NT[g]	NT[g]	0/6	0/4

[a] Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs ($n = 3$–6); OVA challenge 1 h after oral dosing of a test compound.
[b] See corresponding footnotes from Table 1.
[c] Inhibition of gastric emptying in rats ($n = 5$).
[d] Inhibition of LPS-induced TNF-α production in human whole blood. IC_{50} represent a mean of $n = 3$.
[e] Vomiting test in fasted ferrets.
[f] Inhibition% at 0.3 mg/kg, po.
[g] Not tested.
[h] Inhibition% at 10 mg/kg, po.
[i] Inhibition% at 3 mg/kg, po.

as IC_{50} values (i.e., the test compound concentration that caused 50% inhibition relative to the vehicle).

The effect of these compounds on SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs was not always consistent with their inhibition of LPS-induced TNF-α production in rats, probably because of differences in pharmacokinetics due to cross-species comparison. For example, **2a**–**b**, and **12b** exhibited much lower inhibition of SRS-A-mediated bronchoconstriction in guinea pigs than expected from their effects on TNF-α production, while **11b** was equipotent in each species.

To assess an adverse effect in the same species, inhibition of gastric emptying by **2b**, **11b**–**14b**, and **17a** was evaluated in rats. The ID_{50} values of these compounds for an effect on gastric emptying were found to be higher than the values at which TNF-α production was inhibited, which is one of their beneficial effects.

To assess the safety of these compounds in another species, a ferret emesis model was used, which is an established method for evaluating the side effect profile of PDE4 inhibitors. Compounds **2b** and **11b**–**13b** did not cause emesis at oral doses up to 0.3 mg/kg, while **14b** and **17a** did not cause emesis at oral doses up to 1 and 10 mg/kg, respectively.

Compounds **2b**, **11b**–**14b**, and **17a** demonstrated more potency than Ariflo **1** against SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs and LPS-induced TNF-α production in rats. Based on their far stronger effect on LPS-induced TNF-α production in HWB compared with Ariflo **1**, these compounds were considered to show improved potential for clinical application.

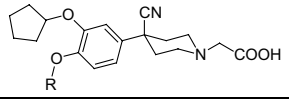
4. Pharmacokinetic study

Pharmacokinetic data for compounds **11b**, **12b**, and **17a** were investigated after administration of single doses to rats (Table 7). Intravenous administration of compounds **11b**, **12b**, and **17a** to rats (3 mg/kg, $N=3$) resulted in detectable plasma levels ($t_{1/2}=0.49$, 0.17, and 1.6 h), respectively, while oral administration of **17a** to rats (10 mg/kg, $N=3$) resulted in a $t_{1/2}$ of 4.4 h. The AUC values of **11b**, **12b**, and **17a** were, respectively 1.78, 1.18, and 20.6 μg h/mL after intravenous administration versus 0.0131, 0.343, and 14.1 μg h/mL after oral administration. The steady state volume of distribution (V_{ss}) was calculated to be 329, 439, and 286 mL/kg, respectively, indicating that these compounds showed moderate distribution to the tissues. Systemic clearance (CL) was 1770, 2650, and 183 mL/h/kg, respectively. The C_{max} values after oral dosing were 0.0225, 0.267, and 3.61 μg/mL, respectively while the t_{max} values were 0.50, 1.2, and 1.0 h, respectively. Bioavailability of **11b**, **12b**, and **17a** was 0.2%, 8.7%, and 68%, respectively.

5. Conclusion

Based on the hypothesis that the dose-limiting side effects of PDE4 inhibitors such as nausea and emesis could be caused by an effect on the CNS, design, and synthesis of hydrophilic inhibitors, which were predicted to show limited penetration of the CNS, was carried out. Several compounds (**2b**, **11b**–**14b**, and **17a**) demonstrated more potency than Ariflo **1** against SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs and LPS-induced TNF-α production in rats. In addition, the potency of **17a** relative to **2a** was increased by replacing the methoxy group of **2a** with a difluoromethoxy group (Table 8). These compounds were also evaluated for inhibition of gastric emptying in rats and emesis in ferrets to assess potential side effects. On the basis of these comparisons, all of the compounds were estimated to show an improved side effect profile. Because of a much stronger effect on LPS-induced TNF-α production in HWB compared with Ariflo **1**, these compounds could show improved potential for clinical

Table 8. Increase of the lipophilicity of **17a** by replacing the methoxy moiety of **2a** with a difluoromethoxy moiety



Compound	*C*Log *P*	Mol vol (cm^3/mol)	Log *D* (octanol/pH 7.4)
2a (R = Me)	0.07	343	0.1
17a (R = CHF_2)	0.51	350	1.0
16a (R = Et)	0.60	364	NT
18a (R = *i*-Pr)	0.90	385	NT

Table 7. Single-dose rat pharmacokinetic data for compounds **11b**, **12b**, and **17a**

Parameter	11b		12b		17a	
	iv (3 mg/kg)	po (10 mg/kg)	iv (3 mg/kg)	po (10 mg/kg)	iv (3 mg/kg)	po (3 mg/kg)
C_{max} (μg/mL)		0.0225		0.267 ± 0.118		3.61 ± 1.56
$t_{1/2}$ (h)	0.49 ± 0.27	ND	0.17 ± 0.02	ND	1.6 ± 0.7	4.4 ± 4.7
t_{max} (h)		0.50		1.2 ± 1.6		1.0 ± 0.0
AUC (μg h/mL)	1.78 ± 0.44	0.0131	1.18 ± 0.28	0.343 ± 0.25	20.6 ± 13.1	14.1 ± 3.7
V_{ss} (mL/kg)	329 ± 179		439 ± 178		286 ± 41	
CL_{total} (mL/h/kg)	1770 ± 510		2650 ± 725		183 ± 89	
%Bioavailabitity		0.2		8.7		68.3

application. The hydroxamic acid group, a well-known metal chelator, was again demonstrated to show a strong interaction with PDE4, as illustrated by data for **2b**, **17b**, and other compounds. The configurational isomerism of Ariflo was abolished without loss of activity. Pharmacokinetic data for compounds **11b**, **12b**, and **17a** were also obtained in rats.

6. Experimental

6.1. General chemical procedures

Analytical samples were homogeneous as confirmed by thin-layer chromatography (TLC), and yielded spectroscopic data consistent with the assigned structures. All ^{1}H NMR spectra were obtained with a Varian Gemini-200 or MERCURY-300 spectrometer. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionization (EI) mass spectra were obtained with a JEOL JMS-DX303HF or JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by a Hitachi M-1200H spectrometer. IR spectra were measured using a Perkin–Elmer FTIR 1760X or JASCO FTIR-430 spectrometer. Elemental analyses were performed with a Perkin–Elmer PE2400 Series II CHNS/O Analyzer and are only indicated as the elements within ±0.4% of the theoretical values unless otherwise noted. Column chromatography was carried out using silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako Gel C200, Fuji Silysia FL60D, or Fuji Silysia BW-235]. TLC was also performed on silica gel (Merck TLC plate, silica gel 60 F_{254}).

6.2. Synthesis of compounds 33a–k

6.2.1. *tert*-Butyl bis(2-hydroxyethyl)carbamate (28). To a stirred solution of bis(2-hydroxyethyl)amine **27** (20.0 g, 190 mmol) in CH_2Cl_2 (200 mL) was added a solution of Boc_2O (45.6 g, 209 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After being stirred for 1.5 h, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2/1–1/2–0/1) to give **28** (41.0 g, quant.) as colorless oil: TLC R_f = 0.56 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 4H), 3.43 (s, 4H), 3.60–3.00 (br, 2H), 1.47 (s, 9H).

6.2.2. [(*tert*-Butoxycarbonyl)imino]diethane-2,1-diyl dimethanesulfonate (29). To a stirred solution of **28** (7.85 g, 383.3 mmol) and Et_3N (16.0 mL, 115 mmol) in CH_2Cl_2 (80 mL) was added MsCl (8.89 mL, 115 mmol) at −78 °C. After being stirred for 10 min, the reaction mixture was poured into H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **29** (13.2 g, 36.6 mmol, 95% in two steps) as a pale yellow oil: TLC R_f = 0.64 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ. 4.40–4.25 (m, 4H), 3.62 (br t, J = 5.4 Hz, 4H), 3.04 (s, 6H), 1.48 (s, 9H).

6.2.3. *tert*-Butyl 4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidine-1-carboxylate (31a) (Method A). To a stirred solution of **30a** (2.50 g, 10.8 mmol) in THF (150 mL) was added LiHMDS (1.0 M solution in THF, 24.0 mL, 24.0 mmol) at −78 °C under argon atmosphere. After being stirred at −78 °C for 30 min, a solution of **29** (2.17 g, 6.00 mmol) in THF (50 mL) was added dropwise. After being stirred at room temperature for 1.5 h, the reaction mixture was poured into brine, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/Et_2O, 2/1–1/2–0/1) to give **31a** (1.71 g, crude 71%) as a yellow oil: TLC R_f = 0.56 (*n*-hexane/EtOAc, 2/1).

6.2.4. *tert*-Butyl 4-cyano-4-[3,4-dimethoxyphenyl]piperidine-1-carboxylate (31b). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a yellow oil: Yield crude 53%; TLC R_f = 0.34 (*n*-hexane/EtOAc, 2/1).

6.2.5. *tert*-Butyl 4-cyano-4-[3-ethoxy-4-methoxyphenyl]piperidine-1-carboxylate (31c). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a yellow oil: Yield crude quant; TLC R_f = 0.46 (*n*-hexane/EtOAc, 2/1).

6.2.6. *tert*-Butyl 4-cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidine-1-carboxylate (31d). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a yellow oil: Yield crude 91%; TLC R_f = 0.60 (*n*-hexane/EtOAc, 2/1).

6.2.7. *tert*-Butyl 4-cyano-4-[3-(isopropyloxy)-4-methoxyphenyl]piperidine-1-carboxylate (31e). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a yellow oil: Yield crude 69%; TLC R_f = 0.64 (*n*-hexane/EtOAc, 2/1).

6.2.8. *tert*-Butyl 4-cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidine-1-carboxylate (31f). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a yellow oil: Yield crude 80%; TLC R_f = 0.44 (*n*-hexane/EtOAc, 2/1).

6.2.9. *tert*-Butyl 4-cyano-4-[3-(cyclopentyloxy)-4-ethoxyphenyl]piperidine-1-carboxylate (31g). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a brown oil: Yield crude 65%; TLC R_f = 0.79 (*n*-hexane/EtOAc, 2/1).

6.2.10. *tert*-Butyl 4-cyano-4-[3-(cyclopentyloxy)-4-difluoromethoxyphenyl]piperidine-1-carboxylate (31h). The title compound was prepared from the corresponding benzyl cyanide according to Method A to give a brown oil: Yield crude 77%; TLC R_f = 0.76 (*n*-hexane/EtOAc, 2/1).

6.2.11. *tert*-Butyl 4-cyano-4-[3-(cyclopentyloxy)-4-isopropoxyphenyl]piperidine-1-carboxylate (31i). The title compound was prepared from the corresponding benzyl

cyanide according to Method A to give a yellow oil: Yield crude 83%; TLC R_f=0.76 (*n*-hexane/EtOAc, 1/1).

6.2.12. 4-[3-Cyclopentyloxy)-4-methoxyphenyl]piperidine-4-carbonitrile hydrochloride (32a) (Method B). To a stirred solution of **31a** (1.71 g) in CH_2Cl_2 (5 mL) were added PhSMe (5.0 mL) and TFA (3.0 mL). After being stirred at room temperature for 1 h, the reaction mixture was poured into H_2O, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. After addition of 4 N HCl/EtOAc (1.4 mL), the resulting mixture was evaporated. The residue was triturated with Et_2O to give **32a** (971 mg, 2.89 mmol, 48% in two steps) as a white powder: TLC R_f=0.33 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) *m*/*z*=301 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.02 (br s, 2H), 7.10–7.00 (m, 2H), 6.88 (d, J=9.0 Hz, 1H), 4.82 (m, 1H), 3.86 (s, 3H), 3.80–3.60 (m, 2H), 3.50–3.30 (m, 2H), 2.80–2.60 (m, 2H), 2.40–2.20 (m, 2H), 2.10–1.80 (m, 6H), 1.80–1.60 (m, 2H).

6.2.13. 4-[3,4-Dimethoxyphenyl]piperidine-4-carbonitrile hydrochloride (32b). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 34% in two steps; TLC R_f=0.23 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Pos. 40 V) *m*/*z*=247 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.00 (br s, 2H), 7.08 (dd, J=8.4, 2.4 Hz, 1H), 7.05 (d, J=2.4 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H), 3.75–3.65 (m, 2H), 3.50–3.30 (m, 2H), 2.80–2.65 (m, 2H), 2.35–2.20 (m, 2H).

6.2.14. 4-[3-Ethoxy-4-methoxyphenyl]piperidine-4-carbonitrile hydrochloride (32c). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 45% in two steps; TLC R_f=0.30 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos. 20 V) *m*/*z*=261 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.05–9.95 (m, 2H), 7.08–7.04 (m, 2H), 6.90 (d, J=8.4 Hz, 1H), 4.15 (q, J=6.9 Hz, 2H), 3.88 (s, 3H), 3.75–3.65 (m, 2H), 3.45–3.35 (m, 2H), 2.71 (br t, J=12.6 Hz, 2H), 2.27 (br d, J=14.1 Hz, 2H), 1.48 (t, J=6.9 Hz, 3H).

6.2.15. 4-[3-Cyclopropylmethoxy-4-methoxyphenyl]piperidine-4-carbonitrile hydrochloride (32d). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 61% in two steps; TLC R_f=0.42 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Pos. 40 V) *m*/*z*=287 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.50–9.00 (br, 2H), 7.10–7.00 (m, 2H), 6.90 (d, J=8.7 Hz, 1H), 3.90 (d, J=6.3 Hz, 2H), 3.89 (s, 3H), 3.75–3.65 (m, 2H), 3.45–3.30 (m, 2H), 2.75–2.60 (m, 2H), 2.35–2.20 (m, 2H), 1.34 (m, 1H), 0.75–0.55 (m, 2H), 0.45–0.30 (m, 2H).

6.2.16. 4-[3-Isopropyloxy-4-methoxyphenyl]piperidine-4-carbonitrile hydrochloride (32e). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 29% in two steps; TLC R_f=0.42 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Pos. 40 V) *m*/*z*=275 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.40–9.60 (br, 2H), 7.10–7.00 (m, 2H), 6.90 (d, J=9.0 Hz, 1H), 4.58 (sept, J=6.0 Hz, 1H), 3.86 (s, 3H), 3.80–3.60 (m, 2H), 3.45–3.35 (m, 2H), 2.80–2.60 (m, 2H), 2.35–2.20 (m, 2H), 1.38 (d, J=6.0 Hz, 6H).

6.2.17. 4-[3-Cyclobutyloxy-4-methoxyphenyl]piperidine-4-carbonitrile hydrochloride (32f). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 60% in two steps; TLC R_f=0.31 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos. 20 V) *m*/*z*=287 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.20–9.80 (m, 2H), 7.10–7.00 (m, 1H), 6.90–6.85 (m, 2H), 4.70 (quintet, J=7.2 Hz, 1H), 3.88 (s, 3H), 3.75–3.65 (m, 2H), 3.45–3.30 (m, 2H), 2.78–2.60 (m, 2H), 2.60–2.50 (m, 2H), 2.35–2.15 (m, 4H), 1.95–1.60 (m, 2H).

6.2.18. 4-[3-Cyclopentyloxy-4-ethoxyphenyl]piperidine-4-carbonitrile hydrochloride (32g). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 45% in two steps; TLC R_f=0.31 ($CHCl_3$/MeOH, 10/1); MS (APCI, Pos. 20 V) *m*/*z*=315 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 9.99 (br s, 2H), 7.05–6.95 (m, 2H), 6.88 (d, J=8.4 Hz, 1H), 4.81 (m, 1H), 4.06 (q, J=7.2 Hz, 2H), 3.75–3.60 (m, 2H), 3.50–3.30 (m, 2H), 2.80–2.60 (m, 2H), 2.35–2.20 (m, 2H), 2.00–1.70 (m, 6H), 1.70–1.50 (m, 2H), 1.43 (t, J=7.2 Hz, 3H).

6.2.19. 4-[3-Cyclopentyloxy-4-difluoromethoxyphenyl]piperidine-4-carbonitrile hydrochloride (32h). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 34% in two steps; TLC R_f=0.42 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Pos. 40 V) *m*/*z*=275 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.40–9.60 (br, 2H), 7.10–7.00 (m, 2H), 6.90 (d, J=9.0 Hz, 1H), 4.58 (sept, J=6.0 Hz, 1H), 3.86 (s, 3H), 3.80–3.60 (m, 2H), 3.45–3.35 (m, 2H), 2.80–2.60 (m, 2H), 2.35–2.20 (m, 2H), 1.38 (d, J=6.0 Hz, 6H).

6.2.20. 4-[3-Cyclopentyloxy-4-isopropoxyphenyl]piperidine-4-carbonitrile hydrochloride (32i). The title compound was prepared from the corresponding *tert*-butylcarbonate according to Method B to give a white powder: Yield 32% in two steps; TLC R_f=0.35 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos. 20 V) *m*/*z*=329 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 10.20–9.85 (m, 2H), 7.27–6.90 (m, 3H), 4.83–4.75 (m, 1H), 4.45 (sept, J=6.0 Hz, 1H), 3.75–3.63 (m, 2H), 3.45–3.30 (m, 2H), 2.75–2.60 (m, 2H), 2.35–2.22 (m, 2H), 1.95–1.75 (m, 6H), 1.70–1.40 (m, 2H), 1.33 (d, J=6.0 Hz, 6H).

6.2.21. Ethyl {4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}acetate (33a) (Method C). To a stirred solution of **32a** (300 mg, 0.893 mmol) in DMF (4.0 mL) were added K_2CO_3 (246 mg, 1.79 mmol) and $BrCH_2COOEt$ (0.15 mL, 1.3 mmol). After being stirred at room temperature for 20 h, the reaction mixture was diluted with EtOAc and washed with H_2O and then brine. The organic layer was dried over $MgSO_4$ and

concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/Et_2O, 2/1–1/1–0/1) to give **33a** (341 mg, 0.883 mmol, 99%) as a colorless oil: TLC R_f=0.36 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–6.95 (m, 2H), 6.86 (d, J=8.8 Hz, 1H), 4.79 (m, 1H), 4.22 (q, J=7.0 Hz, 2H), 3.85 (s, 3H), 3.31 (s, 2H), 3.15–3.00 (m, 2H), 2.75–2.55 (m, 2H), 2.30–2.05 (m, 2H), 2.20–2.00 (m, 2H), 2.00–1.80 (m, 6H), 1.80–1.50 (m, 2H), 1.30 (t, J=7.0 Hz, 3H).

6.2.22. Ethyl [4-cyano-4-(3,4-dimethoxyphenyl)piperidin-1-yl]acetate (33b). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 97%; TLC R_f=0.33 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (dd, J=8.4, 2.4 Hz, 1H), 6.99 (d, J=2.4 Hz, 1H), 6.87 (d, J=8.4 Hz, 1H), 4.22 (q, J=7.4 Hz, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.31 (s, 2H), 3.15–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.30–2.15 (m, 2H), 2.15–2.00 (m, 2H), 1.30 (t, J=7.4 Hz, 3H).

6.2.23. Ethyl [4-cyano-4-(3-ethoxy-4-methoxyphenyl)piperidin-1-yl]acetate (33c). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 100%; TLC R_f=0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–6.95 (m, 2H), 6.87 (d, J=8.4 Hz, 1H), 4.22 (q, J=7.2 Hz, 2H), 4.10 (q, J=7.2 Hz, 2H), 3.88 (s, 3H), 3.31 (s, 2H), 3.13–3.05 (m, 2H), 2.73–2.60 (m, 2H), 2.26–2.15 (m, 2H), 2.12–2.05 (m, 2H), 1.47 (t, J=7.2 Hz, 3H), 1.30 (t, J=7.2 Hz, 3H).

6.2.24. Ethyl {4-cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}acetate (33d). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 93%; TLC R_f=0.36 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, J=8.7, 2.7 Hz, 1H), 6.99 (d, J=2.7 Hz, 1H), 6.87 (d, J=8.7 Hz, 1H), 4.22 (q, J=7.2 Hz, 2H), 3.88 (s, 3H), 3.86 (d, J=6.9 Hz, 2H), 3.30 (s, 2H), 3.15–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.25–2.10 (m, 2H), 2.15–2.00 (m, 2H), 1.30 (t, J=7.2 Hz, 3H), 1.40–1.20 (m, 1H), 0.70–0.60 (m, 2H), 0.40–0.30 (m, 2H).

6.2.25. Ethyl [4-cyano-4-(3-isopropoxy-4-methoxyphenyl)piperidin-1-yl]acetate (33e). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 93%; TLC R_f=0.34 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.00 (m, 2H), 6.87 (d, J=8.4 Hz, 1H), 4.54 (sept, J=6.0 Hz, 1H), 4.22 (q, J=7.2 Hz, 2H), 3.85 (s, 3H), 3.30 (s, 2H), 3.20–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.25–2.10 (m, 2H), 2.15–2.00 (m, 2H), 1.37 (d, J=6.0 Hz, 6H), 1.30 (t, J=7.2 Hz, 3H).

6.2.26. Ethyl {4-cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}acetate (33f). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 99%; TLC R_f=0.95 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02–7.00 (m, 1H), 6.90–6.80 (m, 2H), 4.67 (quint, J=7.2 Hz, 1H), 4.22 (q, J=7.2 Hz, 2H), 3.87 (s, 3H), 3.31 (s, 2H), 3.12–3.05 (m, 2H), 2.73–2.60 (m, 2H), 2.55–2.43 (m, 2H), 2.33–2.00 (m, 6H), 1.93–1.78 (m, 1H), 1.76–1.60 (m, 1H), 1.30 (t, J=7.2 Hz, 3H).

6.2.27. Ethyl {4-cyano-4-[3-(cyclopentyloxy)-4-ethoxyphenyl]piperidin-1-yl}acetate (33g). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 96%; TLC R_f=0.80 ($CHCl_3$/MeOH, 10/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05–6.95 (m, 2H), 6.86 (d, J=9.0 Hz, 1H), 4.78 (m, 1H), 4.22 (q, J=7.2 Hz, 2H), 4.06 (q, J=7.2 Hz, 2H), 3.30 (s, 2H), 3.10–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.25–2.10 (m, 2H), 2.15–2.00 (m, 2H), 1.95–1.75 (m, 6H), 1.70–1.55 (m, 2H), 1.42 (t, J=7.2 Hz, 3H), 1.30 (t, J=7.2 Hz, 3H).

6.2.28. Ethyl {4-cyano-4-[3-(cyclopentyloxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetate (33h). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 94%; TLC R_f=0.20 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, J=8.4 Hz, 1H), 7.10 (d, J=2.4 Hz, 1H), 7.03 (dd, J=8.4, 2.4 Hz, 1H), 6.54 (t, J=75.3 Hz, 1H), 4.83 (m, 1H), 4.22 (q, J=7.2 Hz, 2H), 3.31 (s, 2H), 3.20–3.00 (m, 2H), 2.80–2.60 (m, 2H), 2.30–2.10 (m, 2H), 2.15–2.00 (m, 2H), 2.00–1.70 (m, 6H), 1.70–1.55 (m, 2H), 1.30 (t, J=7.2 Hz, 3H).

6.2.29. Ethyl {4-cyano-4-[3-(cyclopentyloxy)-4-isopropoxyphenyl]piperidin-1-yl}acetate (33i). The title compound was prepared from the corresponding piperidine according to Method C to give a pale yellow oil: Yield 90%; TLC R_f=0.95 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01–6.97 (m, 2H), 6.89 (d, J=7.8 Hz, 1H), 4.80–4.74 (m, 1H), 4.42 (sept, J=6.0 Hz, 1H), 4.22 (q, J=7.0 Hz, 2H), 3.30 (s, 2H), 3.12–3.02 (m, 2H), 2.72–2.61 (m, 2H), 2.26–2.14 (m, 2H), 2.12–2.05 (m, 2H), 1.90–1.75 (m, 2H), 1.65–1.50 (m, 6H), 1.31 (d, J=6.0 Hz, 6H), 1.28 (t, J=7.0 Hz, 3H).

6.2.30. Methyl (2*R*)-2-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoate (33j). The following reaction was carried out under argon atmosphere. To a stirred solution of methyl-(*S*)-(−)-lactate (0.34 mL, 3.56 mmol) in CH_2Cl_2 (3.0 mL) were added Tf_2O (0.66 mL, 3.93 mmol) and 2,6-lutidine (0.46 mL, 3.93 mmol) at 0 °C. After being stirred at room temperature for 30 min, a solution of **32a** (400 mg, 1.19 mmol) in CH_2Cl_2 (2.5 mL) and Et_3N (0.36 mL, 2.38 mmol) were added to the reaction mixture. After being stirred at room temperature for 18 h, the reaction mixture was poured into H_2O, extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 3/1) to give **33j** (492 mg, quant.) as a colorless oil: TLC R_f=0.90 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05–6.97 (m, 2H), 6.87–6.83 (m, 1H), 4.85–4.75 (m, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.39 (q, J=7.0 Hz, 1H), 3.10–2.95 (m,

2H), 2.85–2.68 (m, 2H), 2.15–2.05 (m, 4H), 2.00–1.75 (m, 6H), 1.65–1.45 (m, 2H), 1.35 (d, $J=7.0$ Hz, 3H).

6.2.31. Methyl (2*S*)-2-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoate (33k). The title compound was prepared according to the same procedures as described for the preparation of **33k** from **32a** using methyl-(*R*)-(+)-lactate instead of methyl-(*S*)-(−)-lactate to give a colorless oil: TLC $R_f=0.90$ ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05–6.97 (m, 2H), 6.87–6.83 (m, 1H), 4.85–4.75 (m, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.39 (q, $J=7.0$ Hz, 1H), 3.10–2.95 (m, 2H), 2.85–2.68 (m, 2H), 2.15–2.05 (m, 4H), 2.00–1.75 (m, 6H), 1.65–1.45 (m, 2H), 1.35 (d, $J=7.0$ Hz, 3H).

6.3. Synthesis of compounds 33l–n

6.3.1. 1-[3-Cyclopentyloxy)-4-methoxyphenyl]cyclopent-3-ene-1-carbonitrile (35e) (Method D). The following reaction was carried out under argon atmosphere. To a stirred solution of **30i** (4.00 g, 17.3 mmol) in THF (75 mL) was added LiHMDS (1.0 M solution in THF, 40.4 mL, 40.4 mmol) at −78 °C. After stirring for 1 h, *cis*-2-butenyl-1,4-dichloride (1.20 mL, 11.5 mmol) was added to the reaction mixture at −78 °C. After being stirred for 1.5 h, the reaction mixture was poured into saturated aqueous NH_4Cl, and extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 8/1) to give **35e** (3.05 g, 10.8 mmol, 94%) as a yellow oil: TLC $R_f=0.39$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.95 (m, 2H), 6.82 (d, $J=8.7$ Hz, 1H), 5.82 (s, 2H), 4.78 (m, 1H), 3.84 (s, 3H), 3.35–3.20 (m, 2H), 3.00–2.85 (m, 2H), 2.00–1.75 (m, 6H), 1.70–1.55 (m, 2H).

6.3.2. 1-[3-Benzyloxy-4-methoxyphenyl]cyclopent-3-ene-1-carbonitrile (35a). The title compound was prepared from the corresponding benzylcyanide according to Method D to give a white powder: Yield 93%; TLC $R_f=0.70$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.20 (m, 5H), 7.02 (dd, $J=8.4$, 2.1 Hz, 1H), 6.94 (d, $J=2.1$ Hz, 1H), 6.85 (d, $J=8.4$ Hz, 1H), 5.76 (s, 2H), 5.15 (s, 2H), 3.88 (s, 3H), 3.15–3.30 (m, 2H), 2.90–2.75 (m, 2H).

6.3.3. 1-[3-Benzyloxy-4-difluoromethoxyphenyl]cyclopent-3-ene-1-carbonitrile (35b). The title compound was prepared from the corresponding benzylcyanide according to Method D to give a pale yellow powder: Yield 79%; TLC $R_f=0.62$ (*n*-hexane/EtOAc, 3/1); MS (APCI, Pos. 20 V) $m/z=342$ $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.31 (m, 5H), 7.16 (d, $J=8.4$ Hz, 1H), 7.12 (d, $J=2.1$ Hz, 1H), 7.02 (dd, $J=8.4$, 2.1 Hz, 1H), 6.57 (t, $J=75.0$ Hz, 1H), 5.83–5.77 (m, 2H), 5.15 (s, 2H), 3.33–3.24 (m, 2H), 2.91–2.83 (m, 2H).

6.3.4. 1-[3-Methoxymethoxy-4-methoxyphenyl]cyclopent-3-ene-1-carbonitrile (35c). The title compound was prepared from the corresponding benzylcyanide according to Method D to give a pale yellow oil: Yield 84%; TLC $R_f=0.42$ (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) $m/z=260$ $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (d, $J=2.1$ Hz, 1H), 7.12 (dd, $J=8.4$, 2.1 Hz, 1H), 6.87 (d, $J=8.4$ Hz, 1H), 5.85–5.78 (m, 2H), 5.22 (s, 2H), 3.88 (s, 3H), 3.52 (s, 3H), 3.32–3.23 (m, 2H), 2.97–2.88 (m, 2H).

6.3.5. 1-[3-Ethoxy-4-methoxyphenyl]cyclopent-3-ene-1-carbonitrile (35d). The title compound was prepared from the corresponding benzylcyanide according to Method D to give a pale yellow oil: Yield 67%; TLC $R_f=0.35$ (*n*-hexane/EtOAc, 5/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (dd, $J=8.4$, 2.1 Hz, 1H), 6.96 (d, $J=2.1$ Hz, 1H), 6.84 (d, $J=8.4$ Hz, 1H), 5.86–5.78 (m, 2H), 4.11 (q, $J=6.9$ Hz, 2H), 3.87 (s, 3H), 3.34–3.22 (m, 2H), 2.99–2.87 (m, 2H), 1.47 (t, $J=6.9$ Hz, 3H).

6.3.6. Benzyl 2-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-2-methylpropanoate (33l) (Method E). To a solution of **35e** (460 mg, 1.63 mmol) in CH_2Cl_2 (10 mL) was bubbled ozone (O_2 containing ca. 3% O_3) at −78 °C for 25 min. To the reaction mixture was added Ph_3P (513 mg, 1.96 mmol), and the reaction mixture was stirred at −78 °C for 30 min. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo to give **36e** (1.27 g, quant.) as a yellow amorphous solid. The product was used for the next reaction without further purification.

To a stirred solution of **36e** (1.27 g) and benzyl-2-amino-2-methylpropanoate (374 mg, 1.63 mmol) in $ClCH_2CH_2Cl$ (10 mL) were added $NaBH(OAc)_3$ (1.03 g, 4.88 mmol) and AcOH (0.56 mL, 9.77 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with EtOAc. The mixture was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4/1) to give **33l** (196 mg, 0.412 mmol, 25%) as a pale yellow oil: TLC $R_f=0.62$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.30 (m, 5H), 7.05–6.95 (m, 2H), 6.85 (d, $J=9.0$ Hz, 1H), 5.19 (s, 2H), 4.80 (m, 1H), 3.84 (s, 3H), 3.05–2.95 (m, 2H), 2.80–2.60 (m, 2H), 2.10–2.00 (m, 4H), 2.05–1.80 (m, 6H), 1.80–1.50 (m, 2H), 1.38 (s, 6H).

6.3.7. Benzyl 1-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylate (33m). The title compound was prepared from the corresponding cyclopentene according to Method E to give a pale yellow oil: Yield 20% in two steps; TLC $R_f=0.50$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.30 (m, 5H), 7.00 (d, $J=2.1$ Hz, 1H), 6.98 (dd, $J=9.0$, 2.1 Hz, 1H), 6.84 (d, $J=9.0$ Hz, 1H), 5.19 (s, 2H), 4.80 (m, 1H), 3.84 (s, 3H), 3.65–3.50 (m, 2H), 3.00–2.90 (m, 2H), 2.10–2.00 (m, 2H), 2.00–1.75 (m, 8H), 1.70–1.55 (m, 2H), 1.40–1.35 (m, 2H), 1.00–0.95 (m, 2H).

6.3.8. Benzyl 1-[4-cyano-4-(3-ethoxy-4-methoxyphenyl)piperidin-1-yl]cyclopropanecarboxylate (33n). The title compound was prepared from the corresponding cyclopentene according to Method E to give a pale

yellow oil: Yield 15% in two steps; TLC R_f = 0.48 (*n*-hexane/EtOAc, 3/1); MS (APCI, Pos. 20 V) *m*/*z* = 435 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.28 (m, 5H), 7.02–6.97 (m, 2H), 6.85 (d, *J* = 9.0 Hz, 1H), 5.20 (s, 2H), 4.12 (q, *J* = 7.0 Hz, 2H), 3.87 (s, 3H), 3.62–3.51 (m, 2H), 3.00–2.91 (m, 2H), 2.08–2.00 (m, 2H), 1.90–1.79 (m, 2H), 1.47 (t, *J* = 7.0 Hz, 3H), 1.38–1.34 (m, 2H), 0.99–0.94 (m, 2H).

6.4. Synthesis of compounds 33o–w

6.4.1. Ethyl {4-[3-(benzyloxy)-4-methoxyphenyl]-4-cyanopiperidin-1-yl}acetate (37a). The title compound was prepared from the corresponding cyclopentene according to Method E to give a pale yellow oil: Yield 47% in two steps; TLC R_f = 0.31 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.40 (m, 2H), 7.45–7.25 (m, 3H), 7.10–7.00 (m, 2H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.15 (s, 2H), 4.22 (q, *J* = 7.2 Hz, 2H), 3.88 (s, 3H), 3.30 (s, 2H), 3.15–3.00 (m, 2H), 2.70–2.55 (m, 2H), 2.20–2.05 (m, 2H), 2.15–1.95 (m, 2H), 1.30 (t, *J* = 7.2 Hz, 3H).

6.4.2. Methyl {4-[3-(benzyloxy)-4-(difluoromethoxy)phenyl]-4-cyanopiperidin-1-yl}acetate (37b). The title compound was prepared from the corresponding cyclopentene according to Method E to give a brown oil: Yield 64% in two steps; TLC R_f = 0.31 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m*/*z* = 431 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.30 (m, 5H), 7.20 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 2.4 Hz, 1H), 7.07 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.58 (t, *J* = 75.0 Hz, 1H), 5.15 (s, 2H), 3.76 (s, 3H), 3.32 (s, 2H), 3.08 (dt, *J* = 12.0, 2.7 Hz, 2H), 2.66 (td, *J* = 12.0, 2.7 Hz, 2H), 2.18 (td, *J* = 12.0, 3.9 Hz, 2H), 2.09–2.01 (m, 2H).

6.4.3. Ethyl [4-cyano-4-(3-hydroxy-4-methoxyphenyl)piperidin-1-yl]acetate (38a) (Method F). A solution of **37a** (892 mg, 2.18 mmol) in EtOH (12 mL) was hydrogenated under atmospheric pressure of H_2 gas in the presence of 10% Pd/C (100 mg) for 2.5 h. The catalyst was removed by filtration through a pad of Celite, and washed with MeOH. The filtrates were concentrated in vacuo to give **38a** (642 mg, 2.02 mmol, 92%) as a white powder: TLC R_f = 0.43 (*n*-hexane/EtOAc, 1/2); MS (APCI, Neg. 20 V) *m*/*z* = 317 $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (d, *J* = 2.4 Hz, 1H), 7.00 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 5.66 (br, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 3.90 (s, 3H), 3.30 (s, 2H), 3.11–3.03 (m, 2H), 2.72–2.62 (m, 2H), 2.20–2.04 (m, 4H), 1.30 (t, *J* = 7.2 Hz, 3H).

6.4.4. Methyl {4-cyano-4-[4-(difluoromethoxy)-3-hydroxyphenyl]piperidin-1-yl}acetate (38b). The title compound was prepared from the corresponding benzyl ether according to Method F to give an ivory powder: Yield 92%; TLC R_f = 0.55 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos. 20 V) *m*/*z* = 341 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 2.4 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.02 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.54 (t, *J* = 73.5 Hz, 1H), 3.76 (s, 3H), 3.32 (s, 2H), 3.07 (d, *J* = 12.0 Hz, 2H), 2.66 (td, *J* = 12.0, 2.7 Hz, 2H), 2.16 (td, *J* = 13.5, 3.9 Hz, 2H), 2.12–2.03 (m, 3H).

6.4.5. Ethyl {4-cyano-4-[3-(2,3-dihydro-1*H*-inden-2-yloxy)-4-methoxyphenyl]piperidin-1-yl}acetate (33o) (Method G). To a stirred solution of **38a** (642 mg, 2.02 mmol) in THF (10 mL) were added PPh_3 (794 mg, 3.03 mmol), diethylazodicarboxylate (0.60 mL, 3.03 mmol) and 2-indanol (406 mg, 3.03 mmol). After being stirred at room temperature for 15 h, the reaction mixture was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2/1) to give **33o** (671 mg, 1.54 mmol, 77%) as a yellow oil: TLC R_f = 0.62 (*n*-hexane/EtOAc, 1/2); MS (APCI, Pos. 20 V) *m*/*z* = 435 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.16 (m, 4H), 7.09–7.05 (m, 2H), 6.90–6.86 (m, 1H), 5.20 (m, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 3.44–3.35 (m, 2H), 3.31 (s, 2H), 3.27–3.19 (m, 2H), 3.14–3.05 (m, 2H), 2.73–2.63 (m, 2H), 2.26–2.16 (m, 2H), 2.15–2.06 (m, 2H), 1.30 (t, *J* = 7.2 Hz, 3H).

6.4.6. Methyl {4-cyano-4-[3-(cyclobutyloxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetate (33t). The title compound was prepared from the corresponding phenol and alcohol according to Method G to give an orange oil: Yield 77%; TLC R_f = 0.54 (toluene/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m*/*z* = 395 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.1 Hz, 1H), 7.02 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.97 (d, *J* = 2.1 Hz, 1H), 6.58 (t, *J* = 75.0 Hz, 1H), 4.69 (m, 1H), 3.76 (s, 3H), 3.33 (s, 2H), 3.09 (dt, *J* = 12.3, 2.7 Hz, 2H), 2.66 (td, *J* = 12.3, 2.7 Hz, 2H), 2.54–2.43 (m, 2H), 2.29–2.13 (m, 4H), 2.11–2.02 (m, 2H), 1.89 (m, 1H), 1.72 (m, 1H).

6.4.7. Methyl {4-cyano-4-[4-(difluoromethoxy)-3-(2,3-dihydro-1*H*-inden-2-yloxy)phenyl]piperidin-1-yl}acetate (33w). The title compound was prepared from the corresponding phenol and alcohol according to Method G to give a pale yellow oil: Yield 63%; TLC R_f = 0.29 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.16 (m, 6H), 7.07 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.38 (t, *J* = 75.3 Hz, 1H), 5.26–5.20 (m, 1H), 3.76 (s, 3H), 3.41 (dd, *J* = 16.5, 6.3 Hz, 2H), 3.34 (s, 2H), 3.20 (dd, *J* = 16.5, 3.3 Hz, 2H), 3.11 (d, *J* = 12.0 Hz, 2H), 2.69 (dt, *J* = 12.0, 2.4 Hz, 2H), 2.28–2.19 (m, 2H), 2.14–2.04 (m, 2H).

6.4.8. Methyl {4-cyano-4-[4-(difluoromethoxy)-3-ethoxyphenyl]piperidin-1-yl}acetate (33p) (Method H). To a stirred solution of **38b** (904 mg, 2.66 mmol) in DMF (10 mL) were added EtI (0.32 mL, 4.0 mmol) and K_2CO_3 (919 mg, 6.65 mmol). After being stirred at 60 °C for 3 h, the reaction mixture was poured into ice H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, concentrated in vacuo, and purified by silica gel column chromatography (*n*-hexane/EtOAc, 1/1–2/1) to give **33p** (951 mg, 2.58 mmol, 97%) as a pale yellow oil: TLC R_f = 0.33 (*n*-hexane/EtOAc, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 2.1 Hz, 1H), 7.04 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.57 (t, *J* = 75.3 Hz, 1H), 4.11 (q, *J* = 6.9 Hz, 2H), 3.75 (s, 3H), 3.32 (s, 2H), 3.08 (d,

J = 12.0 Hz, 2H), 2.66 (dt, J = 12.0, 2.7 Hz, 2H), 2.26–2.16 (m, 2H), 2.10–2.05 (m, 2H), 1.46 (t, J = 6.9 Hz, 3H).

6.4.9. Methyl {4-cyano-4-[4-(difluoromethoxy)-3-propoxyphenyl]piperidin-1-yl}acetate (33q). The title compound was prepared from the corresponding alkyl halide and **38b** according to Method H to give a pale brown powder: Yield 99%; TLC R_f = 0.56 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.1 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 7.05 (dd, J = 8.1, 2.4 Hz, 1H), 6.57 (t, J = 75.0 Hz, 1H), 3.99 (t, J = 7.0 Hz, 2H), 3.76 (s, 3H), 3.33 (s, 2H), 3.15–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.30–2.15 (m, 2H), 2.15–2.00 (m, 2H), 1.86 (sext, J = 7.0 Hz, 2H), 1.06 (t, J = 7.0 Hz, 3H).

6.4.10. Methyl {4-[3-butoxy-4-(difluoromethoxy)phenyl]-4-cyanopiperidin-1-yl}acetate (33r). The title compound was prepared from the corresponding alkyl halide and **38b** according to Method H to give a pale brown oil: Yield 100%; TLC R_f = 0.57 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) m/z = 397 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, J = 8.5 Hz, 1H), 7.09 (d, J = 2.2 Hz, 1H), 7.05 (dd, J = 8.5, 2.2 Hz, 1H), 6.56 (t, J = 75.0 Hz, 1H), 4.03 (t, J = 6.5 Hz, 2H), 3.76 (s, 3H), 3.33 (s, 2H), 3.13–3.05 (m, 2H), 2.72–2.62 (m, 2H), 2.27–2.17 (m, 2H), 2.11–2.04 (m, 2H), 1.86–1.76 (m, 2H), 1.60–1.45 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H).

6.4.11. Methyl {4-cyano-4-[4-(difluoromethoxy)-3-isobutoxyphenyl]piperidin-1-yl}acetate (33s). The title compound was prepared from the corresponding alkyl halide and **38b** according to Method H to give a pale brown powder: Yield 90%; TLC R_f = 0.40 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 7.8 Hz, 1H), 7.10–7.00 (m, 2H), 6.56 (t, J = 75.3 Hz, 1H), 3.78 (d, J = 6.6 Hz, 2H), 3.76 (s, 3H), 3.33 (s, 2H), 3.20–3.00 (m, 2H), 2.80–2.60 (m, 2H), 2.30–2.15 (m, 2H), 2.25–2.00 (m, 1H), 2.20–2.00 (m, 2H), 1.05 (d, J = 6.6 Hz, 6H).

6.4.12. Methyl {4-cyano-4-[3-(cyclobutylmethoxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetate (33u). The title compound was prepared from the corresponding alkyl halide and **38b** according to Method H to give a pale brown oil: Yield 84%; TLC R_f = 0.84 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 7.05 (dd, J = 8.4, 2.1 Hz, 1H), 6.57 (t, J = 75.3 Hz, 1H), 3.99 (d, J = 6.6 Hz, 2H), 3.76 (s, 3H), 3.33 (s, 2H), 3.09 (dt, J = 11.7, 2.4 Hz, 2H), 2.81 (m, 1H), 2.67 (td, J = 11.7, 2.4 Hz, 2H), 2.28-1.82 (m, 10H).

6.4.13. Methyl {4-cyano-4-[3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetate (33v). The title compound was prepared from the corresponding alkyl halide and **38b** according to Method H to give a pale yellow oil: Yield 93%; TLC R_f = 0.80 ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.1 Hz, 1H), 7.09–7.03 (m, 2H), 6.63 (t, J = 75.6 Hz, 1H), 3.88 (d, J = 6.9 Hz, 2H), 3.76 (s, 3H), 3.32 (s, 2H), 3.08 (dt, J = 12.0, 2.7 Hz, 2H), 2.66 (td, J = 12.0, 2.7 Hz, 2H), 2.27–2.15 (m, 2H), 2.12–2.05 (m, 2H), 1.28 (m, 1H), 0.69–0.63 (m, 2H), 0.40–0.33 (m, 2H).

6.5. Synthesis of compounds 33x–y

6.5.1. Benzyl 1-{4-cyano-4-[4-methoxy-3-(methoxymethoxy)phenyl]piperidin-1-yl}cyclopropanecarboxylate (39). The title compound was prepared from the corresponding cyclopentene according to Method E to give a beige solid: Yield 65% in two steps; TLC R_f = 0.45 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) m/z = 451 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.29 (m, 5H), 7.22 (d, J = 2.4 Hz, 1H), 7.14 (dd, J = 8.4, 2.4 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 5.23 (s, 2H), 5.19 (s, 2H), 3.88 (s, 3H), 3.61–3.50 (m, 2H), 3.53 (s, 3H), 3.00–2.92 (m, 2H), 2.08–2.00 (m, 2H), 1.89–1.78 (m, 2H), 1.38–1.34 (m, 2H), 1.00–0.95 (m, 2H).

6.5.2. Benzyl 1-[4-cyano-4-(3-hydroxy-4-methoxyphenyl)piperidin-1-yl]cyclopropanecarboxylate hydrochloride (40). To a stirred solution of **39** (1.80 g, 3.99 mmol) in CH_2Cl_2 (10 mL) was added 4 N HCl/EtOAc (10 mL). After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was triturated with EtOAc to give **40** (1.51 g, 3.41 mmol, 85%) as a pale beige powder: TLC R_f = 0.38 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) m/z = 407 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.32 (m, 5H), 7.19 (d, J = 2.7 Hz, 1H), 7.07 (dd, J = 8.4, 2.7 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 5.90–5.83 (br s, 1H), 5.22 (s, 2H), 4.50–4.36 (m, 2H), 3.89 (s, 3H), 3.56–3.47 (m, 2H), 3.27–3.09 (m, 2H), 2.30–2.23 (m, 2H), 2.22–2.12 (m, 2H), 1.72–1.65 (m, 2H), 1.70–1.50 (br, 1H).

6.5.3. Benzyl 1-{4-cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylate (33x). The title compound was prepared from the corresponding alkyl haride and **40** according to Method H to give a colorless oil: Yield quant; TLC R_f = 0.57 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) m/z = 461 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.28 (m, 5H), 7.02–6.96 (m, 2H), 6.87–6.82 (m, 1H), 5.19 (s, 2H), 3.88–3.84 (m, 5H), 3.61–3.49 (m, 2H), 3.00–2.90 (m, 2H), 2.08–1.99 (m, 2H), 1.90–1.77 (m, 2H), 1.38–1.32 (m, 2H), 0.98–0.96 (m, 2H), 0.69–0.61 (m, 2H), 0.40–0.33 (m, 3H).

6.5.4. Benzyl 1-{4-cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylate (33y). The title compound was prepared from the corresponding alcohol and **40** according to Method G to give a white powder: Yield quant; TLC R_f = 0.52 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) m/z = 461 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.29 (m, 5H), 6.97 (dd, J = 8.4, 2.4 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.19 (s, 2H), 4.69 (quint, J = 7.0 Hz, 1H), 3.86 (s, 3H), 3.61–3.50 (m, 2H), 3.00–2.90 (m, 2H), 2.55–2.43 (m, 2H), 2.33–2.18 (m, 2H), 2.08–1.99 (m, 2H), 1.89–1.60 (m, 4H), 1.38–1.34 (m, 2H), 0.99–0.94 (m, 2H).

6.6. Synthesis of compounds 2a–14a, 15, 16a–18a, 19–26, 2b–14b, and 16b–18b

6.6.1. {4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}acetic acid (2a) (Method I). To a stirred solution of **33a** (330 mg, 0.855 mmol) in EtOH (5.0 mL) was added 2 N NaOH (0.86 mL, 1.71 mmol). After being stirred at room temperature for 35 min, the reaction mixture was acidified with 2 N HCl (0.86 mL, 1.71 mmol), and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O, 10/2/0.1) to give **2a** (278 mg, 0.77 mmol, 91%) as a white powder: TLC R_f = 0.22 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); IR (KBr) 3446, 2959, 2870, 2233, 1721, 1631, 1517, 1444, 1417, 1361, 1260, 1169, 1151, 1088, 1025, 985; MS (MALDI, Pos.) *m*/*z* = 359 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.00 (m, 2H), 6.88 (d, *J* = 9.0 Hz, 1H), 4.83 (m, 1H), 4.30–4.00 (br, 1H), 3.85 (s, 3H), 3.56 (br d, *J* = 12.6 Hz, 2H), 3.46 (s, 2H), 2.99 (br t, *J* = 12.6 Hz, 2H), 2.51 (br t, *J* = 12.6 Hz, 2H), 2.19 (br d, *J* = 12.6 Hz, 2H), 2.05–1.75 (m, 6H), 1.70–1.55 (m, 2H); Anal. Found $C_{20}H_{26}N_2O_4$3/7H_2O (C, H, N); HRMS (EI) calcd for $C_{20}H_{26}N_2O_4$ 358.1893. Found 358.1901.

6.6.2. (2*R*)-2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoic acid (3a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 54%; TLC R_f = 0.20 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) *m*/*z* = 373 $(M+H)^+$, 395 $(M+Na)^+$, 411 $(M+K)^+$; IR (KBr) 3453, 2958, 2348, 2232, 1617, 1519, 1449, 1365, 1261, 1150, 1023; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.00 (m, 2H), 6.90–6.85 (m, 1H), 4.88–4.80 (m, 1H), 3.85 (s, 3H), 3.50 (br q, *J* = 7.0 Hz, 1H), 3.28–3.04 (m, 3H), 3.00–2.90 (m, 1H), 2.50–2.15 (m, 5H), 2.05–1.80 (m, 6H), 1.70–1.55 (m, 2H), 1.48 (br d, *J* = 7.0 Hz, 3H); Optical rotation $[\alpha]_D^{30} + 10.69$ (*c* 0.305, DMSO); Anal. Found $C_{21}H_{28}N_2O_4$2/3H_2O (C, H, N).

6.6.3. (2*S*)-2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoic acid (4a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 91%; TLC R_f = 0.20 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) *m*/*z* = 373 $(M+H)^+$, 395 $(M+Na)^+$; (KBr) 3443, 2959, 2370, 2231, 1616, 1519, 1449, 1366, 1262, 1151, 1023.; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.00 (m, 2H), 6.90–6.80 (m, 1H), 4.86–4.58 (m, 1H), 3.84 (s, 3H), 3.54–3.44 (m, 1H), 3.34–3.20 (m, 2H), 3.14–3.02 (m, 1H), 3.00–2.86 (m, 1H), 2.50–1.75 (m, 11H), 1.75–1.55 (m, 2H), 1.45 (br d, *J* = 7.0 Hz, 3H); Optical rotation $[\alpha]_D^{30} - 10.40$ (c 0.245, DMSO); Anal. Found $C_{21}H_{28}N_2O_4$4/3H_2O (C, H, N).

6.6.4. [4-Cyano-4-(3,4-dimethoxyphenyl)piperidin-1-yl]acetic acid (10a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 94%; TLC R_f = 0.38 ($CHCl_3$/MeOH/AcOH, 10/2/1); MS (MALDI, Pos.) *m*/*z* = 305 $(M+H)^+$, 259; IR (KBr) 3494, 3006, 2963, 2840, 2594, 2241, 1614, 1520, 1443, 1412, 1381, 1335, 1265, 1243, 1201, 1174, 1151, 1090, 1025; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.10–7.00 (m, 2H), 6.97 (d, *J* = 9.3 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 4.00–3.00 (br, 1H), 3.23 (s, 2H), 3.05–2.95 (m, 2H), 2.65–2.50 (m, 2H), 2.20–1.95 (m, 4H); Anal. Found $C_{16}H_{20}N_2O_4$-2H_2O (C, H, N).

6.6.5. {4-Cyano-4-[3-ethoxy-4-methoxyphenyl]piperidin-1-yl}acetic acid (11a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield quant; TLC R_f = 0.30 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) *m*/*z* = 319 $(M+H)^+$; IR (KBr) 3436, 2982, 2242, 1637, 1520, 1400, 1264, 1176, 1152, 1025, 907, 635; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.05 (m, 2H), 6.95–6.85 (m, 1H), 4.15 (q, *J* = 6.9 Hz, 2H), 3.88 (s, 3H), 3.50–3.40 (m, 4H), 3.10–2.95 (m, 2H), 2.60–2.40 (m, 3H), 2.25–2.15 (m, 2H), 1.49 (t, *J* = 6.9 Hz, 3H); Anal. Found $C_{17}H_{22}N_2O_4$3/4H_2O (C, H, N).

6.6.6. {4-Cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}acetic acid (12a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 74%; TLC R_f = 0.59 ($CHCl_3$/MeOH/AcOH, 10/2/1); MS (MALDI, Pos.) *m*/*z* = 345 $(M+H)^+$, 367 $(M+Na)^+$, 299; IR (KBr) 3436, 3082, 3004, 2932, 2837, 2237, 1637, 1522, 1462, 1398, 1356, 1287, 1249, 1176, 1153, 1142, 1090, 1026, 1013, 966; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.05–6.95 (m, 3H), 3.83 (d, *J* = 6.9 Hz, 2H), 3.76 (s, 3H), 3.60–2.90 (br, 1H), 3.27 (s, 2H), 3.05–2.95 (m, 2H), 2.70–2.50 (m, 2H), 2.20–1.95 (m, 4H), 1.20 (m, 1H), 0.65–0.55 (m, 2H), 0.40–0.25 (m, 2H); Anal. Found $C_{19}H_{24}N_2O_4 \cdot H_2O$ (C, H, N).

6.6.7. [4-Cyano-4-(3-isopropoxy-4-methoxyphenyl)piperidin-1-yl]acetic acid (13a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield quant; TLC R_f = 0.10 (EtOAc); MS (MALDI, Pos.) *m*/*z* = 333 $(M+H)^+$; IR (KBr) 3650, 3401, 2980, 2838, 2550, 2232, 1638, 1518, 1446, 1400, 1371, 1331, 1263, 1178, 1155, 1104; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15–7.00 (m, 2H), 6.95–6.85 (m, 1H), 4.59 (sept, *J* = 6.0 Hz, 1H), 3.86 (s, 3H), 3.60–3.50 (m, 2H), 3.46 (s, 2H), 3.05–2.93 (m, 2H), 2.85–2.60 (m, 1H), 2.60–2.40 (m, 2H), 2.24–2.12 (m, 2H), 1.37 (d, *J* = 6.0 Hz, 6H); Anal. Found $C_{18}H_{24}N_2O_4 \cdot 2/3H_2O$ (C, H, N).

6.6.8. {4-Cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}acetic acid (14a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 60%; TLC R_f = 0.10 (EtOAc); MS (MALDI, Pos.) *m*/*z* = 345 $(M+H)^+$; IR (KBr) 3449, 2940, 2856, 2369, 2242, 1637, 1521, 1403, 1265, 1152, 1078; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.00 (m, 1H), 6.95–6.85 (m, 2H), 4.71 (quint, *J* = 7.5 Hz, 1H), 3.87 (s, 3H), 3.70–3.40 (m, 2H), 3.49 (s, 2H), 3.10–2.95 (m, 2H), 2.70–2.00 (m, 9H), 2.00–1.80 (m, 1H), 1.80–1.60(m, 1H); Anal. Found $C_{19}H_{24}N_2O_4 \cdot 2/3H_2O$ (C, H, N).

6.6.9. {4-Cyano-4-[3-(2,3-dihydro-1*H*-inden-2-yloxy)-4-methoxyphenyl]piperidin-1-yl}acetic acid (15). The title

compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 76%; TLC R_f=0.68 ($CHCl_3$/MeOH/AcOH, 30/2/1); MS (APCI, Pos. 20 V) m/z=407 $(M+H)^+$; IR (KBr) 3653, 3392, 2956, 2836, 2234, 1633, 1521, 1481, 1441, 1421, 1401, 1357, 1322, 1268, 1248, 1198, 1174, 1138, 1018, 992, 977; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.28–7.23 (m, 2H), 7.19–7.13 (m, 2H), 7.10 (d, J=1.8 Hz, 1H), 7.06 (dd, J=8.6, 1.8 Hz, 1H), 6.98 (d, J=8.6 Hz, 1H), 5.28 (m, 1H), 3.69 (s, 3H), 3.80–2.60 (br, 1H), 3.39–3.30 (m, 2H), 3.25 (s, 2H), 3.16–2.98 (m, 4H), 2.66–2.53 (m, 2H), 2.15–1.97 (m, 4H); Anal. Found $C_{24}H_{26}N_2O_4 \cdot H_2O$ (C, H, N); HRMS (EI) calcd for $C_{24}H_{26}N_2O_4$ 406.1893. Found 406.1888.

6.6.10. {4-Cyano-4-[3-(cyclopentyloxy)-4-ethoxyphenyl]piperidin-1-yl}acetic acid (16a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 82%; TLC R_f=0.39 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Neg. 40 V) m/z=371 $(M-H)^-$; IR (KBr) 3453, 2968, 2874, 2236, 1640, 1520, 1485, 1450, 1418, 1374, 1352, 1333, 1297, 1262, 1244, 1154, 1125, 1089, 1039, 1007, 989; ^{1}H NMR (300 MHz, DMSO-d_3) δ 7.05–6.90 (m, 3H), 4.83 (m, 1H), 4.00 (q, J=6.9 Hz, 2H), 4.00–3.00 (br, 1H), 3.23 (s, 2H), 3.05–2.95 (m, 2H), 2.65–2.50 (m, 2H), 2.15–1.90 (m, 4H), 1.95–1.80 (m, 2H), 1.80–1.60 (m, 4H), 1.65–1.50 (m, 2H), 1.29 (t, J=6.9 Hz, 3H); Anal. Found $C_{21}H_{28}N_2O_4 \cdot 2/3H_2O$ (C, H, N).

6.6.11. {4-Cyano-4-[3-(cyclopentyloxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetic acid (17a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield quant; TLC R_f=0.35 ($CHCl_3$/MeOH/AcOH, 10/1/0.2); MS (APCI, Neg. 40 V) m/z=393 $(M-H)^-$; IR (KBr) 3427, 2965, 2875, 2239, 1611, 1516, 1420, 1271, 1226, 1126, 1041, 988; ^{1}H NMR (300 MHz, DMSO-d_3) δ 7.25–7.15 (m, 2H), 7.09 (dd, J=8.1, 2.1 Hz, 1H), 7.01 (t, J=75.0 Hz, 1H), 4.98 (m, 1H), 3.60–3.00 (br, 1H), 3.26 (s, 2H), 3.10–2.95 (m, 2H), 2.70–2.50 (m, 2H), 2.20–2.00 (m, 4H), 2.00–1.80 (m, 2H), 1.80–1.60 (m, 4H), 1.65–1.50 (m, 2H); Anal. Found $C_{20}H_{24}F_2N_2O_4$ (C, H, N).

6.6.12. {4-Cyano-4-[3-(cyclopentyloxy)-4-isopropoxyphenyl]piperidin-1-yl}acetic acid (18a). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 69%; TLC R_f=0.20 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) m/z=387 $(M+H)^+$, 409 $(M+Na)^+$; IR (KBr) 3433, 2970, 2882, 2232, 1632, 1513, 1404, 1370, 1265, 1172, 1136, 1109; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, J=2.1 Hz, 1H), 7.00 (dd, J=8.4, 2.1 Hz, 1H), 6.91 (d, J=8.4 Hz, 1H), 4.84–4.78 (m, 1H), 4.44 (sept, J=6.0 Hz, 1H), 3.48–3.34 (m, 4H), 3.02–2.90 (m, 2H), 2.48–2.30 (m, 2H), 2.20–1.75 (m, 9H), 1.70–1.60 (m, 2H), 1.32 (d, J=6.0 Hz, 6H).

6.6.13. {4-Cyano-4-[4-(difluoromethoxy)-3-ethoxyphenyl]piperidin-1-yl}acetic acid (19). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 88%; TLC R_f=0.15 ($CHCl_3$/MeOH/AcOH, 9/1/0.1); MS (APCI, Neg. 40 V) m/z=353 $(M-H)^-$; IR (KBr) 3442, 2984, 2235, 1631, 1519, 1487, 1422, 1349, 1300, 1274, 1226, 1177, 1110, 1029, 970; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.22–7.19 (m, 2H), 7.09 (dd, J=8.4, 2.1 Hz, 1H), 7.06 (t, J=74.4 Hz, 1H), 4.13 (q, J=6.9 Hz, 2H), 4.00–3.00 (br, 1H), 3.21 (s, 2H), 3.01 (d, J=12.0 Hz, 2H), 2.57 (dt, J=11.7, 3.0 Hz, 2H), 2.13–1.99 (m, 4H), 1.32 (t, J=6.9 Hz, 3H); HRMS (EI) calcd for $C_{17}H_{20}F_2N_2O_4$ 354.1391. Found 354.1383.

6.6.14. {4-Cyano-4-[4-(difluoromethoxy)-3-propoxyphenyl]piperidin-1-yl}acetic acid (20). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 60%; TLC R_f=0.60 ($CHCl_3$/MeOH/AcOH, 10/2/1); MS (MALDI, Pos.) m/z=369 $(M+H)^+$; IR (KBr) 3438, 2967, 2237, 1631, 1511, 1473, 1421, 1390, 1344, 1297, 1273, 1226, 1179, 1113, 1072, 1033, 978; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40–11.00 (br, 1H), 7.30–7.15 (m, 2H), 7.11 (dd, J=8.7, 2.7 Hz, 1H), 7.05 (t, J=74.4 Hz, 1H), 4.04 (t, J=6.6 Hz, 2H), 3.23 (s, 2H), 3.10–2.95 (m, 2H), 2.70–2.50 (m, 2H), 2.20–2.00 (m, 4H), 1.74 (sext, J=6.6 Hz, 2H), 0.98 (t, J=6.6 Hz, 3H); Anal. Found $C_{18}H_{22}F_2N_2O_4$ (C, H, N).

6.6.15. {4-[3-Butoxy-4-(difluoromethoxy)phenyl]-4-cyanopiperidin-1-yl}acetic acid (21). The title compound was prepared from the corresponding ester according to Method I to give a pale brown powder: Yield 78%; TLC R_f=0.56 ($CHCl_3$/MeOH/AcOH, 10/2/1); MS (APCI, Neg. 20 V) m/z=381 $(M-H)^-$; IR (KBr) 3451, 2987, 2981, 2879, 2696, 2547, 2236, 1611, 1521, 1423, 1298, 1124, 1034; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 7.17 (d, J=8.1 Hz, 1H), 7.09 (dd, J=8.1, 2.1 Hz, 1H), 7.04 (d, J=2.1 Hz, 1H), 6.57 (t, J=75.2 Hz, 1H), 4.03 (t, J=6.5 Hz, 2H), 3.57 (br, 1H), 3.29 (s, 2H), 3.20–3.10 (m, 2H), 2.72–2.61 (m, 2H), 2.30–2.19 (m, 2H), 2.13–2.05 (m, 2H), 1.86–1.76 (m, 2H), 1.58–1.44 (m, 2H), 0.99 (t, J=7.5 Hz, 3H); Anal. Found $C_{19}H_{24}F_2N_2O_4$ (C, H, N).

6.6.16. {4-Cyano-4-[4-(difluoromethoxy)-3-isobutoxyphenyl]piperidin-1-yl}acetic acid (22). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 74%; TLC R_f=0.63 ($CHCl_3$/MeOH/AcOH, 10/2/1); MS (MALDI, Pos.) m/z=83 $(M+H)^+$, 405 $(M+Na)^+$; IR (KBr) 3071, 2965, 2235, 1640, 1518, 1473, 1420, 1407, 1389, 1370, 1338, 1316. 1290, 1271, 1256, 1227, 1177, 1106, 1046, 1028, 963; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20–10.80 (br, 1H), 7.25–7.20 (m, 2H), 7.11 (dd, J=8.7, 2.4 Hz, 1H), 7.04 (t, J=74.4 Hz, 1H), 3.86 (d, J=6.3 Hz, 2H), 3.23 (s, 2H), 3.10–2.95 (m, 2H), 2.65–2.50 (m, 2H), 2.20–2.00 (m, 5H), 0.98 (d, J=6.6 Hz, 6H); Anal. Found $C_{19}H_{24}F_2N_2O_4 \cdot H_2O$ (C, H, N).

6.6.17. {4-Cyano-4-[3-(cyclobutyloxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetic acid (23). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 89%; TLC R_f=0.67 ($CHCl_3$/MeOH, 3/1); MS (MALDI, Pos.) m/z=381 $(M+H)^+$, 403 $(M+Na)^+$; IR (KBr) 3430,

2239, 1636, 1516, 1408, 1371, 1318, 1275, 1111, 1074, 1050; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21 (d, J = 8.1 Hz, 1H), 7.10 (dd, J = 8.1, 2.1 Hz, 1H), 7.08 (t, J = 74.4 Hz, 1H), 7.06 (d, J = 2.1 Hz, 1H), 4.85 (m, 1H), 4.25–2.60 (br s, 1H), 3.24 (s, 2H), 3.01 (br d, J = 12.0 Hz, 2H), 2.58 (br t, J = 12.0 Hz, 2H), 2.48–2.34 (m, 2H), 2.16–1.94 (m, 6H), 1.77 (m, 1H), 1.62 (m, 1H); Anal. Found $C_{19}H_{22}F_2N_2O_4 \cdot 1/4H_2O$ (C, H, N).

6.6.18. {4-Cyano-4-[3-(cyclobutylmethoxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetic acid (24). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 98%; TLC R_f = 0.53 ($CHCl_3$/MeOH, 3/1); MS (MALDI, Pos.) m/z = 395 $(M+H)^+$, 417 $(M+Na)^+$; IR (KBr) 2976, 2229, 1638, 1518, 1408, 1385, 1368, 1338, 1316, 1271, 1258, 1100, 1047; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25 (d, J = 2.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.11 (dd, J = 8.4, 2.4 Hz, 1H), 7.03 (t, J = 74.4 Hz, 1H), 4.06 (d, J = 6.6 Hz, 2H), 4.00–2.80 (br s, 1H), 3.24 (s, 2H), 3.02 (br d, J = 12.0 Hz, 2H), 2.72 (m, 1H), 2.59 (td, J = 12.0, 2.7 Hz, 2H), 2.17–1.95 (m, 6H), 2.00–1.75 (m, 4H); HRMS (EI) calcd for $C_{20}H_{24}F_2N_2O_4$ 394.1704. Found 394.1715.

6.6.19. {4-Cyano-4-[3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}acetic acid hydrochloride (25). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 74%; TLC R_f = 0.61 ($CHCl_3$/MeOH, 2/1); MS (MALDI, Pos.) m/z = 381 $(M+H)^+$, 403 $(M+Na)^+$; IR (KBr) 3482, 2943, 2672, 2239, 1750, 1521, 1407, 1297, 1237, 1187, 1132, 1051; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21–7.14 (m, 2H), 7.02 (t, J = 74.4 Hz, 1H), 7.01 (dd, J = 8.7, 2.1 Hz, 1H), 4.12 (s, 2H), 3.95–2.95 (br s, 2H), 3.85 (d, J = 6.9 Hz, 2H), 3.58 (br d, J = 12.0 Hz, 2H), 3.23 (br t, J = 12.0 Hz, 2H), 2.54–2.31 (m, 4H), 1.14 (m, 1H), 0.47 (m, 2H), 0.24 (m, 2H); HRMS (EI) calcd for $C_{19}H_{22}F_2N_2O_4$ 380.1548. Found 380.1550.

6.6.20. {4-Cyano-4-[4-(difluoromethoxy)-3-(2,3-dihydro-1*H*-inden-2-yloxy)phenyl]piperidin-1-yl}acetic acid (26). The title compound was prepared from the corresponding ester according to Method I to give a white powder: Yield 86%; TLC R_f = 0.24 ($CHCl_3$/MeOH/AcOH, 9/1/0.1); MS (APCI, Neg. 40 V) m/z = 441 $(M-H)^-$; IR (KBr) 3132, 2237, 1637, 1515, 1407, 1388, 1367, 1311, 1271, 1221, 1177, 1118, 1035, 960; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32–7.14 (m, 7H), 6.91 (t, J = 74.4 Hz, 1H), 5.43–5.37 (m, 1H), 4.00–2.60 (br, 1H), 3.39 (dd, J = 16.8, 6.0 Hz, 2H), 3.24 (s, 2H), 3.07–3.00 (m, 4H), 2.60 (dt, J = 11.7, 3.0 Hz, 2H), 2.17–2.03 (m, 4H); Anal. Found $C_{24}H_{24}F_2N_2O_4 \cdot 1/2H_2O$ (C, H, N).

6.6.21. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-2-methylpropanoic acid (5a) (Method J). A solution of **33l** (180 mg, 0.378 mmol) in MeOH (4.0 mL) and THF (4.0 mL) was hydrogenated under atmospheric pressure of H_2 gas in the presence of 10% Pd/C (20 mg) for 1.5 h. The catalyst was removed by filtration through a pad of Celite, and washed with MeOH. The filtrates were concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O, 10/2/0.1) to give **5a** (140 mg, 0.363 mmol, 96 %) as a white powder: TLC R_f = 0.34 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z = 387 $(M+H)^+$, 409 $(M+Na)^+$; IR (KBr) 3571, 3422, 2955, 2853, 2685, 2606, 2238, 1626, 1591, 1519, 1497, 1466, 1420, 1406, 1389, 1365, 1351, 1286, 1257, 1211, 1200, 1170, 1149, 1123, 1084, 1063, 1019, 988; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.05–6.90 (m, 3H), 4.84 (m, 1H), 3.74 (s, 3H), 3.80–3.00 (br, 1H), 3.15–3.00 (m, 2H), 2.65–2.50 (m, 2H), 2.20–2.05 (m, 2H), 2.10–1.95 (m, 2H), 2.00–1.80 (m, 2H), 1.80–1.60 (m, 4H), 1.65–1.55 (m, 2H), 1.25 (s, 6H); HRMS (FAB) calcd for $C_{22}H_{31}N_2O_4$ 387.2284. Found 387.2289.

6.6.22. 1-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylic acid (6a). The title compound was prepared from the corresponding benzyl ester according to Method J to give a white powder: Yield 93%; TLC R_f = 0.45 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z = 385 $(M+H)^+$, 407 $(M+Na)^+$; IR (KBr) 3440, 2956, 2870, 2236, 1725, 1605, 1519, 1445, 1418, 1364, 1259, 1170, 1148, 1082, 1027, 996; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.29 (br s, 1H), 7.05–6.90 (m, 3H), 4.82 (m, 1H), 3.73 (s, 3H), 3.45–3.30 (m, 2H), 2.95–2.85 (m, 2H), 2.10–1.95 (m, 2H), 2.00–1.60 (m, 8H), 1.65–1.50 (m, 2H), 1.25–1.10 (m, 2H), 0.95–0.80 (m, 2H); HRMS (EI) calcd for $C_{22}H_{28}N_2O_4$ 384.2046. Found 384.2032.

6.6.23. 1-[4-Cyano-4-(3-ethoxy-4-methoxyphenyl)piperidin-1-yl]cyclopropanecarboxylic acid (7a). The title compound was prepared from the corresponding benzyl ester according to Method J to give a white powder: Yield 68%; TLC R_f = 0.38 ($CHCl_3$/MeOH, 9/1); MS (FAB, Pos.) m/z = 345 $(M+H)^+$; IR (KBr) 3443, 3025, 2930, 2230, 1732, 1621, 1522, 1494, 1451, 1372, 1255, 1151, 1022, 923; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.45–12.15 (br, 1H), 7.03–6.93 (m, 3H), 4.04 (q, J = 6.9 Hz, 2H), 3.75 (s, 3H), 3.45–3.35 (m, 2H), 2.95–2.86 (m, 2H), 2.09–1.98 (m, 2H), 1.86–1.72 (m, 2H), 1.32 (t, J = 6.9 Hz, 3H), 1.21–1.16 (m, 2H), 0.92–0.86 (m, 2H); Anal. Found $C_{19}H_{24}N_2O_4$ (C, H, N).

6.6.24. 1-{4-Cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylic acid (8a). The title compound was prepared from the corresponding benzyl ester according to Method J to give a white powder: Yield 99%; TLC R_f = 0.35 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) m/z = 371 $(M+H)^+$; IR (KBr) 3448, 3012, 2959, 2942, 2910, 2864, 2236, 1700, 1638, 1607, 1519, 1473, 1442, 1420, 1403, 1335, 1280, 1252, 1182, 1169, 1151, 1125, 1017, 965; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.5–12.0 (br, 1H), 7.04–6.93 (m, 3H), 3.82 (d, J = 7.2 Hz, 2H), 3.77 (s, 3H), 3.46–3.36 (m, 2H), 2.94–2.86 (m, 2H), 2.07–1.97 (m, 2H), 1.85–1.71 (m, 2H), 1.26–1.14 (m, 3H), 0.91–0.85 (m, 2H), 0.60–0.53 (m, 2H), 0.35–0.28 (m, 2H); HRMS (EI) calcd for $C_{21}H_{26}N_2O_4$ 370.1893. Found 370.1873.

6.6.25. 1-{4-Cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}cyclopropanecarboxylic acid (9a). The title compound was prepared from the corresponding benzyl ester according to Method J to give a white powder: Yield 65%; TLC R_f=0.36 ($CHCl_3$/MeOH, 19/1); MS (APCI, Pos. 20 V) m/z=371 $(M+H)^+$; IR (KBr) 3445, 2940, 2236, 1715, 1605, 1520, 1443, 1419, 1357, 1266, 1174, 1150, 1078, 1050, 1023, 984; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.5–12.1 (br, 1H), 7.01–6.93 (m, 2H), 6.84 (d, J=1.8 Hz, 1H), 4.73 (quint, J=7.5 Hz, 1H), 3.75 (s, 3H), 3.44–3.36 (m, 2H), 2.95–2.86 (m, 2H), 2.46–2.33 (m, 2H), 2.11–1.96 (m, 4H), 1.83–1.55 (m, 4H), 1.21–1.15 (m, 2H), 0.92–0.86 (m, 2H); HRMS (EI) calcd for $C_{21}H_{26}N_2O_4$ 370.1893. Found 370.1882.

6.6.26. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (2b) (Method K). To a stirred solution of **2a** (239 mg, 0.668 mmol) in DMF (4.0 mL) were added EDC (192 mg, 1.00 mmol), HOBt (135 mg, 1.00 mmol) and (1-methoxy-1-methyethyl)oxyamine (351 mg, 3.34 mmol). After being stirred at room temperature for 3 h, the mixture was poured into H_2O, and extracted with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1/1–0/1) to give **2c** (289 mg, 0.649 mmol, 97%) as a pale yellow oil: TLC R_f=0.26 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (br s, 1H), 7.05–6.90 (m, 2H), 6.87 (d, J=8.4 Hz, 1H), 4.81 (m, 1H), 3.86 (s, 3H), 3.36 (s, 3H), 3.23 (s, 2H), 3.10–3.00 (m, 2H), 2.80–2.65 (m, 2H), 2.20–2.00 (m, 2H), 2.10–1.75 (m, 8H), 1.70–1.55 (m, 2H), 1.46 (s, 6H).

To a stirred solution of **2c** (280 mg, 0.629 mmol) in MeOH (3.0 mL) was added 2 N HCl (0.35 mL, 0.692 mmol). After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was triturated with *i*-Pr_2O/MeOH to give **2b** (189 mg, 0.462 mmol, 73%) as a white powder: TLC R_f=0.38 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z=374 $(M+H)^+$; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.09 (d, J=2.4 Hz, 1H), 6.99 (dd, J=8.4, 2.4 Hz, 1H), 6.88 (d, J=8.4 Hz, 1H), 6.08 (br s, 3H), 4.75 (m, 1H), 3.72 (s, 3H), 3.30 (s, 2H), 3.02 (br d, J=14.4 Hz, 2H), 2.75–2.60 (m, 2H), 2.20–1.95 (m, 4H), 2.00–1.65 (m, 6H), 1.60–1.40 (m, 2H); Anal. Found $C_{20}H_{26}N_2O_4 \cdot 3/7H_2O$ (C, H, N).

6.6.27. (2*R*)-2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxypropanamide hydrochloride (3b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 75% in two steps; TLC R_f=0.45 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) m/z=388 $(M+H)^+$; IR (KBr) 3371, 3164, 2939, 2674, 2232, 1665, 1519, 1465, 1443, 1420, 1382, 1355, 1259, 1179, 1149, 1130, 1106, 1067, 1016; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.08–7.00 (m, 1H), 7.00–6.95 (m, 1H), 6.85 (d, J=8.7 Hz, 1H), 5.95–5.50 (m, 3H), 4.80–4.72 (m, 1H), 3.73 (s, 3H), 3.38–3.25 (m, 1H), 3.08–2.98 (m, 2H), 2.90–2.75 (m, 1H), 2.75–2.60 (m, 1H), 2.15–2.00 (m, 4H), 1.95–1.65 (m, 6H), 1.55–1.45 (m, 2H), 1.43 (br d, J=6.6 Hz, 3H); Optical rotation $[\alpha]_D^{30}$ + 8.8 (*c* 0.37, DMSO); Anal. Found $C_{21}H_{29}N_3O_4$6/5HCl·1/5H_2NOH (C, H, N).

6.6.28. (2*S*)-2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxypropanamide hydrochloride (4b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 44% in two steps; TLC R_f=0.45 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) m/z=388 $(M+H)^+$; IR (KBr) 3370, 3167, 2940, 2714, 2232, 1685, 1519, 1465, 1443, 1420, 1382, 1356, 1259, 1179, 1149, 1130, 1106, 1016; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.11 (d, J=2.1 Hz, 1H), 7.02 (dd, J=2.1, 8.7 Hz, 1H), 6.88 (d, J=8.7 Hz, 1H), 6.80–6.20 (m, 3H), 4.80–4.72 (m, 1H), 3.72 (s, 3H), 3.40 (br q, J=6.9 Hz, 1H), 3.14–3.02 (m, 2H), 3.00–2.88 (m, 1H), 2.82–2.70 (m, 1H), 2.20–2.05 (m, 4H), 1.95–1.65 (m, 6H), 1.55–1.45 (m, 2H), 1.41 (d, J=6.9 Hz, 3H); Optical rotation $[\alpha]_D^{30}$ − 8.7 (*c* 0.15, DMSO); Anal. Found $C_{21}H_{29}N_3O_4$6/5HCl·1/5H_2 NOH (C, H, N).

6.6.29. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxy-2-methylpropanamide hydrochloride (5b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 62% in two steps; TLC R_f=0.38 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) m/z=402 $(M+H)^+$; IR (KBr) 3423, 3235, 2959, 2871, 2709, 2564, 2237, 1732, 1677, 1604, 1590, 1524, 1465, 1446, 1374, 1328, 1307, 1274, 1248, 1218, 1151, 1123, 1063, 1028, 993; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.14 (d, J=2.1 Hz, 1H), 6.99 (dd, J=8.4, 2.1 Hz, 1H), 6.91 (d, J=8.4 Hz, 1H), 5.95 (br s, 3H), 4.72 (m, 1H), 3.73 (s, 3H), 3.10–3.00 (m, 2H), 2.80–2.65 (m, 2H), 2.20–2.00 (m, 4H), 2.00–1.70 (m, 6H), 1.60–1.40 (m, 2H), 1.39 (s, 6H); Anal. Found $C_{22}H_{31}N_3O_4 \cdot HCl \cdot H_2O$ (C, H, N).

6.6.30. 1-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxycyclopropanecarboxamide hydrochloride (6b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 84% in two steps; TLC R_f=0.45 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z=400 $(M+H)^+$, 422 $(M+Na)^+$; IR (KBr) 3437, 3198, 2960, 2871, 2642, 2600, 2553, 2240, 1731, 1662, 1519, 1444, 1419, 1361, 1334, 1305, 1257, 1171, 1151, 1133, 1055, 1027, 993; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.12 (d, J=2.4 Hz, 1H), 6.99 (dd, J=8.4, 2.4 Hz, 1H), 6.87 (d, J=8.4 Hz, 1H), 6.90–6.00 (br, 3H), 4.75 (m, 1H), 3.73 (s, 3H), 3.00–2.90 (m, 2H), 2.90–2.70 (m, 2H), 2.20–2.00 (m, 4H), 2.00–1.60 (m, 6H), 1.60–1.40 (m, 2H), 1.35–1.25 (m, 2H), 1.10–1.00 (m, 2H); Anal. Found $C_{22}H_{29}N_3O_4 \cdot HCl \cdot H_2O$ (C, H, N).

6.6.31. 1-[4-Cyano-4-(3-ethoxy-4-methoxyphenyl)piperidin-1-yl]-*N*-hydroxycyclopropanecarboxamide hydrochloride (7b). The title compound was prepared from the corresponding carboxylic acid according to Method K

to give a pale yellow powder: Yield 89% in two steps; TLC R_f=0.42 ($CHCl_3$/MeOH, 9/1); MS (FAB, Pos.) m/z=360 $(M+H)^+$; IR (KBr) 3439, 3138, 2980, 2907, 2385, 1656, 1523, 1446, 1419, 1386, 1264, 1251, 1173, 1151, 1129, 1043, 1025, 961; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.00–7.20 (br, 3H), 7.09 (d, J=1.8 Hz, 1H), 7.04 (dd, J=8.4, 1.8 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 3.91 (q, J=6.9 Hz, 2H), 3.74 (s, 3H), 2.99–2.79 (m, 4H), 2.19–2.10 (m, 4H), 1.37–1.27 (m, 5H), 1.09–1.03 (m, 2H); Anal. Found $C_{19}H_{25}N_3O_4$·HCl (C, H, N).

6.6.32. 1-{4-Cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxycyclopropanecarboxamide hydrochloride (8b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 91% in two steps; TLC R_f=0.60 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos. 20 V) m/z=386 $(M+H)^+$; IR (KBr) 3432, 3127, 3005, 2872, 2632, 2593, 2538, 2411, 2389, 2238, 1654, 1605, 1592, 1521, 1467, 1444, 1421, 1409, 1360, 1331, 1251, 1176, 1153, 1135, 1051, 1024, 1010, 965; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.60–6.80 (br, 3H), 7.15 (d, J=2.0 Hz, 1H), 7.05 (dd, J=9.0, 2.0 Hz, 1H), 6.91 (d, J=9.0 Hz, 1H), 3.81 (d, J=6.9 Hz, 2H), 3.73 (s, 3H), 2.99–2.79 (m, 4H), 2.20–2.02 (m, 4H), 1.37–1.31 (m, 2H), 1.31–1.20 (m, 1H), 1.08–1.03 (m, 2H), 0.55–0.47 (m, 2H), 0.32–0.26 (m, 2H); Anal. Found $C_{21}H_{27}N_3O_4$·HCl (C, H, N).

6.6.33. 1-{4-Cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxycyclopropanecarboxamide hydrochloride (9b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 99% in two steps; TLC R_f=0.64 ($CHCl_3$/MeOH, 9/1); MS (APCI, Pos.20 V) m/z=386 $(M+H)^+$; IR (KBr) 3450, 3099, 2983, 2878, 2632, 2590, 2505, 2412, 2233, 1650, 1593, 1521, 1464, 1448, 1419, 1384, 1352, 1262, 1249, 1189, 1171, 1151, 1134, 1080, 1053, 1042, 1025, 978; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.00–7.10 (br, 3H), 7.04 (d, J=2.1 Hz, 1H), 7.00 (dd, J=8.4, 2.1 Hz, 1H), 6.90 (d, J=8.4 Hz, 1H), 4.62 (quint, J=7.5 Hz, 1H), 3.75 (s, 3H), 3.00–2.80 (m, 4H), 2.42–2.30 (m, 2H), 2.23–2.02 (m, 6H), 1.74–1.60 (m, 1H), 1.58–1.40 (m, 1H), 1.38–1.32 (m, 2H), 1.09–1.03 (m, 2H); HRMS (FAB) calcd for $C_{21}H_{28}N_3O_4$ 386.2080. Found 386.2072.

6.6.34. 2-[4-Cyano-4-(3,4-dimethoxyphenyl)piperidin-1-yl]-*N*-hydroxyacetamide hydrochloride (10b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 42% in two steps; TLC R_f=0.21 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z=320 $(M+H)^+$; IR (KBr) 3431, 3120, 2999, 2959, 2841, 2244, 1701, 1609, 1523, 1469, 1445, 1415, 1365, 1340, 1320, 1263, 1249, 1161, 1140, 1091, 1023, 969; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.05–6.95 (m, 2H), 6.85 (d, J=9.0 Hz, 1H), 6.80–6.00 (br, 3H), 3.75 (s, 6H), 3.28 (s, 2H), 3.05–2.95 (m, 2H), 2.70–2.55 (m, 2H), 2.20–1.90 (m, 4H); HRMS (FAB) calcd for $C_{16}H_{22}N_3O_4$ 320.1610. Found 320.1627.

6.6.35. 2-[4-Cyano-4-(3-ethoxy-4-methoxyphenyl)piperidin-1-yl]-*N*-hydroxyacetamide hydrochloride (11b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 64% in two steps; TLC R_f=0.15 (EtOAc); MS (APCI, Neg. 20 V) m/z=332 $(M-H)^-$; IR (KBr) 3421, 3107, 2987, 2839, 2242, 1698, 1608, 1525, 1457, 1423, 1396, 1366, 1340, 1318, 1262, 1250, 1177, 1158, 1138, 1084, 1034; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.30–7.00 (m, 5H), 6.93–6.87 (m, 1H), 3.95 (q, J=6.9 Hz, 2H), 3.74 (s, 3H), 3.34 (s, 2H), 3.10–3.00 (m, 2H), 2.75–2.60 (m, 2H), 2.15–1.95 (m, 4H), 1.33 (t, J=6.9 Hz, 3H); Anal. Found $C_{17}H_{23}N_3O_4$·HCl·1/4H_2O (C, H, N).

6.6.36. 2-{4-Cyano-4-[3-(cyclopropylmethoxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (12b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 52% in two steps; TLC R_f=0.31 ($CHCl_3$/MeOH, 10/1); MS (MALDI, Pos.) m/z=360 $(M+H)^+$; IR (KBr) 3126, 3002, 2239, 1699, 1607, 1593, 1521, 1466, 1423, 1407, 1363, 1340, 1314, 1254, 1201, 1177, 1153, 1139, 1093, 1014, 966; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.80–7.50 (br, 3H), 7.08 (d, J=2.4 Hz, 1H), 7.02 (dd, J=8.4, 2.4 Hz, 1H), 6.87 (d, J=8.4 Hz, 1H), 3.83 (d, J=6.9 Hz, 2H), 3.73 (s, 3H), 3.30 (s, 2H), 3.10–2.90 (m, 2H), 2.70–2.55 (m, 2H), 2.15–1.95 (m, 4H), 1.26 (m, 1H), 0.55–0.45 (m, 2H), 0.35–0.25 (m, 2H); Anal. Found $C_{19}H_{25}N_3O_4$·HCl (C, H, N).

6.6.37. 2-[4-Cyano-4-(3-isopropoxy-4-methoxyphenyl)piperidin-1-yl]-*N*-hydroxyacetamide hydrochloride (13b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 25% in two steps; TLC R_f=0.22 (EtOAc); MS (APCI, Neg. 20 V) m/z=346 $(M-H)^-$; IR (KBr) 3431, 3153, 2976, 2232, 1686, 1518, 1445, 1419, 1264, 1175, 1152, 1108, 1026; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.10 (d, J=2.1 Hz, 1H), 7.02 (dd, J=6.0, 2.1 Hz, 1H), 6.88 (d, J=6.0 Hz, 1H), 6.25–5.50 (m, 3H), 4.51 (sept, J=6.0 Hz, 1H), 3.73 (s, 3H), 3.28 (s, 2H), 3.05–2.95 (m, 2H), 2.75–2.55 (m, 2H), 2.15–1.95 (m, 4H), 1.28 (d, J=6.0 Hz, 6H); Anal. Found $C_{18}H_{25}N_3O_4$·HCl·H_2O (C, H, N).

6.6.38. 2-{4-Cyano-4-[3-(cyclobutyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (14b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 58% in two steps; TLC R_f=0.20 (EtOAc); MS (MALDI, Pos.) m/z=360 $(M+H)^+$; IR (KBr) 3393, 3171, 2939, 2654, 2571, 2242, 1697, 1521, 1443, 1420, 1355, 1261, 1175, 1152, 1137, 1074, 1024; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.05–7.00 (m, 2H), 6.95–6.88 (m, 1H), 6.80–6.20 (m, 3H), 4.65 (quint, J=6.9 Hz, 1H), 3.75 (s, 3H), 3.36 (s, 2H), 3.10–3.00 (m, 2H), 2.75–2.65 (m, 2H), 2.40–2.30 (m, 2H), 2.20–1.90 (m, 6H), 1.75–1.40 (m, 2H); Anal. Found $C_{19}H_{25}N_3O_4$·HCl (C, H, N).

6.6.39. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-ethoxyphenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (16b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 58% in two steps; TLC R_f = 0.36 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) m/z = 388 $(M+H)^+$; IR (KBr) 3293, 3120, 2972, 2659, 2610, 2565, 2254, 1694, 1604, 1516, 1477, 1451, 1420, 1361, 1317, 1262, 1184, 1129, 1092, 1046, 975; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 8.10–7.20 (br, 3H), 7.10 (d, J = 2.1 Hz, 1H), 7.00 (dd, J = 8.4, 2.1 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 4.76 (m, 1H), 3.97 (q, J = 6.9 Hz, 2H), 3.31 (s, 2H), 3.10–2.95 (m, 2H), 2.75–2.60 (m, 2H), 2.20–1.95 (m, 4H), 2.00–1.65 (m, 6H), 1.60–1.40 (m, 2H), 1.31 (t, J = 6.9 Hz, 3H); Anal. Found $C_{21}H_{29}N_3O_4$·HCl (C, H, N).

6.6.40. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-(difluoromethoxy)phenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (17b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 81% in two steps; TLC R_f = 0.36 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) m/z = 410 $(M+H)^+$; IR (KBr) 3296, 3119, 2973, 2659, 2612, 2568, 2253, 1692, 1606, 1508, 1479, 1452, 1420, 1385, 1317, 1291, 1267, 1236, 1183, 1147, 1107, 1056, 1003, 976; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.23 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 1.8 Hz, 1H), 6.97 (dd, J = 8.4, 1.8 Hz, 1H), 6.97 (t, J = 75.0 Hz, 1H), 6.60–5.60 (br, 3H), 4.74 (m, 1H), 3.31 (s, 2H), 3.10–3.00 (m, 2H), 2.70–2.60 (m, 2H), 2.20–2.00 (m, 4H), 1.85–1.60 (m, 6H), 1.60–1.40 (m, 2H); Anal. Found $C_{20}H_{25}F_2N_3O_4$·HCl (C, H, N).

6.6.41. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-isopropoxyphenyl]piperidin-1-yl}-*N*-hydroxyacetamide hydrochloride (18b). The title compound was prepared from the corresponding carboxylic acid according to Method K to give a white powder: Yield 71% in two steps; TLC R_f = 0.40 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) m/z = 402 $(M+H)^+$; IR (KBr) 3291, 3118, 2971, 2658, 2561, 2253, 1693, 1601, 1513, 1451, 1418, 1370, 1319, 1262, 1183, 1130, 1101, 1045; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.15–7.10 (m, 1H), 7.05–6.90 (m, 2H), 5.80–5.35 (m, 3H), 4.78–4.72 (m, 1H), 4.46 (sept, J = 6.0 Hz, 1H), 3.28 (s, 2H), 3.04–2.96 (m, 2H), 2.68–2.58 (m, 2H), 2.15–1.95 (m, 4H), 1.95–1.65 (m, 6H), 1.58–1.45 (m, 2H), 1.28 (d, J = 6.0 Hz, 6H); Anal. Found $C_{22}H_{31}N_3O_4$·HCl·1/3H_2O (C, H, N).

6.7. Assay of human PDE4 activity

The method of Reeves et al.[32] was modified to isolate phosphodiesterase type 4 isozyme (PDE4). The enzyme was prepared from U937 cells derived from human monocytes, and was stored at −20 °C after preparation. Measurement of PDE4 activity was performed using this stored enzyme after it was diluted with distilled water containing bovine serum albumin. The substrate solution was prepared by adding ^{3}H–*c*AMP (300,000 dpm (5000 Bq)/assay) and 100 μmol/L *c*AMP solution to 100 mmol/L Tris–HCl (pH 8.0) containing 5 mmol/L ethylene glycol-bis (β-aminoethyl ether) and *N*,*N*,*N*′,*N*′-tetraacetic acid. The substrate solution was mixed with the enzyme solution containing a test compound dissolved in dimethylsulfoxide (DMSO), and incubation was done for 30 min at 30 °C. Assays were performed in duplicate at three to four different concentrations of each test compound, and the IC_{50} values were determined.

6.8. Inhibition of LPS-induced plasma TNF-α production in rats

Male Crj:CD(SD)IGS rats aged 6 weeks (n = 7) were fasted overnight, and the test compounds (0.01–0.1 mg/10 mL/kg) were administered orally at 1 h before intravenous injection of 1 μg/kg of LPS (*Escherichia coli* Serotype 055 B5). The plasma TNF-α level was measured with a commercially available ELISA kit (R&D Systems) at 90 min after LPS challenge. The percent inhibition (the dosage required to inhibit plasma TNF-α production by 50%) was determined by the following formula:

$$\%\text{Inhibition} = 100 - (C - S)/(L - S) \times 100$$

C: Plasma TNF-α concentration in LPS-treated animals pretreated with a test compound. *L*: Plasma TNF-α concentration in LPS-treated animals pretreated with saline. *S*: Plasma TNF-α concentration in saline-treated animals pretreated with saline.

6.9. SRS-A mediated bronchoconstriction in guinea pigs

Male Hartley guinea pigs aged 7 weeks (n = 5) were actively sensitized by intraperitoneal administration of 1 mg of ovalbumin (OVA) containing 5×10^9 killed *Bordetella pertussis* organisms on day 0. On day 14, the bronchoconstrictor response was measured using a modified version of the method of Konzett and Rössler. Bronchoconstriction was induced by an intravenous injection of OVA (0.15–0.5 mg/kg). Sensitized animals were treated with both a cyclooxygenase inhibitor (indomethacin at 5 mg/kg i.v., 3 min before OVA) and an antihistamine (pyrilamine at 1 mg/kg i.v., 1 min before OVA) to ensure that endogenous SRS-A was solely responsible for bronchoconstriction. Test compounds were administered orally at 1 h before antigen challenge. Bronchoconstrictor response was measured for 15 min and the result was represented as the area under the curve (AUC 0–15 min).

6.10. Gastric emptying in rats

Male Sprague-Dawley rats were fasted overnight and were orally administered test compounds or 0.5 w/v% methylcellulose (10 mL/kg). In addition, 0.05 mg/mL of phenol red solution was orally administered in a volume of 1.5 mL at twenty minutes after dosing with the test compounds. Forty minutes after administration of the test compounds, both the cardia and pylorus of the stomach were ligated under anesthesia with sodium pentobarbital (75 mg/kg, i.p.), and then the stomach was isolated without leakage of phenol red. The stomach was cut open and the phenol red solution was drained into a beaker containing 100 mL of 0.1 N

NaOH. Part of the solution was filtrated (pore size: 0.45 μm) and the absorbance at 546 nm was measured to determine the amount of dye remaining in the stomach. Then the gastric emptying rate was calculated by the following formula:

$$\text{Gastric emptying rate} = 100 \times (0.75 - \text{concentration of dye in the stomach})/0.75$$

A value of 0.75 μg/mL was equal to a concentration of 0.05 mg/mL, which was achieved by adding 1.5 mL of phenol red to 100 mL of 0.1 N NaOH. The 50% inhibition rate for gastric emptying by the test compounds was calculated by defining gastric emptying after vehicle administration as 100%.

6.11. Inhibitory activity on LPS-induced TNF-α production in human whole blood

Under the supervision of a physician, blood was collected into a heparinized tube (final concentration: 10 U/mL heparin sodium) from a forearm vein in three healthy male donors. A solution of the test compound (10 μL) dissolved in DMSO was added to 180 μL of whole blood, and the mixture was pre-incubated for 30 min at 37 °C. Then 10 μL of 2 μg/mL of LPS was added and incubated for 6 h at 37 °C, after which the plasma TNF-α concentration was measured with a human TNF-α ELISA kit (DIACLONE). Assays were performed in duplicate at three to four different concentrations of each test compound, and the IC_{50} values were determined.

6.12. Ferret emetic study

Male ferrets (weighting about 1.2 kg) were fasted overnight and test compounds were administered orally. Their behavior was observed throughout a 1 h period after gavage. Results were expressed as the number of animals that vomited relative to the animals tested.

6.13. Pharmacokinetic study

Pharmacokinetic parameters were determined in rats after intravenous (3 mg/mL/kg, iv) or oral (10 mg/5 mL/kg, po) administration. Rats ($n = 3$) were given the test compound intravenously in 20% HP-δ-CD solution, or orally in 0.5% methylcelluslose suspension. Blood samples were collected from jugular veins into a heparinized syringe at 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 h after dosing. Plasma samples were analyzed after extraction of the test compounds by simple liquid–liquid extraction. LC/MS/MS analysis was performed with a Quattro II (Micromass Co., Ltd). HPLC was carried out using a HP1100 (Agilent Co., Ltd) apparatus equipped with YMC-Pack Pro C18 (2.1×150 mm, YMC Corporation).

References and notes

1. Houslay, M. D. *Prog. Nucl. Acid Res. Mol. Biol.* **2001**, *69*, 249.
2. Torphy, T. J. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 351.
3. Essayan, D. M. *J. Allergy Clin. Immunol.* **2001**, *108*, 671.
4. Soderling, S. H.; Beavo, J. A. *Curr. Opin. Cell Biol.* **2000**, *12*, 174.
5. Teixeira, M. M.; Gristwood, R. W.; Cooper, N.; Hellewell, P. G. *Trends Pharmacol. Sci.* **1997**, *18*, 164.
6. Dyke, H. J.; Montana, J. G. *Exp. Opin. Invest. Drugs* **2002**, *11*, 1.
7. Christensen, S. B.; Guider, A.; Forster, C. F.; Gleason, J. G.; Bender, P. E.; Karponski, J. M.; Dewolf, W. E.; Barnette, M. S.; Underwood, D. C.; Griswold, D. E.; Cieslinski, L. B.; Burman, M.; Bochnowicz, S.; Osborn, R. R.; Manning, C. D.; Grous, M.; Hillegas, L. M.; Bartus, J. O.; Ryan, M. D.; Eggleston, D. S.; Haltiwanger, R. C.; Torphy, T. J. *J. Med. Chem.* **1998**, *41*, 821.
8. Schneider, H. H.; Schmiechen, R.; Brezinski, M.; Seidler, J. *Eur. J. Pharmacol.* **1986**, *127*, 105.
9. Duplantier, A. J.; Biggers, M. S.; Chambers, R. J.; Cheng, J. B.; Cooper, K.; Damon, D. B.; Eggler, J. F.; Kraus, K. G.; Marfat, A.; Masamune, H.; Pillar, J. S.; Shirley, J. T.; Umland, J. P.; Watson, J. W. *J. Med. Chem.* **1996**, *39*, 120.
10. Hughes, B.; Owens, R.; Perry, M.; Warvellow, G.; Allen, R. *Drug Discov. Today* **1997**, *2*, 89.
11. Kleinman, E. F.; Campbell, E.; Giordano, L. A.; Cohan, V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, E. R.; Salter, E. D.; Hibbs, T. A.; Dicapua, F. M.; Bordner, J. *J. Med. Chem.* **1998**, *41*, 266.
12. Freyne, E. J.; Diels, G. S.; Matesanz-Ballesteros, M. E.; Diaz-Martinez, A. WO 9950262, 1999.
13. Hersperger, R.; Bray-French, K.; Mazzoni, L.; Muller, T. *J. Med. Chem.* **2000**, *43*, 675.
14. Hersperger, R.; Dawson, J.; Mueller, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 233.
15. Giembycz, M. A. *Expert Opin. Invest. Drugs* **2001**, *10*, 1361.
16. Dyke, H. J.; Montana, J. G. *Expert Opin. Invest. Drugs* **1999**, *8*, 1301.
17. Ajay; Bemis, G. W.; Murcko, M. A. *J. Med. Chem.* **1999**, *42*, 4942.
18. Levin, V. A. *J. Med. Chem.* **1980**, *23*, 682.
19. Norinder, U.; Haeberlein, M. *Adv. Drug Deliv. Rev.* **2002**, *54*, 291.
20. Mickelson, J. W.; Belonga, K. L.; Jacobsen, E. J. *J. Org. Chem.* **1995**, *60*, 4177.
21. Verardo, G.; Giumanini, A. G.; Favret, G.; Strazzolini, P. *Synthesis* **1991**, *6*, 447.
22. Torphy, T. J.; Zhou, H.; Cieslinski, L. B. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1195.
23. Tracey, K. J.; Cerami, A. *Annu. Rev. Med.* **1994**, *45*, 491.
24. Cheng, J. B.; Cooper, K.; Duplantier, A. J.; Eggler, J. F.; Kraus, K. G.; Marshall, S. C.; Marfat, A.; Masamune, H.; Shirley, J. T.; Tickner, J. E.; Umland, J. P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1969.
25. Chauret, N.; Guay, D.; Li, C.; Day, S.; Silva, J.; Blouin, M.; Ducharme, Y.; Yergey, J. A.; Nicoll-Griffith, D. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2149.
26. Schroeder, X. Estimation of the Liquid Molal Volume at the Normal Boiling Point Additive Method. In *An Advanced Treatise on Physical Chemistry*; Partington, J., Ed.; Fundamental Principles: The Properties of Gases; Longmans: Green, New York, 1949.
27. Agoram, B.; Woltosz, W. S.; Bolger, M. B. *Adv. Drug Deliv. Rev.* **2001**, *50*, S41.
28. Nakagawa, N.; Obata, T.; Kobayashi, T.; Okada, Y.; Nambu, F.; Terawaki, T.; Aishita, H. *Jpn. J. Pharmacol.* **1992**, *60*, 217.
29. Nakagawa, N.; Obata, T.; Kobayashi, T.; Okada, Y.; Nambu, F.; Terawaki, T.; Furuya, T.; Muryobayashi, K.;

Sawada, C. C.; Aishita, H. *Eur. J. Pharmacol.* **1993**, *235*, 211.
30. Andres, J. I.; Alonso, J. M.; Diaz, A.; Fernandez, J.; Iturrino, L.; Martinez, P.; Matesanz, E.; Freyne, E. J.; Deroose, F.; Boeckx, G.; Petit, D.; Diels, G.; Megens, A.; Somers, M.; Wauwe, J. V.; Stoppie, P.; Cools, M.; Clerck, F. D.; Peeters, D.; Chaffoy, D. D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 653.
31. Brideau, C.; Staden, C. V.; Sthyler, A.; Rodger, I. W.; Chan, C. C. *Br. J. Pharmacol.* **1999**, *126*, 979.
32. Reeves, M. L.; Leigh, B. K.; England, P. J. *Biochem. J.* **1987**, *241*, 535.