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Design, synthesis, and biological evaluation of new phosphodiesterase type 4 inhibitors

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Abstract—The design, synthesis, and biological evaluation of new phosphodiesterase type 4 inhibitors, which possess new templates instead of a cyclohexane ring, are described. The mode of interaction with the enzyme is discussed based on the structure–activity relationship (SAR) data obtained for the synthesized inhibitors. Furthermore, the roles of three pharmacophores, a catechol moiety, a nitrile moiety, and acidic moieties, are discussed using in silico docking studies. More detailed biological evaluations of selected compounds are also presented.

1. Introduction

Phosphodiesterase type 4 (PDE4) is an enzyme that is mainly expressed in airway smooth muscle, immune cells, and inflammatory cells, where it catalyzes the hydrolysis of the second messenger adenosine 3′,5′-monophosphate (cAMP) to form the inactive nucleotide 5′-monophosphate.[1–4] An anti-inflammatory effect of PDE4 inhibitors has been demonstrated in various animal models,[5,6] and inhibition of PDE4 has been proposed as a new therapeutic approach to the treatment of chronic inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis. Common side effects of PDE4 inhibitors are nausea and emesis. Ariflo™ (cilomilast **1**) is the PDE4 inhibitor for which development is most advanced and it is currently in pre-registration for COPD.[7–9] This drug seems to have an acceptable therapeutic index.

Although many efforts have been made to develop PDE4 inhibitors with acceptable therapeutic indexes, their common side effects of nausea and emesis have still not been overcome. Among the innumerable structures reported so far for PDE4 inhibitors, we focused our attention on the unique arrangement of three pharmacophores (carboxylic acid, benzylic nitrile, and 3-cyclopentyloxy-4-methoxyphenyl moieties) on the cyclohexane ring of Ariflo **1**. It is especially interesting that the *cis*-isomer (Ariflo) exhibits more potent PDE4 inhibitory activity than the corresponding *trans*-isomer, and that the methyl ester of Ariflo shows reduced inhibitory activity relative to Ariflo.[7] Thus, the carboxylic acid moiety may play an important role in increasing the affinity of the inhibitor for the target enzyme. The nitrile group could also play a role in promoting this interaction because the decyanated analog of Ariflo **1** exhibits weaker activity.[7]

To improve the above-described side-effect profiles, we reported on the design and synthesis for two series of PDE4 inhibitors, that is, piperidines[10] and bicyclo[3.3.0]octanes,[11] both of which have these three pharmacophores. The piperidines were designed so as to decrease their penetration into the central nervous system (CNS), while the bicyclo[3.3.0]octanes were expected to improve subtype selectivity by restricting a spatial arrangement of these three pharmacophores.

It is of interest to estimate the probable mode of interaction of the new inhibitors with diverse stereochemistries of these three pharmacophores as shown in Figure 1.

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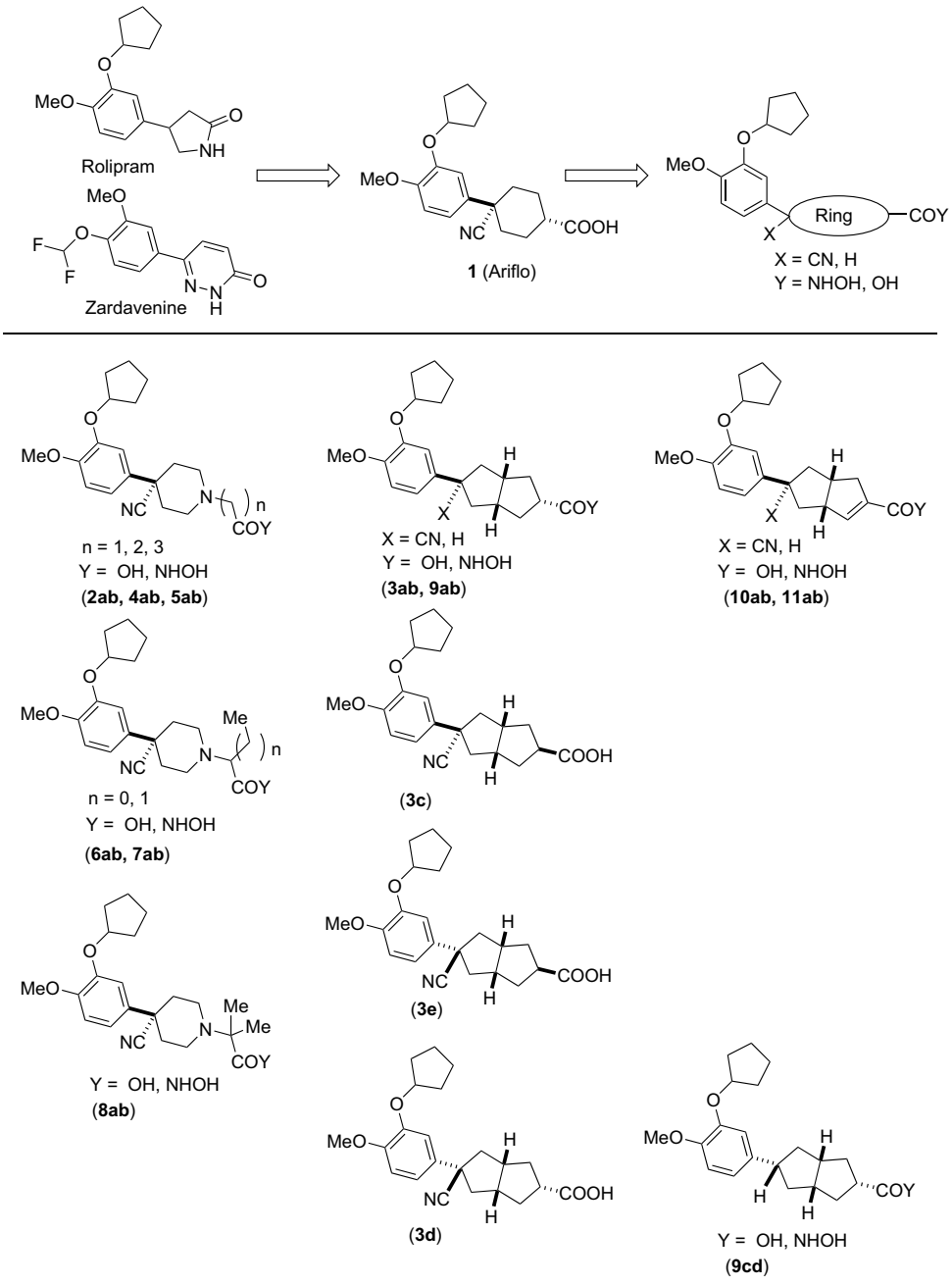


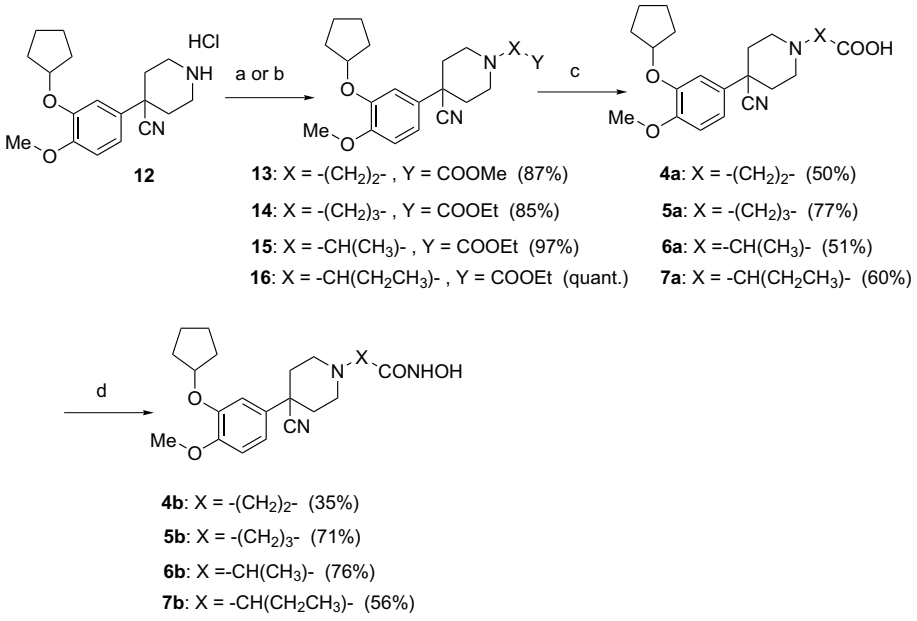
Figure 1. New templates with stereochemical diversity.

Here we report on the probable mode of interaction of newly found PDE4 inhibitors with the target enzyme, based on their SAR and in silico docking studies. Further biological evaluation of selected inhibitors is also presented.

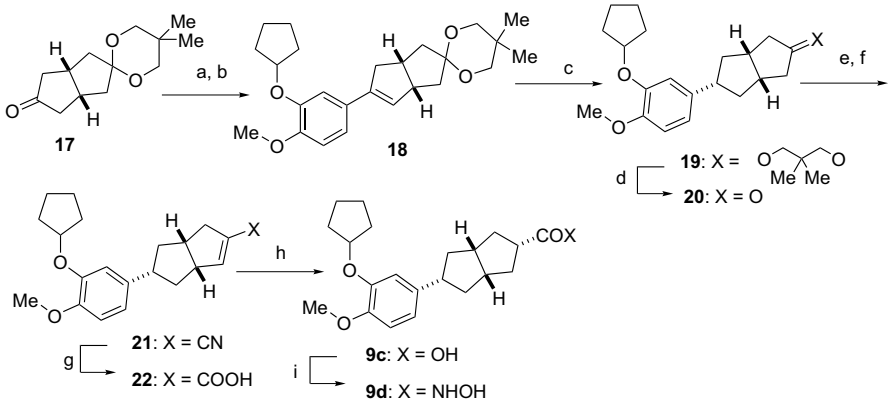
2. Chemistry

Synthesis of **2a**–**b** and **8a**–**b** was reported in the preceding paper,[10] while synthesis of **4**–**7** is outlined in Scheme 1. 1,4-Addition of **12**[10] to methyl acrylate provided **13**. *N*-Alkylation of piperidine **12** with an appropriate alkyl halide in the presence of potassium carbonate afforded **14**–**16**. Alkaline hydrolysis of esters **13**–**16** gave carboxylic acids **4a**–**7a**, respectively. Condensation of **4a**–**7a** with the *O*-protected hydroxylamine, followed by acidic deprotection in methanol, produced the corresponding hydroxamic acids **4b**–**7b**, respectively.

Synthesis of **9c**–**d** is described in Scheme 2. Monoprotected bicyclo[3.3.0]octane-3,7-dione **17**[12,13] was converted to a bicyclo[3.3.0]octene intermediate **18** by nucleophilic addition of the appropriately substituted phenyl lithium, followed by dehydration through methanesulfonylation in the presence of triethyl amine. Catalytic hydrogenation of **18** in the presence of palladium on carbon provided *endo*-configuration **19**, acidic deprotection of which afforded a ketone **20**. Nucleophilic addition of trimethylsilyl cyanide to **20** in the



Scheme 1. Synthesis of compounds **4**–**7**. Reagents: (a) $CH_2CHCOOMe$, Et_3N, THF, 45 °C; (b) alkylbromide, K_2CO_3, DMF, rt to 80 °C; (c) 1 N NaOH, MeOH, rt to 80 °C; (d) EDC, HOBt, DMF, Et_3N, $NH_2OC(CH_3)_2OCH_3$ then 2 N HCl, MeOH.



Scheme 2. Synthesis of compounds **9c**–**d**. Reagents: (a) *n*BuLi, 3-cyclopentyloxy-4-methoxy phenylbromide, $CeCl_3$, THF, −78 °C; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt (56% in two steps); (c) H_2, 10% Pd/C, dioxane (100%); (d) TsOH, acetone (95%); (e) TMSCN, ZnI_2, CH_2Cl_2; (f) $POCl_3$, py, reflux (91% in two steps); (g) 40% KOHaq, $HOCH_2CH_2OH$, 200 °C (64%); (h) H_2, 10% Pd/C, MeOH (54%); (i) EDC, HOBt, DMF, Et_3N, $H_2NOC(CH_3)_2OCH_3$ then 2 N HCl, MeOH (75%).

presence of zinc iodide followed by dehydration afforded the conjugated nitrile **21**, alkaline hydrolysis of which produced a carboxylic acid **22**. Catalytic hydrogenation of **22** in the presence of palladium on carbon resulted in **9c**, which was converted to the corresponding hydroxamic acid **9d** by condensation with *O*-protected hydroxamine followed by acidic deprotection. The stereochemistry of **22** and **9c** was determined by X-ray crystallography (Fig. 2) and NMR analysis (Fig. 3), respectively. As shown in Figure 3, NOESY correlation of the three protons Ha–c, which are attached to the benzylic position, the ring juncture, and the carbon atom adjacent to the carboxylic acid moiety, respectively, was observed.

Synthesis of **9a**–**b** and **11a**–**b** is outlined in Scheme 3. Stereoselective synthesis of **9a**–**b** starting from **18** was one of the challenging problems to be solved because of the *exo*-configuration of the aromatic moiety. However, synthesis was successfully carried out by the catalytic hydrogenation of a key intermediate **24** in the presence of an iridium(I) catalyst,[14–16] as described below. Compound **23** was obtained by acidic deprotection of **18**. First, hydride reduction of **23** from the less hindered side gave the hindered alcohol **24**. Second, stereoselective catalytic hydrogenation of **24** from the hindered side was achieved without contamination by the unwanted *endo*-isomer using an iridium(I) catalyst[14,15] to produce the *exo*-isomer **25** exclusively.[16] The hindered hydroxy residue of **24** served as an excellent scaffold for the iridium(I) catalyst.[16] Oxidation of the alcohol **25** with tetrapropylammonium perruthenate (TPAP) in methylene chloride in the presence of 4-methylmorpholine *N*-oxide gave **26**. Compound **26** was

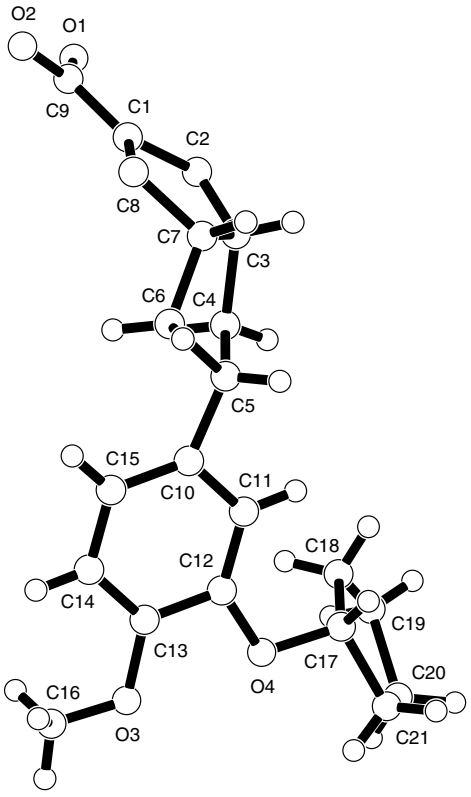


Figure 2. X-ray crystallographic study of **22**.

converted to a conjugated nitrile **27** by formation of a cyanohydrin with TMS cyanide in the presence of zinc iodide, followed by dehydration with phosphorus oxychloride in the presence of pyridine. Alkaline hydrolysis of **27** gave a conjugated carboxylic acid **11a** (Fig. 4), which was converted to the corresponding hydroxamic acid **11b** according to the same procedures as described for the conversion of **9c** to **9d**. Stereochemistry of **11a** was determined by comparison of its ^{1}H NMR spectrum with that of **22**. The two spectra were clearly different with regard to the δ values of the protons at C-4 (H-4αβ) and C-6 (H-6αβ). Catalytic hydrogenation of **11a** produced saturated carboxylic acid **9a** (Fig. 5), which was converted to the corresponding hydroxamic acid analog **9b** by the method described above. Stereochemistry of **9a** was determined by comparison of its ^{1}H NMR spectrum with that of **9c**. The two spectra were clearly different with regard to the δ values of the protons at C-2 (H-2), C-4 (H-4β), and C-6 (H-6β). Synthesis of **3a**–**e** was reported in the preceding paper.[11]

Synthesis of **10a**–**b** from **28**[11] is described in Scheme 4. Trapping an enolate anion prepared from **28** with *N*,*N*-bis(trifluoromethylsulfonyl)aniline afforded an enol triflate **29**. Insertion reaction of a carbon monoxide into **29** in the presence of the palladium catalyst and methanol resulted in an α,β-unsaturated ester **30**, alkaline hydrolysis of which produced a carboxylic acid **10a**. Compound **10a** was converted to the corresponding hydroxamic acid **10b** according to the same procedures as described above.

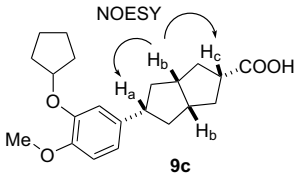
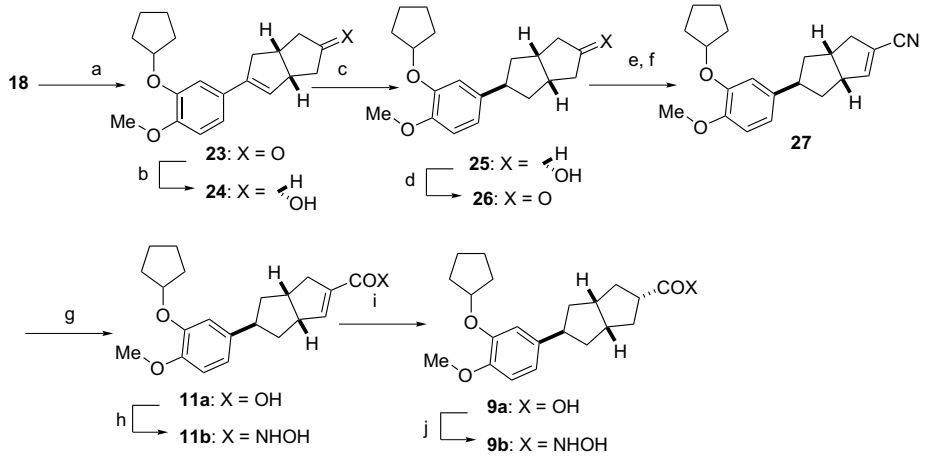


Figure 3. Observed key NOESY correlations of **9c**.

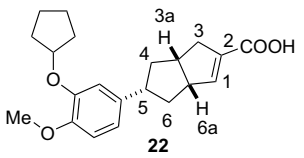
3. Results and discussion

3.1. Inhibition of PDE4

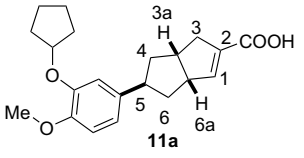
All the test compounds listed in Tables 1–4 were synthesized and evaluated for their ability to inhibit PDE4 prepared from U937 cells[17] (derived from human monocytes). The assay data are expressed as IC_{50} values,



Scheme 3. Synthesis of compounds **11a**–**b** and **9a**–**b**. Reagents: (a) TsOH, acetone (90%); (b) $NaBH_4$, MeOH, THF, 0 °C (100%); (c) H_2, CH_2Cl_2, Ir(cod)py(PCy_3)PF_3 (89%); (d) TPAP, NMO, CH_2Cl_2, MS4A (92%); (e) TMSCN, ZnI_2, CH_2Cl_2; (f) $POCl_3$, pyridine, 90 °C (55% in two steps); (g) 40% KOHaq, $HOCH_2CH_2OH$, 200 °C (93%); (h) EDC, HOBt, DMF, Et_3N, $H_2NOC(CH_3)_2OMe$ then 2 N HCl, MeOH (56%); (i) H_2, 10% Pd/C, MeOH (81%); (j) EDC, HOBt, DMF, Et_3N, $H_2NOC(CH_3)_2OCH_3$ then 2 N HCl, MeOH (70%).

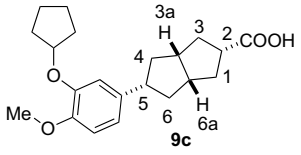


^{1}H NMR δ (ppm)
3.39 (1H, m, H-6a)
2.91 (1H, m, H-5)
2.90-2.75 (2H, m, H-3a, 3β)
2.50-2.35 (2H, m, H-3α, 6β)
2.30 (1H, m, H-4β)
1.98-1.70 (7H, m, *c*Pen X 3/4 + COOH)
1.70-1.50 (2H, m, *c*Pen X 1/4)
1.45-1.27 (2H, m, H-4α, 6α)

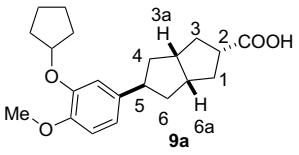


^{1}H NMR δ (ppm)
3.52 (1H, m, H-6a)
3.10-2.89 (3H, m, H-5, 3a, 3β)
2.39 (1H, m, H-3α)
2.00-1.74 (10H, m, H-6αβ,4αβ, *c*Pen X 3/4)
1.68-1.53 (3H, m, *c*Pen X 1/4 + COOH)

Figure 4. ^{1}H NMR comparison of **11a** and **22**.

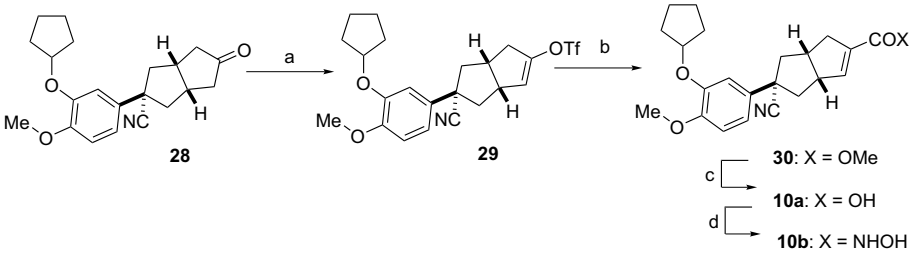


^{1}H NMR δ (ppm)
3.08 (1H, m, H-5)
2.92 (1H, m, H-2)
2.66-2.55 (2H, m, H-3a, 6a)
2.30-2.19 (4H, m, H-1β, 3β, 4β, 6β)



^{1}H NMR δ (ppm)
3.10 (1H, m, H-5)
2.73-2.57 (3H, m, H-2, 3a, 6a)
2.34-2.24 (2H, m, H-1β, 3β)

Figure 5. ^{1}H NMR comparison of **9a** and **9c**.



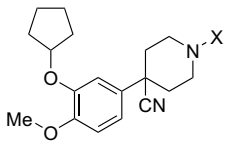
Scheme 4. Synthesis of compound **10a**–**b**. Reagents: (a) LiHMDS, Tf_2NPh, THF, −78 to 0 °C; (b) $Pd(OAc)_2$, Et_3N, PPh_3, CO, MeOH, DMF (94% in two steps); (c) 2 N NaOH, MeOH, THF (85%); (d) EDC, HOBt, Et_3N, $NH_2OC(CH_3)_2OMe$, DMF then 2 N HCl, MeOH (56%).

that is, the test compound concentration that achieved 50% inhibition of PDE4 relative to the effect of the vehicle. In preceding papers,[10,11] we reported on the discovery of two new series of PDE4 inhibitors with therapeutic potential, starting from the chemical modification of Ariflo (Fig. 1). During those studies, the carboxylic acid or hydroxamic acid in addition to another two pharmacophores (a catechol and a benzylic nitrile) were found to play an important role in the interaction of these inhibitors with the target enzyme. In the present report, we discuss the critical role of these pharmacophores (carboxylic/hydroxamic acid, benzylic nitrile, and catechol) based on SAR data and in silico docking studies for newly found inhibitors.

As shown in Table 1, C1 and C2 homologation of inhibitors **2a**–**b** gave **4a**–**b** and **5a**–**b**, respectively, with a gradual decrease in inhibitory activity for each series. Thus, the chain length between the nitrogen of the piperizine ring and the acidic group was found to be optimized at the monomethylene moiety, as illustrated by **2a**–**b**, which shows the strongest activity in the SAR study.

Secondly, substitution of the α-position of the carbonyl group to block rapid hydrolysis of hydroxamic acid was carried out. Introduction of a methyl group at the α-position of **2a**–**b** afforded **6a**–**b**, respectively, with more than 4.5-fold and 5-fold decreases in inhibitory activity, respectively. Replacement of the methyl moiety of **6a**–**b** with a more hindered ethyl moiety produced **7a**–**b**, respectively, with a further decrease in inhibitory activity for each series. Introduction of another methyl moiety at the α-position of **6a**–**b** gave **8a**–**b**, respectively, also

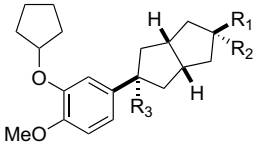
Table 1. Activity profiles of piperidine analogs



Compd	X =	PDE4[a] IC_{50} (nM)	Inhibition of TNF-α[b] ID_{50} (mg/kg, po)
2a (Y=OH)	COY	66	(74%)[c]
2b (Y=NHOH)		0.080	0.04
4a (Y=OH)	$-(CH_2)_2COY$	>300	NE[d]
4b (Y=NHOH)		18	NE[d]
5a (Y=OH)	$-(CH_2)_3COY$	>300	NT[e]
5b (Y=NHOH)		>100	NT[e]
6a (Y=OH)	Me COY	>300	NT[e]
6b (Y=NHOH)		0.41	0.4
7a (Y=OH)	Me COY	>1000	NT[e]
7b (Y=NHOH)		37	NT[e]
8a (Y=OH)	MeMe COY	>1000	NT[e]
8b (Y=NHOH)		34	NT[e]

[a] Inhibition of PDE4 prepared from U937 cells (a cell line derived from human monocytes). IC_{50} values 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] Not effective at 1 mg/kg, po.
[e] Not tested.

Table 2. Activity profiles of bicyclo[3.3.0]octane analogs

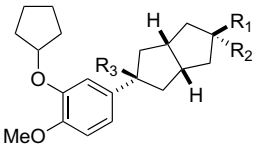


Compd	R_1	R_2	R_3	PDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
3a	H	COOH	CN	9.0	0.4
3b	H	CONHOH	CN	9.7	(52%)[b]
3c	COOH	H	CN	150	3.8
9a	H	COOH	H	150	(79%)[b]
9b	H	CONHOH	H	5.8	(80%)[b]

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

showing a further decrease in inhibitory activity. Thus, as the acidic groups became more sterically hindered, the inhibitory activity decreased. These findings strongly support our hypothesis that the structures around the acidic group are critical for the interaction between these inhibitors and the target enzyme. The SAR data indicated that inhibitory activity is impaired if the interaction of the acidic groups with the enzyme is disturbed by steric repulsion and/or incorrect positioning. The marked increase in the inhibitory activity of the

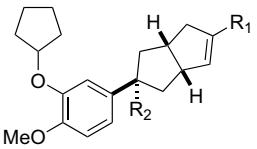
Table 3. Activity profiles of bicyclo[3.3.0]octane analogs



Compd	R_1	R_2	R_3	PDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
3d	H	COOH	CN	>300	NT[c]
3e	COOH	H	CN	>300	NT[c]
9c	H	COOH	H	80	2
9d	H	CONHOH	H	26	(37%)[b]

[a] See corresponding footnotes from Table 1.
[b] Inhibition % at 3 mg/kg, po.
[c] Not tested.

Table 4. Activity profiles of bicyclo[3.3.0]octene analogs



Compd	R_1	R_2	PDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
10a	COOH	CN	42	0.6
10b	CONHOH	CN	0.32	(41%)[b]
11a	COOH	H	100	(84%)[b]
11b	CONHOH	H	2.5	0.2

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

hydroxamic acid analogs relative to their corresponding carboxylic acid analogs may be due to the greater affinity of the hydroxamic acid group for the metal ion at the catalytic site of PDE relative to that of the carboxylic acid group.[18]

Among the four possible isomers (**3a**, **3c**, **3d**, and **3e**) of bicyclo[3.3.0]octane with three pharmacophores, **3a** showed the most strong PDE4 inhibitory activity. As shown in Table 2, relatively more active bicyclo[3.3.0]octane analogs **3a**–**b**[11] and their corresponding decyanated analogs **9a**–**b** were synthesized and evaluated. The cyanated analog **3a** exhibited more potent activity than the decyanated analog **9a**. In fact, an almost 16-fold increase in inhibitory activity was obtained by the conversion of **9a** into **3a**. This clearly shows that the newly introduced benzylic nitrile was useful for promoting the interaction of the inhibitor with the enzyme. However, the hydroxamic acid analog **3b** unexpectedly showed no increase in inhibitory activity relative to that of the corresponding carboxylic acid analog **3a**. Additionally, **3b** did not show an increased activity relative to **9b**. This suggested that there may be two different modes of interaction between the inhibitor and the enzyme. With regard to the series of carboxylic acid analogs **3a** and **9a**, the benzylic nitrile plays an important

role in the increase of activity. Alternatively, for the series of hydroxamic acid analogs **3b** and **9b**, the hydroxamic acid group plays the dominant role in the increase in activity.

As shown in Table 3, *endo*-phenyl isomers **3d**–**e** and their decyanated analogs **9c**–**d** were synthesized and evaluated. Cyanated analogs **3d** and **3e** did not show PDE4 inhibitory activity at a concentration of 300 nM, regardless of the stereochemistry of their carboxylic acid residue, while decyanated analogs **9c** and **9d** exhibited IC_{50} values of 80 and 26 nM, respectively. These findings support our speculation that the benzylic nitrile has an appropriate interaction site in the enzyme and prevents an inhibitor from docking into the enzyme if it has undesirable stereochemistry. Regarding decyanated analogs **9c** and **9d**, which show weaker in vitro activity than the cyanated analogs **3a**–**b**, respectively, they are considered to have relatively more flexible interactions with the enzyme regardless of their stereochemistry.

These analyses are also consistent with the SAR study of α,β-unsaturated analogs **10a**–**b** and **11a**–**b**. As can be seen in Table 4, **10a** was more potent than the decyanated analog **11a**. Hydroxamic acid analog **10b** was also much more potent than expected, presumably because of the better positioning of the hydroxamic acid group relative to those of **3b**, **9b**, and **11b**. The smaller increase in inhibitory activity by conversion of **9a** to **9b** relative to conversion of **11a** to **11b** (25-fold vs 40-fold) was ascribed to relatively less favorable positioning of the hydroxamic acid group.

3.2. In silico docking study

Molecular modeling and graphic manipulations of the complexes consisting of the enzyme and the inhibitor were performed using the INSIGHT II (ver. 2000) software packages, running on a Silicon Graphics OCTANE work station (Figs. 6A and B, 7A and B and 8A and B). Proposed main interactions of the PDE4/**2a** and PDE4/**2b** complexes are briefly described in Figure 9. Model building and conformational analysis were accomplished with the CHARMm (ver. 23.2) force field.[19] Adopted-Basis Newton Rapson method was applied for CHARMm minimization step. During the

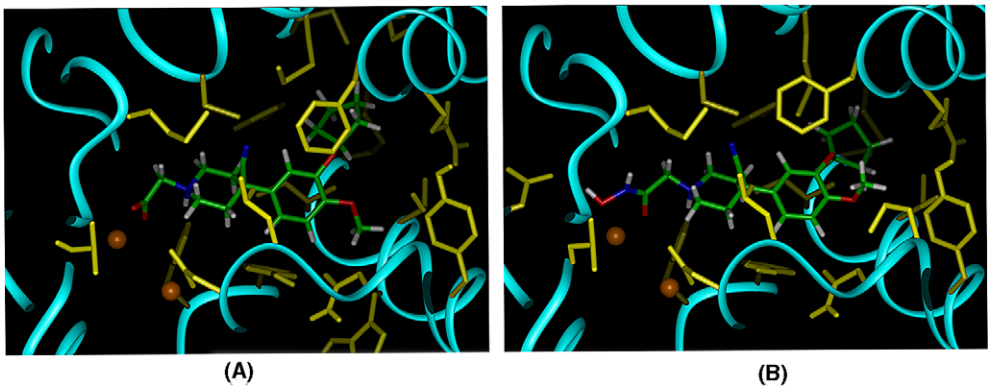


Figure 6. (A, B) The carboxylic acid residue can coordinate with Mg^{2+} without distortion of the piperidine ring. A hydrogen bond is formed between the tertiary amine part of the piperidine ring and the nitrogen atom of the imidazole ring. Replacement of the carboxylic acid residue of **2a** with a hydroxamic acid residue afforded **2b** with the increased bidentate coordination with Mg^{2+}. As a result, **2b** exhibited a remarkable increase in its inhibitory activity.

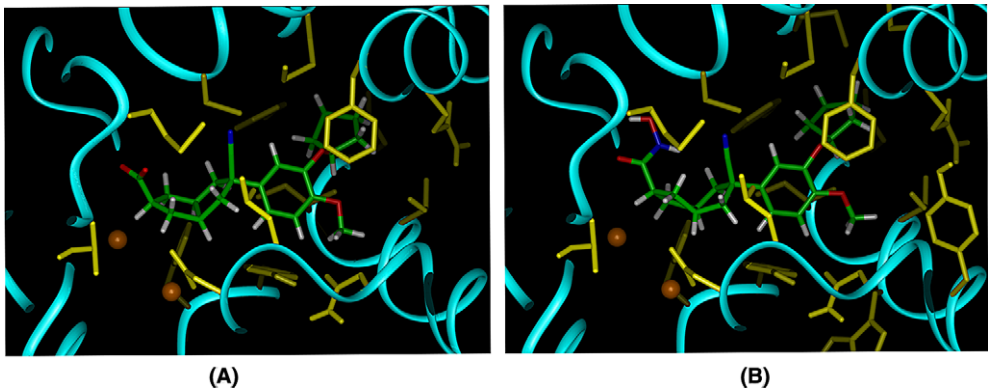


Figure 7. (A, B) Severe distortion of the bicyclo[3.3.0]octane ring of **3a** is needed for the carboxylic acid residue to coordinate with Mg^{2+} because the carboxylic acid residue has a relatively more hindered endo-stereochemistry. As a result, the corresponding hydroxamic acid analog **3b** did not show increased inhibitory activity.

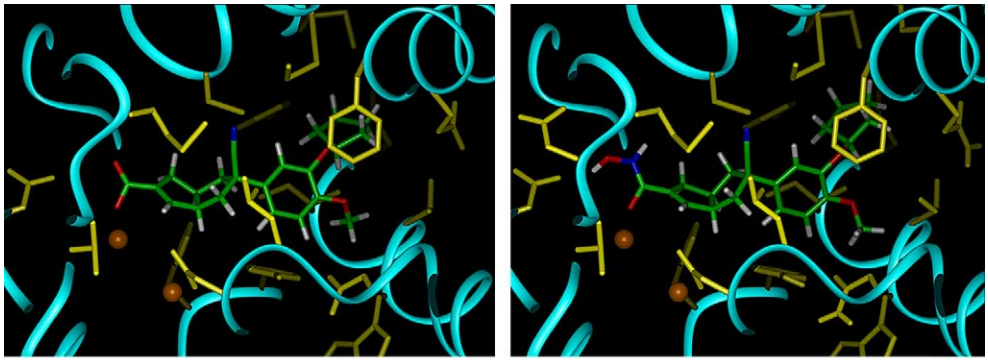


Figure 8. (A, B) The carboxylic acid residue of **10a** can coordinate with the Mg^{2+} without distortion of the bicyclo[3.3.0]octane ring. Thus the corresponding hydroxamic acid analog **10b** can coordinate strongly with Mg^{2+}. Consequently, a remarkable increase (nearly 100-fold) in the inhibitory activity was obtained.

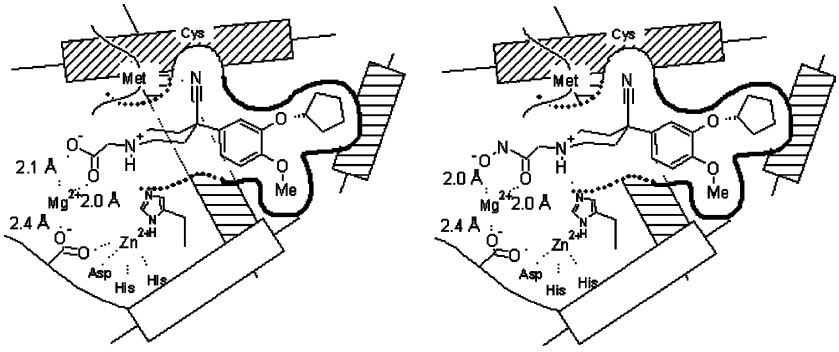


Figure 9. Scheme of the main interactions observed in the molecular modeling of the PDE4/**2a** and PDE4/**2b** complexes.

calculations, the inhibitor atoms and side chain atoms, which directly interact with the inhibitors were refined, and other atoms including metal ions and water molecules were fixed at the positions reported in the crystal structure.[20–23]

Manipulations regarding crystallographic analysis of rolipram/PDE4D complex (PDB ID:1Q9M)[23] and zardaverine/PDE4D complex (PDB ID:1OYN)[22] were applied to our in silico docking study because of the structural analogy among rolipram, zardaverine, and the new inhibitors (Fig. 1). The aromatic moiety of these inhibitors was speculated to dock into the same site of the enzyme as that of rolipram and zardaverine does.

Modeling was performed so that the aromatic part of the inhibitors overlapped the corresponding part of the reported PDE-bound conformation of rolipram. As a result, the benzylic nitrile was exposed to the solvent region.

The Apo-PDE4B crystal structure (PDB ID:1F0J) was reported by Xu et al.,[21] and two independent PDEs were in the crystal structure. In one of these PDE structures, the substrate binding site was covered by an α-helix. Xu et al. speculated that the conformation of this helix was caused by its crystal packing, however our inhibitors are nicely docked into this structure. A modeling study was conducted regarding this α-helix as another recognition site, the presence of which was strongly suggested by the in vitro SAR study of nitrile or nonnitrile compounds, as described in Tables 2–4. Docking of the inhibitors into Apo-PDE4B (PDB ID:1F0J) followed by optimization of the amino acid side chains around the recognition site was performed. The α-helix has a small hydrophobic pocket consisting of methionine, cysteine, and valine for the benzylic nitrile to bind. The cyclopentyloxy moiety occupies the region, which can avoid the methionine residue attached to the α-helix, while the aromatic moiety occupies the predicted region.

The cyclopentyloxy moiety is slightly rotated from the corresponding region in the PDE4D/rolipram complex structure (PDB ID:1OYN).[22] This displacement can avoid the methionine residue attached to the α-helix while the aromatic moiety occupies the same region as that in the PDE4D/rolipram complex structure.

The acidic residues of the inhibitors were postulated to coordinate with a metal cation in one of the recognition sites of the enzyme. However, the most plausible target metal cation, Zn^{2+}, is already coordinated to the imidazoles of the two histidine residues and the carboxylic acid of the aspartic acid residue. Thus, Zn^{2+} is not thought to be a target metal, which acidic residues can coordinate with, while another metal cation, Mg^{2+}, which is located

close to Zn^{2+} and is loosely bound to aspartic acid residues of the enzyme, is considered to be a possible target metal instead. The role of this Mg^{2+} cation is reported in detail.[20] As shown in Figure 6A and B, two oxgen atoms of the acidic residues of compounds **2a**–**b** are coordinated with Mg^{2+} ion. As shown in Figure 9, the nitrogen atoms of piperidines **2a**–**b** were expected to perform the hydrogen bond to the imidazole of the histidine residue (His160). Consequently, the conformation of the piperidine ring of compound **2a** was slightly more distorted than that of compound **2b**. Docking study of **3a**–**b** was described in Figure 7A and B. Both of the acidic residues of **3a**–**b** occupy the space, which is not able to coordinate with Mg^{2+} ion. Docking study of **10a**–**b** was also conducted as described in Figure 8A and B. Acidic residues of **10a**–**b** are able to occupy closer position to Mg^{2+} ion relative to those of **3a**–**b** because of the α,β-unsaturated flat structures.

Thus, modeling and graphic manipulations of the complexes PDE4/**2a**, PDE4/**2b**, PDE4/**3a**, PDE4/**3b**, PDE4/**10a**, and PDE4/**10b** were performed as shown in Figures 6A and B, 7A and B, and 8A and B, respectively.

3.3. Inhibition of TNF-α production

Test compounds, which were selected based on their LPDE4 inhibitory activity, were also evaluated for their potency to inhibit lipopolysaccharide (LPS)-induced production of tumor necrosis factor-α (TNF-α) in rats.[24] The results were expressed as ID_{50} values, that is, the dose that resulted in 50% inhibition relative to the vehicle. In contrast to our prediction based on their in vitro results, a series of hydroxamic acid analogs did not always exhibit more potency than their corresponding carboxylic acid analogs in this assay, presumably because of their pharmacodynamic problems with oral absorption, metabolism etc. A series of carboxylic acid analogs also exhibited results that were distinct from their LPDE4 inhibitory activity. Hydroxamic acid analogs **2b**, **6b**, and **11b** exhibited relatively potent activity while other analogs **9b**, **9d**, and **10b** showed unexpectedly weak potency in comparison to their potent LPDE4 inhibitory activity. With regard to carboxylic acid analogs, compounds **9a** and **10a** exhibited unexpectedly potent in vivo activity in comparison to their relatively weak in vitro activity.

3.4. Further biological evaluations

Further biological evaluations of **2b** and **3a** have already been reported in our previous paper.[10,11]

Further biological evaluations of **2b**, **3a**,[10,11] **10a**, and **11b**, which were selected based on their oral activity against LPS-induced TNF-α production assay, were carried out as shown in Table 5. These compounds were evaluated for their potency to inhibit the slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction.[25,26] The results were obtained as ID_{50} values, that is, the dose that resulted in 50% inhibition relative to the vehicle. These compounds were also evaluated as to the concentration required to inhibit TNF-α production in human whole blood (HWB)[27] to estimate their clinical potential. The results of the assays were obtained as IC_{50} values, that is, the test compound concentration that resulted in 50% inhibition relative to the vehicle. The potency of these compounds for inhibiting SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs was not always consistent with their potency for inhibiting LPS-induced TNF-α production in rats, probably because of differences in the pharmacokinetics due to cross-species comparison. Compound **3a**, the stereochemistry of which is close to that of Ariflo, showed 50% inhibition of SRS-A-induced bronchoconstriction at ID_{50} values of 9.6 mg/kg while it showed an ID_{50} value for TNF-α production of 0.4 mg/kg and an IC_{50} value for TNF-α production in HWB of 21 μM. Compounds **2b** and **11b** demonstrated 50% inhibition of SRS-A-induced bronchoconstriction at ID_{50} values of 0.3 and 0.2 mg/kg, respectively, while their ID_{50} values for TNF-α production were 0.043 and 0.2 mg/kg, respectively. With regard to gastric emptying in rats,[28] the inhibitory activities of **2b** and **11b** were weak in comparison to their much more potent IC_{50} values relative to Ariflo **1** for LPS-induced TNF-α

Table 5. Further biological evaluation of **2b**, **3a**, **10a**, and **11b**

Compd	Inhibition of 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 in HWB[d] IC_{50} (μM)	Ferret emesis[e] (vomiting/tested) (mg/kg, po)		
					1	3	10
1 (Ariflo)	4.5	1.7	5.7	18	NT[g]	NT[g]	NT[g]
2b	0.3	0.04	0.3	0.0089	2/2	NT[g]	NT[g]
3a	9.6	0.4	(73%)[f]	21	NT[g]	0/3	0/3
10a	7.7	0.6	(38%)[f]	NT[g]	NT[g]	NT[g]	NT[g]
11b	0.2	0.2	(71%)[f]	0.043	0/1	0/2	NT[g]

[a] Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs (n = 3–6); OVA challenge 0.15 mg/kg 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} values represent a mean of n = 3.
[e] Vomiting test in fasted ferrets.
[f] Inhibition % at 10 mg/kg, po.
[g] Not tested.

production in HWB. Based on the above-mentioned biological data, **2b** and **11b** are likely to have improved therapeutic potential and an improved side-effect profile. In a ferret emesis model, which is known as an established evaluation method for the side effects of PDE4 inhibitors, **3a** and **11b** did not cause emesis up to oral doses of 10 and 3 mg/kg, respectively, although **2b** caused emesis at an oral dose of 1 mg/kg. Regarding inhibition of the SRS-A-induced bronchoconstriction assay, **10a** showed the highest ID_{50} value (7.7 mg/kg, po), which was more than 10-fold less potent than that of LPS-induced TNF-α production in rats, while it showed the least potent inhibition against gastric emptying in rats among the compounds **2b**, **3a**, **10a**, and **11b** selected for further evaluation.

4. Conclusion

In summary, the possible mode of interaction of the new inhibitors of PDE4 with the target enzyme was discussed based on both the SAR of the synthesized compounds and their in silico docking studies. The cyanated analogs seem to interact with the target enzyme via three pharmacophores, which are a catechol moiety, a benzylic nitrile moiety, and acidic moieties. There seem to be two different modes of interaction between these inhibitors and PDE4. With the carboxylic acid analogs, the benzylic nitrile plays a dominant role in the increase in PDE4 inhibitory activity, while the hydroxamic acid group plays a dominant role in the hydroxamic acid analogs. Appropriate spatial positioning and reduced steric hindrance around the acidic group were found to be critical for achieving potent inhibitory activity, as illustrated by the SAR data in Tables 1–4. As illustrated in Tables 2 and 3, decyanated carboxylic acid analogs **9a** and **9c**, which could not have an interaction with the enzyme via a benzylic nitrile moiety, appeared to have a relatively loose interaction with the enzyme regardless of their stereochemistry. The hydroxamic acid group, a well-known metal chelator, achieved a striking increase in PDE4 inhibitory activity in both the analogs.

Further biological evaluation of selected compounds based on their inhibition of TNF-α production assay identified another two orally active inhibitors, **2b** and **11b**, with therapeutic potential.

5. Experimental

5.1. General procedure

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. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were obtained on a PerSeptive Voyager Elite 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}).

5.2. Synthesis of compounds 4a–7a and 4b–7b

5.2.1. Methyl 3-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanate (13). To a stirred solution of **12** (0.45 g, 1.34 mmol) in THF (5.0 mL) were added Et_3N (0.38 mL, 2.68 mmol) and methylacrylate (0.36 mL, 4.0 mmol). After being stirred at 45 °C for 24 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and then brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1/1) to give **13** (0.45 g, 1.17 mmol, 87%) as colorless oil: TLC $R_f = 0.42$, (*n*-hexane/EtOAc, 2/3); MS (APCI, Pos. 20 V) $m/z = 387$ $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02–6.97 (m, 2H), 6.87–6.83 (m, 1H), 4.83–4.76 (m, 1H), 3.85 (s, 3H), 3.70 (s, 3H), 3.05–2.96 (m, 2H), 2.81 (t, $J = 7.2$ Hz, 2H), 2.59–2.49 (m, 2H), 2.55 (t, $J = 7.2$ Hz, 2H), 2.12–2.02 (m, 4H), 2.02–1.76 (m, 6H), 1.70–1.50 (m, 2H).

5.2.2. Ethyl 4-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}butanate (14) (Method A). To a stirred solution of **12** (0.45 g, 1.34 mmol) in DMF (10 mL) were added K_2CO_3 (554 mg, 4.01 mmol) and ethyl 4-bromobutyrate (0.21 mL, 1.47 mmol). After being stirred at 50 °C for 24 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and then brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2/1–1/1) to give **14** (0.470 mg, 1.13 mmol, 85%) as a colorless oil: TLC $R_f = 0.48$ (*n*-hexane/EtOAc, 2/3); MS (APCI, Pos. 20 V) $m/z = 415$ $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02–6.98 (m, 2H), 6.87–6.84 (m, 1H), 4.83–4.76 (m, 1H), 4.15 (q, $J = 7.2$ Hz, 2H), 3.85 (s, 3H), 3.05–2.97 (m, 2H), 2.52–2.43 (m, 4H), 2.36 (t, $J = 7.2$ Hz, 2H), 2.12–2.03 (m, 4H), 2.01–1.76 (m, 8H), 1.68–1.55 (m, 2H), 1.27 (t, $J = 7.2$ Hz, 3H).

5.2.3. Ethyl 2-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanate (15). The title compound was prepared from the corresponding alkylbromide according to Method A to give a colorless oil: Yield 97%; TLC $R_f = 0.46$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05–6.95 (m, 2H), 6.85 (d,

J = 8.7 Hz, 1H), 4.79 (m, 1H), 4.30–4.15 (m, 2H), 3.84 (s, 3H), 3.37 (q, J = 7.4 Hz, 1H), 3.10–2.95 (m, 2H), 2.90–2.65 (m, 2H), 2.20–2.00 (m, 4H), 2.00–1.80 (m, 6H), 1.75–1.50 (m, 2H), 1.35 (d, J = 7.4 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H).

5.2.4. Ethyl 2-{4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}butanate (16). The title compound was prepared from the corresponding alkylbromide according to Method A to give a colorless oil: Yield quant; TLC R_f = 0.55 (n-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) m/z = 415 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02–6.98 (m, 2H), 6.88–6.83 (m, 1H), 4.83–4.76 (m, 1H), 4.24 (q, J = 7.4 Hz, 2H), 3.84 (s, 3H), 3.16–3.10 (m, 1H), 3.04–2.95 (m, 2H), 2.95–2.84 (m, 1H), 2.79–2.69 (m, 1H), 2.13–2.01 (m, 4H), 2.00–1.54 (m, 10H), 1.26 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H).

5.2.5. 3-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoic acid (4a) (Method B). To a stirred solution of **13** (451 mg, 1.17 mmol) in MeOH (10 mL) was added 1 N NaOH (2.33 mL, 2.33 mmol). After being stirred at room temperature for 3.5 h, the reaction mixture was acidified with 1 N HCl (2.33 mL, 2.33 mmol), and extracted with EtOAc/THF. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was triturated with EtOAc/Et_2O/n-hexane to give **4a** (217 mg, 0.58 mmol, 50%) as a white powder: TLC R_f = 0.43 ($CHCl_3$/MeOH, 9/1); IR (KBr) 3431, 2957, 2872, 2657, 2570, 2231, 1731, 1608, 1519, 1455, 1416, 1373, 1336, 1258, 1171, 1147, 1133, 1073, 1026, 993; MS (APCI, Neg. 20 V) m/z = 371 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03–6.95 (m, 3H), 4.87–4.80 (m, 1H), 3.74 (s, 3H), 3.31 (br, 1H), 3.24–3.19 (m, 2H), 2.96–2.85 (m, 2H), 2.66–2.53 (m, 4H), 2.27–2.20 (m, 2H), 2.16–2.05 (m, 2H), 1.96–1.80 (m, 2H), 1.75–1.63 (m, 4H), 1.63–1.48 (m, 2H); Anal. found $C_{21}H_{28}N_2O_4 \cdot 3/2H_2O$ (C, H, N).

5.2.6. 4-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}butanoic acid (5a). The title compound was prepared according to Method B to give a white powder: Yield 77%; TLC R_f = 0.55 ($CHCl_3$/MeOH, 8/1); IR (KBr) 3449, 2958, 2842, 2236, 1944, 1696, 1593, 1519, 1474, 1445, 1421, 1363, 1336, 1255, 1176, 1134, 1025, 963; MS (APCI, Neg. 20 V) m/z = 385 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03–6.94 (m, 3H), 4.87–4.81 (m, 1H), 3.74 (s, 3H), 3.31 (br, 1H), 3.01–2.96 (m, 2H), 2.44–2.38 (m, 2H), 2.33–2.21 (m, 4H), 2.13–2.08 (m, 2H), 2.00–1.94 (m, 2H), 1.92–1.83 (m, 2H), 1.76–1.63 (m, 6H), 1.62–1.54 (m, 2H); Anal. found $C_{22}H_{30}N_2O_4 \cdot 1/4H_2O$ (C, H, N).

5.2.7. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}propanoic acid (6a). The title compound was prepared according to Method B to give a white powder: Yield 51%; TLC R_f = 0.67 ($CHCl_3$/MeOH/AcOH, 15/2/1); IR (KBr) 3439, 2957, 2231, 1615, 1521, 1446, 1397, 1366, 1261, 1151, 1022, 993; MS (APCI, Neg. 20 V) m/z = 371 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03–6.94 (m, 3H), 4.88–4.80 (m, 1H), 3.73 (s, 3H), 3.30 (q, J = 7.1 Hz, 1H), 4.00–2.70 (br, 1H), 3.00–2.90 (m, 2H), 2.78–2.68 (m, 1H), 2.66–2.56 (m, 1H), 2.13–2.04 (m, 2H), 2.02–1.80 (m, 4H), 1.76–1.63 (m, 4H), 1.63–1.50 (m, 2H), 1.19 (d, J = 7.1 Hz, 3H); Anal. found $C_{21}H_{28}N_2O_4 \cdot HCl$ (C, H, N).

5.2.8. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}butanoic acid (7a). The title compound was prepared according to Method B to give a white powder: Yield 60%; TLC R_f = 0.52 ($CHCl_3$/MeOH, 9/1); IR (KBr) 3457, 2957, 2231, 1619, 1523, 1467, 1446, 1419, 1374, 1303, 1262, 1152, 1107, 1065, 1022, 957; MS (APCI, Neg. 20 V) m/z = 385 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.02–6.93 (m, 3H), 4.87–4.80 (m, 1H), 3.72 (s, 3H), 3.31 (br, 1H), 3.05 (t, J = 7.4 Hz, 1H), 2.97–2.86 (m, 2H), 2.81–2.70 (m, 1H), 2.64–2.54 (m, 1H), 2.13–2.04 (m, 2H), 1.99–1.80 (m, 4H), 1.75–1.63 (m, 6H), 1.63–1.51 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H); Anal. found $C_{22}H_{30}N_2O_4 \cdot 2/3HCl \cdot 1/3H_2O$ (C, H, N).

5.2.9. 3-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxypropanamide hydrochloride (4b) (Method C). To a stirred solution of **4a** (378 mg, 1.01 mmol) in DMF (5.0 mL) were added Et_3N (0.21 mL, 1.5 mmol), EDC·HCl (292 mg, 1.52 mmol), HOBt (206 mg, 1.52 mmol), and (1-methoxy-1-methylethyl)oxyamine (533 mg, 5.07 mmol). After being stirred at room temperature for 24 h, the mixture was poured into H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/MeOH, 1/0–20/1) to give an amide (190 mg, 0.410 mmol) as a pale yellow oil: TLC R_f = 0.50 (EtOAc/MeOH, 15/1).

To a stirred solution of an amide (190 mg, 0.410 mmol) in MeOH (4.0 mL) was added 2 N HCl (0.25 mL, 0.50 mmol). After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was triturated with Et_2O/MeOH to give **4b** (149 mg, 0.350 mmol, 35% in two steps) as a white powder: TLC R_f = 0.52 ($CHCl_3$/MeOH, 9/1); IR (KBr) 3466, 3141, 2954, 2665, 2611, 2596, 2243, 1670, 1607, 1594, 1518, 1469, 1442, 1387, 1369, 1337, 1320, 1290, 1247, 1217, 1163, 1139, 1077, 1025, 992; MS (FAB, Pos.) m/z = 388 $(M+H)^+$; ^{1}H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.23 (d, J = 2.2 Hz, 1H), 7.11 (dd, J = 8.5, 2.2 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.53 (br, 3H), 4.86–4.79 (m, 1H), 3.72 (s, 3H), 3.37–3.28 (m, 2H), 3.28 (t, J = 7.2 Hz, 2H), 2.93–2.83 (m, 2H), 2.85 (t, J = 7.2 Hz, 2H), 2.59–2.48 (m, 2H), 2.15–2.10 (m, 2H), 1.92–1.84 (m, 4H), 1.80–1.65 (m, 2H), 1.54–1.41 (m, 2H); Anal. found $C_{21}H_{29}N_3O_4 \cdot HCl \cdot 3/4H_2O$ (C, H, N).

5.2.10. 4-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxybutanamide hydrochloride (5b). The title compound was prepared according to Method C to give a white powder: Yield 71% in two steps; TLC R_f = 0.63 ($CHCl_3$/MeOH, 9/1); IR (KBr) 3437, 3143, 2975, 2817, 2599, 2246, 1656, 1567, 1517, 1469, 1444, 1421, 1357, 1333, 1305, 1255, 1243, 1157,

1137, 1071, 1058, 1036, 1022, 1009, 995; MS (FAB, Pos.) $m/z = 402$ $(M+H)^+$; ^1H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.29 (d, $J = 2.6$ Hz, 1H), 7.17 (dd, $J = 8.7$, 2.6 Hz, 1H), 6.87 (d, $J = 8.7$ Hz, 1H), 6.04 (br, 3H), 4.89–4.83 (m, 1H), 3.72 (s, 3H), 3.32–3.28 (m, 2H), 2.90–2.63 (m, 6H), 2.46 (t, $J = 6.9$ Hz, 2H), 2.26–2.14 (m, 4H), 1.92–1.85 (m, 4H), 1.80–1.66 (m, 2H), 1.56–1.45 (m, 2H); Anal. found $C_{22}H_{31}N_3O_4 \cdot HCl \cdot 1/4H_2O$ (C, H, N).

5.2.11. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxypropanamide hydrochloride (6b). The title compound was prepared according to Method C to give a white powder: Yield 76% in two steps; TLC $R_f = 0.44$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3439, 3118, 2960, 2720, 2239, 1671, 1640, 1518, 1443, 1417, 1393, 1362, 1266, 1157, 1126, 1038, 991; MS (APCI, Pos. 20 V) $m/z = 388$ $(M+H)^+$; ^1H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.20 (d, $J = 2.3$ Hz, 1H), 7.09 (dd, $J = 8.3$, 2.3 Hz, 1H), 6.94 (d, $J = 8.3$ Hz, 1H), 6.45 (br, 3H), 4.82–4.75 (m, 1H), 3.74 (s, 3H), 3.53 (q, $J = 6.9$ Hz, 1H), 3.23–3.02 (m, 3H), 2.93–2.82 (m, 1H), 2.26–2.10 (m, 4H), 1.95–1.65 (m, 6H), 1.53–1.41 (m, 2H), 1.47 (d, $J = 6.9$ Hz, 3H); HRMS (FAB) calcd for $C_{21}H_{30}N_3O_4$ 388.2236, found 388.2238.

5.2.12. 2-{4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]piperidin-1-yl}-*N*-hydroxybutanamide hydrochloride (7b). The title compound was prepared according to Method C to give a white powder: Yield 56% in two steps; TLC $R_f = 0.54$ ($CHCl_3$/MeOH, 9/1); IR (KBr) 3438, 3107, 2957, 2876, 2750, 2713, 2239, 1672, 1605, 1590, 1519, 1460, 1447, 1420, 1362, 1318, 1258, 1197, 1172, 1136, 1106, 1032, 993; MS (FAB, Pos.) $m/z = 402$ $(M+H)^+$; ^1H NMR (300 MHz, pyridine-d_5+$CDCl_3$) δ 7.20 (d, $J = 2.4$ Hz, 1H), 7.10 (dd, $J = 8.5$, 2.4 Hz, 1H), 6.92 (d, $J = 8.5$ Hz, 1H), 6.35 (br, 3H), 4.83–4.76 (m, 1H), 3.72 (s, 3H), 3.34–3.15 (m, 4H), 3.06–2.96 (m, 1H), 2.27–2.00 (m, 5H), 1.95–1.62 (m, 7H), 1.54–1.39 (m, 2H), 0.99 (t, $J = 7.2$ Hz, 3H); Anal. found $C_{22}H_{31}N_3O_4 \cdot HCl$ (C, H, N).

5.3. Synthesis of compounds 9c–d

5.3.1. An enantiomeric mixture (18) of (1′*S*,5′*R*)-5,5-dimethyl-3′-(3-cyclopentyloxy-4-methoxyphenyl)spiro-[1,3-dioxine-2,7′-bicyclo[3.3.0]octan-2′-en] and (1′*R*,5′*S*)-5,5-dimethyl-3′-(3-cyclopentyloxy-4-methoxyphenyl)spiro-[1,3-dioxine-2,7′-bicyclo[3.3.0]octan-2′-en]. The following reaction was carried out under argon atmosphere. To a stirred solution of 3-cyclopentyloxy-4-methoxy phenylbromide (5.66 g, 20.9 mmol) in THF (80 mL) were added *n*-BuLi (1.53 M solution in *n*-hexane, 14.5 mL, 22.3 mmol) and $CeCl_3$ (5.48 g, 22.3 mmol) at −78 °C. To this suspension was added a solution of **17** (3.12 g, 13.9 mmol) in THF (60 mL) at −78 °C. After being stirred at −78 °C for 3 h, the reaction mixture was quenched with 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/EtOAc, 5/1) to give an alcohol (3.48 g, 8.35 mmol) as a pale yellow oil: TLC $R_f = 0.39$ (*n*-hexane/EtOAc, 2/1). To a stirred solution of an alcohol (2.24 g, 5.38 mmol) in CH_2Cl_2 (30 mL) were added Et_3N (1.80 mL, 12.9 mmol) and MsCl (0.50 mL, 6.45 mmol) at 0 °C. After being stirred at room temperature for 24 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 6/1) to give **18** (1.99 g, 4.99 mmol, 56% in two steps) as a pale yellow oil: TLC $R_f = 0.49$ (*n*-hexane/EtOAc, 5/1); MS (APCI, Pos. 20 V) $m/z = 399$ $(M+H)^+$; ^1H NMR (200 MHz, CD_3OD) δ 6.99 (d, $J = 2.1$ Hz, 1H), 6.93 (dd, $J = 8.4$, 2.1 Hz, 1H), 6.80 (d, $J = 8.4$ Hz, 1H), 5.93 (m, 1H), 4.83–4.75 (m, 1H), 3.84 (s, 3H), 3.53 (s, 2H), 3.33 (m, 1H), 2.97–2.80 (2H, H), 2.51–2.37 (m, 3H), 1.98–1.54 (m, 8H), 0.97 (s, 6H).

5.3.2. (1′*S*,3′*S*,5′*R*)-5,5-Dimethyl-3′-(3-cyclopentyloxy-4-methoxyphenyl)spiro[1,3-dioxine-2,7′-bicyclo[3.3.0]octane] (19). A solution of **18** (683 mg, 1.71 mmol) in dioxane (16 mL) was hydrogenated under atmospheric pressure of H_2 gas in the presence of 10% Pd/C (68 mg) for 3 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 (*n*-hexane/EtOAc, 7/1) to give **19** (689 mg, 1.71 mmol, 100%) as a white powder: TLC $R_f = 0.65$ (*n*-hexane/EtOAc, 7/1); MS (FAB, Pos.) $m/z = 401$ $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 6.82–6.72 (m, 3H), 4.76 (m, 1H), 3.81 (s, 3H), 3.51 (s, 4H), 2.93 (m, 1H), 2.68–2.48 (m, 2H), 2.31–2.14 (m, 4H), 2.00–1.73 (m, 8H), 1.66–1.39 (m, 2H), 0.98 (s, 6H).

5.3.3. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]hexahydro-2(1*H*)-pentalenone (20). To a stirred solution of **19** (2.49 g, 6.22 mmol) in acetone (60 mL) was added $TsOH \cdot H_2O$ (118 mg, 0.62 mmol). After being stirred at room temperature for 24 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 5/1) to give **20** (1.85 g, 5.89 mmol, 95%) as a pale yellow oil: TLC $R_f = 0.48$ (*n*-hexane/EtOAc, 3/1); MS (APCI, Pos. 20 V) $m/z = 315$ $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.82–6.72 (m, 3H), 4.76 (m, 1H), 3.82 (s, 3H), 3.10 (m, 1H), 2.91–2.76 (m, 2H), 2.63–2.52 (m, 2H), 2.49–2.38 (m, 2H), 2.19–2.11 (m, 2H), 1.95–1.76 (m, 6H), 1.68–1.53 (m, 2H), 1.44 (m, 2H).

5.3.4. An enantiomeric mixture (21) of (3*aR*,5*R*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarbonitrile and (3*aS*,5*S*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarbonitrile (Method D). To a stirred solution of **20** (1.41 g, 4.47 mmol) in CH_2Cl_2 (60 mL) were added ZnI_2 (143 mg, 0.447 mmol) and TMSCN (577 mg, 5.81 mmol). After being stirred at room temperature for 2 h, the mixture was concentrated in vacuo. The residue was used for the next step without further purification.

To a stirred solution of cyanohydrin in pyridine (20 mL) was added $P(O)Cl_3$ (1.03 mL, 11.2 mmol). After being stirred at 120 °C for 1.5 h, the reaction mixture was poured into ice water, and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4/1) to give **21** (1.31 g, 4.05 mmol, 91% in two steps) as a white powder: TLC $R_f = 0.62$ (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m*/*z* = 324 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.81–6.77 (m, 1H), 6.75–6.70 (m, 2H), 6.59 (m, 1H), 4.77 (m, 1H), 3.82 (s, 3H), 3.39 (m, 1H), 2.99–2.77 (m, 3H), 2.48–2.24 (m, 3H), 1.96–1.76 (m, 6H), 1.68–1.54 (m, 2H), 1.43–1.31 (m, 2H).

5.3.5. An enantiomeric mixture (22) of (3*aR*,5*R*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarboxylic acid and (3*aS*,5*S*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarboxylic acid (Method E). To a stirred solution of **21** (941 mg, 2.91 mmol) in ethylene glycol (6.5 mL) was added 40% aqueous KOH (5.4 mL, 58.2 mmol). After being stirred at 200 °C for 4 h, the reaction mixture was washed with Et_2O, neutralized by 2 N HCl, and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was triturated with EtOAc to give **22** (635 mg, 1.85 mmol, 64%) as a pale powder: TLC $R_f = 0.46$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3436, 2944, 1981, 1626, 1517, 1424, 1292, 1241, 1143, 1034, 850; MS (APCI, Neg. 20 V) *m*/*z* = 341 $(M-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) δ 6.86 (m, 1H), 6.82–6.70 (m, 1H), 4.77 (m, 1H), 3.81 (s, 3H), 3.39 (m, 1H), 2.91 (m, 1H), 2.90–2.75 (m, 2H), 2.50–2.35 (m, 2H), 2.30 (m, 1H), 1.98–1.70 (m, 7H), 1.70–1.50 (m, 2H), 1.45–1.27 (m, 2H).

5.3.6. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (9c) (Method F). A solution of **22** (670 mg, 1.96 mmol) in MeOH (16 mL) was hydrogenated under atmospheric pressure of H_2 gas in the presence of 10% Pd/C (70 mg) for 4 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, 100/1) to give **9c** (689 mg, 1.11 mmol, 54%) as a white powder: TLC $R_f = 0.78$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3439, 2960, 1698, 1516, 1467, 1421, 1303, 1246, 1161, 1139, 1036, 1001, 855; MS (APCI, Neg. 20 V) *m*/*z* = 343 $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.81–6.73 (m, 3H), 4.81–4.74 (m, 1H), 3.82 (s, 3H), 3.08 (m, 1H), 2.92 (m, 1H), 2.66–2.55 (m, 2H), 2.30–2.19 (m, 4H), 1.97–1.78 (m, 6H), 1.78–1.37 (m, 7H); Anal. found $C_{21}H_{28}O_4 \cdot 1/4H_2O$ (C, H).

5.3.7. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (9d). The title compound was prepared according to Method C to give a pale pink powder: Yield 75%; TLC $R_f = 0.48$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3432, 2946, 1629, 1515, 1464, 1265, 1137, 1030; MS (FAB, Pos.) *m*/*z* = 382 $(M+Na)^+$, 359 $(M)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.64 (br, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 1.8 Hz, 1H), 6.72 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.76 (m, 1H), 3.68 (s, 3H), 3.02 (m, 1H), 2.60–2.40 (m, 3H), 2.17–2.07 (m, 2H), 1.98–1.76 (m, 4H), 1.76–1.61 (m, 4H), 1.61–1.44 (m, 4H), 1.40–1.28 (m, 2H).

5.4. Synthesis of compounds 9a–b and 11a–b

5.4.1. An enantiomeric mixture (23) of (3*aR*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-3,3*a*,4,6*a*-tetrahydro-2(1*H*)-pentalenone and (3*aS*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-3,3*a*,4,6*a*-tetrahydro-2(1*H*)-pentalenone. To a stirred solution of **18** (4.61 g, 11.6 mmol) in acetone (116 mL) was added $TsOH \cdot H_2O$ (220 mg). After being stirred at room temperature for 3.5 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with H_2O and brine, 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 **23** (3.24 g, 10.4 mmol, 90%) as a pale yellow oil: TLC $R_f = 0.48$ (*n*-hexane/EtOAc, 3/1); MS (APCI, Pos. 20 V) *m*/*z* = 313 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, *J* = 2.1 Hz, 1H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 5.89 (m, 1H), 4.86–4.76 (m, 1H), 3.85 (s, 3H), 3.60 (m, 1H), 3.16–3.01 (m, 2H), 2.63–2.47 (m, 3H), 2.33 (m, 1H), 2.14–2.04 (m, 1H), 1.97–1.81 (m, 6H).

5.4.2. An enantiomeric mixture (24) of (2*S*,3*aR*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,2,3,3*a*,4,6*a*-hexahydro-2-pentalenol and (2*R*,3*aS*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,2,3,3*a*,4,6*a*-hexahydro-2-pentalenol. To a stirred solution of **23** (3.24 g, 10.4 mmol) and THF (275 mL) in MeOH (275 mL) was added $NaBH_4$ (395 mg, 10.4 mmol). After being stirred at 0 °C for 1 h, the reaction mixture was quenched with acetone and H_2O. The mixture was extracted with EtOAc and washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **24** (3.27 g, 10.4 mmol, 100%) as a pale yellow oil: TLC $R_f = 0.67$ (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m*/*z* = 315 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, *J* = 2.0 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.02 (m, 1H), 4.79 (m, 1H), 4.23 (m, 1H), 3.84 (s, 3H), 3.40–3.30 (m, 2H), 3.04 (m, 1H), 2.85 (m, 1H), 2.63 (m, 1H), 2.20 (m, 1H), 2.07 (m, 1H), 2.00–1.76 (m, 6H), 1.69–1.53 (m, 4H).

5.4.3. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]octahydro-2-pentalenol (25). A solution of **24** (39 mg, 0.098 mmol) in CH_2Cl_2 (690 mL) was hydrogenated under atmospheric pressure of H_2 gas in the presence of $Ir(cod)py(PCy_3)PF_6$ (16 mg, 0.020 mmol) for 1 h. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 3/2) to give **25** (28 mg, 0.087 mmol, 89%) as a pale yellow oil: TLC $R_f = 0.64$, (*n*-hexane/EtOAc, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 6.84–6.75 (m 3H), 4.78 (m, 1H), 3.95 (m, 1H), 3.76 (s, 3H), 3.15

(m, 1H), 2.58–2.45 (m, 2H), 2.21–2.11 (m, 2H), 1.88–1.56 (m, 12H), 1.27–1.16 (m, 2H).

5.4.4. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]hexahydro-2(1*H*)-pentalenone (26). To a stirred solution of **25** (1.3 g, 4.1 mmol) in CH_2Cl_2 (20 mL) were added NMO (1.44 g, 12.3 mmol), TPAP (2.6 g, 8.2 mmol), and MS4A (1 g). After being stirred at room temperature for 24 h, the reaction mixture was filtrated with column chromatography on silica gel (CH_2Cl_2) and purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4/1) to give **26** (1.18 g, 3.76 mmol, 92%) as a white powder: TLC $R_f = 0.57$, (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82–6.73 (m, 3H), 4.77 (m, 1H), 3.82 (s, 3H), 3.18 (m, 1H), 3.03–2.92 (m, 2H), 2.65–2.54 (m, 2H), 2.16–2.08 (m, 2H), 2.06–1.76 (m, 10H), 1.67–1.55 (m, 2H).

5.4.5. An enantiomeric mixture (27) of (3*aR*,5*S*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarbonitrile and (3*aS*,5*R*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydro-2-pentalenecarbonitrile. The title compound was prepared according to Method D to give a white powder: Yield 55% in two steps.

5.4.6. (3*aR*,5*S*,6*aR*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxylic acid (11a). The title compound was prepared according to Method E to give a white powder: Yield 93%; TLC $R_f = 0.56$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3433, 2933, 1686, 1630, 1518, 1421, 1270, 1139, 1032, 801; MS (APCI, Neg. 20 V) $m/z = 341$ $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82–6.71 (m, 4H), 4.76 (m, 1H), 3.82 (s, 3H), 3.52 (m, 1H), 3.10–2.89 (m, 3H), 2.39 (m, 1H), 2.00–1.74 (m, 10H), 1.68–1.53 (m, 3H); Anal. found $C_{21}H_{26}O_4$ (C, H).

5.4.7. (3*aR*,5*S*,6*aR*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxy-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxamide (11b). The title compound was prepared according to Method C to give a pale brown powder: Yield 56%; TLC $R_f = 0.64$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3424, 2934, 1655, 1514, 1261, 1136, 1030, 795; MS (FAB, Pos.) $m/z = 358$ $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.52 (br, 1H), 8.79 (br, 1H), 6.81 (d, $J = 8.3$ Hz, 1H), 6.77 (d, $J = 1.7$ Hz, 1H), 6.71 (dd, $J = 8.3$, 1.7 Hz, 1H), 6.19 (m, 1H), 4.76 (m, 1H), 3.68 (s, 3H), 3.40–3.26 (m, 1H), 2.96–2.70 (m, 3H), 2.24 (m, 1H), 1.88–1.61 (m, 10H) 1.61–1.48 (m, 2H); Anal. found $C_{21}H_{27}NO_4 \cdot 1/4H_2O$ (C, H, N).

5.4.8. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (9a). The title compound was prepared according to Method F to give a white powder: Yield 81%; TLC $R_f = 0.59$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 2936, 1699, 1588, 1517, 1443, 1357, 1321, 1269, 1226, 1200, 1161, 1137, 1029, 850; MS (APCI, Neg. 20 V) $m/z = 343$ $(M-H)^-$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82–6.74 (m, 3H), 4.80–4.73 (m, 1H), 3.82 (s, 3H), 3.10 (m, 1H), 2.73–2.57 (m, 3H), 2.34–2.24 (m, 2H), 1.96–1.78 (m, 8H), 1.75–1.45 (m, 7H); Anal. found $C_{21}H_{28}O_4$ (C, H).

5.4.9. (3*aR*,6*aS*)-5-[3-(Cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (9b). The title compound was prepared according to Method C to give a pale pink powder: Yield 70%; TLC $R_f = 0.49$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3210, 2940, 1630, 1515, 1442, 1263, 1160, 1138, 1034, 998; MS (FAB, Pos.) $m/z = 360$ $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.67 (s, 1H), 6.84–6.80 (m, 2H), 6.74 (dd, $J = 8.4$, 1.8 Hz, 1H), 4.77 (m, 1H), 3.68 (s, 3H), 3.01 (m, 1H), 2.60–2.40 (m, 1H), 2.29–2.16 (m, 2H), 2.00–1.89 (m, 2H), 1.89–1.77 (m, 2H), 1.77–1.50 (m, 10H), 1.44–1.31 (m, 2H); Anal. found $C_{21}H_{29}NO_4 \cdot 1/2H_2O$ (C, H, N).

5.5. Synthesis of compounds 10a–b

5.5.1. An enantiomeric mixture (29) of (3*aS*,5*S*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-5-cyano-1,3*a*,4,5,6,6*a*-hexahydropentalen-2-yl trifluoromethanesulfonate and (3*aR*,5*R*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-5-cyano-1,3*a*,4,5,6,6*a*-hexahydropentalen-2-yl trifluoromethanesulfonate. The following reaction was carried out under argon atmosphere. To a stirred solution of **28** (587 mg, 1.73 mmol) in THF (10 mL) was added LiHMDS (1.0 M solution in THF, 1.73 mL, 1.73 mmol) at −78 °C. After being stirred at −78 °C for 10 min, Tf_2NPh (618 mg, 1.73 mmol) was added. After being stirred at 0 °C for 30 min, the reaction mixture was diluted with brine, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **29** (1.54 g) as a yellow oil: TLC $R_f = 0.68$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00–6.75 (m, 3H), 5.67 (m, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.50–3.30 (m, 2H), 3.00–2.80 (m, 2H), 2.70–2.40 (m, 2H), 2.40–2.20 (m, 2H), 2.00–1.70 (m, 6H), 1.70–1.50 (m, 2H).

5.5.2. An enantiomeric mixture (30) of methyl (3*aS*,5*S*,6*aS*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-5-cyano-1,3*a*,4,5,6,6*a*-hexahydropentalen-2-carboxylate and methyl (3*aR*,5*R*,6*aR*)-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-5-cyano-1,3*a*,4,5,6,6*a*-hexahydropentalen-2-carboxylate. To a stirred solution of **29** (1.54 g) in DMF/MeOH (2/1, 6 mL) were added PPh_3 (27.3 mg, 1.04 mmol), $Pd(OAc)_2$ (11.7 mg, 0.0519 mmol), and Et_3N (0.48 mL, 3.5 mmol). After being stirred at room temperature for 19 h under CO atmosphere, the reaction mixture was diluted with H_2O and extracted with EtOAc/*n*-hexane. 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, 9/1–3/1) to give **30** (621 mg, 1.63 mmol, 94% in two steps) as a yellow oil: TLC $R_f = 0.50$ (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 40 V) $m/z = 382$ $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.83 (d, $J = 8.4$ Hz, 1H), 6.68 (m, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.51 (m, 1H), 3.00–2.80 (m, 2H), 2.65–2.45 (m, 3H), 2.40–2.10 (m, 2H), 2.00–1.75 (m, 6H), 1.70–1.50 (m, 2H).

5.5.3. An enantiomeric mixture (10a) of (3*aS*,5*S*,6*aS*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxylic acid and (3*aR*,5*R*,6*aR*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxylic acid. To a stirred solution of **30** (488 mg, 1.28 mmol) in THF/MeOH (1/2, 7.5 mL) was added 2 N NaOH (2.0 mL, 4.0 mmol). After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated and washed Et_2O. The water layer was acidified with 2 N HCl, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was triturated with Et_2O to give **10a** (400 mg, 1.09 mmol, 85%) as a white powder: TLC $R_f = 0.43$ ($CHCl_3$/MeOH, 9/1); IR (KBr) 2961, 2871, 2594, 2232, 1687, 1634, 1519, 1517, 1467, 1444, 1416, 1343, 1299, 1254, 1145, 1132, 1026, 982; MS (APCI, Neg. 40 V) $m/z = 366$ $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.90–6.80 (m, 2H), 4.79 (m, 1H), 3.84 (s, 3H), 3.55 (m, 1H), 3.00–2.80 (m, 2H), 2.70–2.50 (m, 3H), 2.32 (dd $J = 13.5$, 5.7 Hz, 1H), 2.22 (dd, $J = 12.9$, 7.2 Hz, 1H), 2.05–1.70 (m, 7H), 1.70–1.55 (m, 2H); HRMS (EI) calcd for $C_{22}H_{25}NO_4$ 367.1784, found 367.1771.

5.5.4. An enantiomeric mixture (10b) of (3*aS*,5*S*,6*aS*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxy-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxamide and (3*aR*,5*R*,6*aR*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxy-1,3*a*,4,5,6,6*a*-hexahydropentalene-2-carboxamide. The title compound was prepared according to Method C to give a white powder: Yield 56%; TLC $R_f = 0.44$ ($CHCl_3$/MeOH, 10/1); IR (KBr) 3208, 2957, 2229, 1644, 1605, 1517, 1444, 1416, 1258, 1143, 1020, 979; MS (APCI, Neg. 40 V) $m/z = 381$ $(M-H)^-$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.80–8.20 (br, 1H), 6.95–6.85 (m, 2H), 6.83 (d, $J = 9.3$ Hz, 1H), 6.42 (m, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.59 (m, 1H), 3.10–2.90 (m, 2H), 2.65–2.20 (m, 5H), 2.00–1.80 (m, 6H), 1.70–1.55 (m, 3H); HRMS (FAB) calcd for $C_{22}H_{27}N_2O_4$ 383.1971, found 38.1996.

5.6. Assay of human PDE4 activity

The method of Reeves et al.[29] 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 ^3H-cAMP (300,000 dpm (5000 Bq)/assay) and 100 μmol/L cAMP solution to 100 mmol/L Tris–HCl (pH 8.0) containing 5 mmol/L ethylene glycol-bis (β-aminoethyl ether) and *O*,*O*′-bis(2-aminoethyl)ethyleneglycol-*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.

5.7. 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 (RD 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.

5.8. 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).

5.9. 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 20 min 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%.

5.10. 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.

5.11. 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.

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