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Original article

Orally active PDE4 inhibitor with therapeutic potential

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Abstract

Based on the promising results obtained by the clinical trial of Ariflo™, further optimization of the spatial arrangement of the three pharmacophores (the carboxylic acid moiety, nitrile moiety and 3-cyclopentyloxy-4-methoxyphenyl moiety) in the structure of Ariflo **1** was attempted using a bicyclo[3·3·0]octane template with more stereochemical diversity than the cyclohexane template of Ariflo **1**. Biological evaluation of the decyanated analogs and further optimization of the cyclopentyloxy moiety of **2a–b** were also performed. Among the compounds tested, **2a, 7a–b** and **12a** were found to be orally active and were estimated to have therapeutic potential based on cross-species and same-species comparisons. The structure–activity relationships (SARs) of these compounds were investigated and pharmacokinetic data for **2a** and **7b** were also obtained by single-dose studies in rats.

Keywords: PDE4 inhibitor; Bicyclo[3·3·0]octane; Spatial arrangement; Three pharmacophores; Orally active

1. Introduction

Phosphodiesterase type 4 enzyme (PDE4) [1,2] is an enzyme involved in the intracellular degradation of cyclic adenosine monophosphate (cAMP) to produce the corresponding 5′-monophosphate, and it has received considerable attention as a molecular target for the treatment of asthma and other inflammatory diseases [3,4]. Previous studies in this field have focused on asthma and chronic obstructive pulmonary disease (COPD), with numerous inhibitors being reported that have reached clinical trials [5,6]. However, clinical use of these pioneer compounds has been limited by side effects such as nausea, emesis and increased gastric acid secretion. In order to obtain more useful PDE4 inhibitors with fewer side effects, two approaches have been attempted. The first is to design compounds with a reduced affinity for the so-called high affinity rolipram-binding site (HPDE4) of PDE4 and a high affinity for its catalytic domain (LPDE4) [7,8]. Ariflo **1** (Fig. 1) has been reported to be 75-fold more selective than (*R*)-rolipram with respect to LPDE4 activity [9], and has shown efficacy with an improved safety margin in clinical assessment. The second approach is to design subtype-selective inhibitors [10–12]. Ariflo **1** is an orally active second-generation PDE4 inhibitor that is reported to be PDE4D-selective.

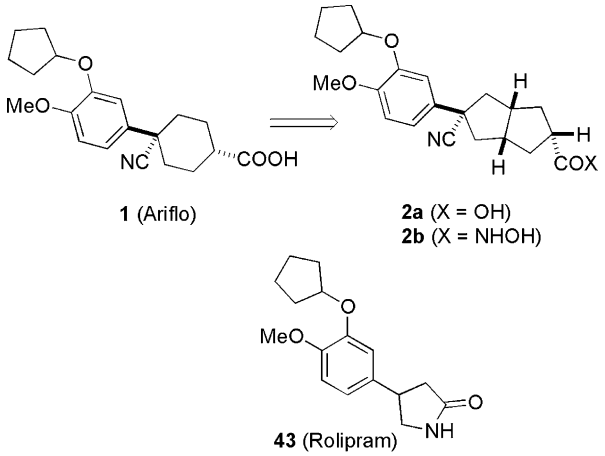

Fig. 1. Structures of Ariflo, rolipram and new inhibitors.

The importance of optimized spatial arrangement of the carboxylic acid moiety relative to the 3-cyclopentyloxy-4-methoxyphenyl and nitrile moieties on the cyclohexane ring is illustrated by comparison of Ariflo **1** (*cis*-isomer) with its

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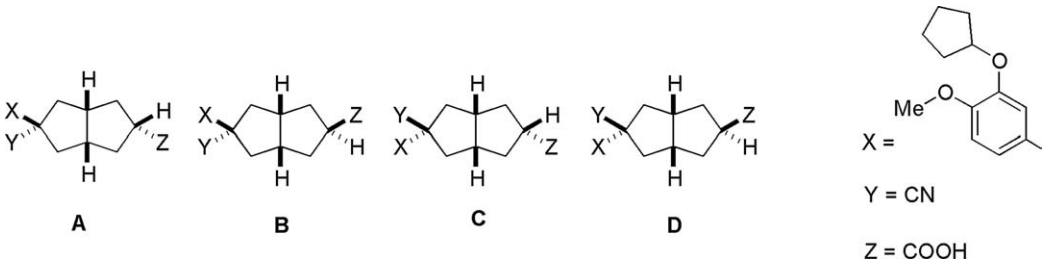


Fig. 2. Stereochemical diversity of bicyclo[3·3·0]octane template.

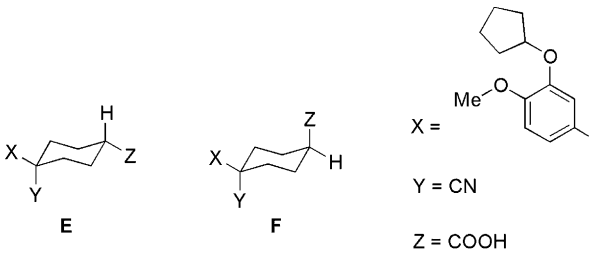


Fig. 3. Stereochemical diversity of cyclohexane template.

trans-isomer [9]. A more restricted spatial arrangement of these three moieties within their optimized stereochemistry using another template instead of the cyclohexane ring was predicted to lead to the discovery of a structurally new inhibitor with improved therapeutic potential. Synthesis and evaluation of a series of bicyclo[3·3·0]octane derivatives was carried out with the expectation of increasing the therapeutic potential because the bicyclo[3·3·0]octane template provides more stereochemical diversity (Fig. 2 four stereoisomers **A–D**) and it was expected that derivatives based on the bicyclo[3·3·0]octane template might provide a more optimal structure than Ariflo **1** and show stronger PDE4 inhibition, higher LPDE4 selectivity, and better subtype selectivity compared with the corresponding cyclohexane derivatives (Fig. 3 two stereoisomers **E** and **F**).

Fig.1 shows the configuration of the above-mentioned three pharmacophores on a new template, the bicyclo[3·3·0] octane ring, which achieved an increase of activity with an improved side effect profile.

2. Chemistry

As outlined in Fig. 4, analogues **2–5** were prepared from a benzylcyanide **20** [9] by the following synthetic pathway. Dialkylation of **20** with *cis*-4,5-bis(bromomethy)cyclohex-1-ene **19**, which was prepared from **16** by three steps as described in Fig. 4, provided **21** at a good yield. Ozonolysis of **21**, followed by oxidation and then esterification, gave the diester **22**. Dieckmann condensation of **22**, followed by demethoxycarbonylation, produced two separable diastereoisomers **23a** and **23b**, which were converted to the ketenedithioacetals **24a** and **24b**, respectively. The stereochemistry of **23a** and **23b** was determined based on X-ray crystallography (Fig. 5). Details are given in Section 5. Deprotection of **24a** yielded two separable isomers **25a** and **25b**, which were converted to the carboxylic acid analogues **2a** and **3**, respectively. The stereochemistry of **2a** and **3** was determined using X-ray crystallography, as described in Fig. 6. Details were also given in Section 5. Condensation of **2a** with *O*-protected hydroxylamine followed by acidic deprotection provided a hydroxamic acid **2b**. According to the procedure described above, **24b** was converted to **25c** and **25d**, which were transformed to **4a** and **5**, respectively.

The synthesis of **6–15** is outlined in Fig. 7. The enol triflate **30** was prepared from a ketone **29a**, which was derived from 3-benzyloxy-4-methoxybenzyl cyanide **26** according to the same procedure as described for the preparation of **23a** from **20**. Palladium-catalyzed insertion of carbon monoxide into **30** in the presence of methanol gave **31**, after which catalytic hydrogenation from less hindered side afforded a single isomer **32**. *O*-Alkylation of **32** by the conventional method gave **33–42**, alkaline hydrolysis of which produced **6a–15a**, respectively. Condensation of **6a–15a** with an *O*-protected hydroxylamine, followed by acidic deprotection, yielded the corresponding hydroxamic acids **6b–15b**, respectively. Synthesis of the decyanated analogs **2c** and **4b** will be reported elsewhere.

3. Results and discussion

All four possible isomers **2–5** were synthesized and evaluated, as shown in Table 1. Compounds **2a–b** and **3**, in which the aromatic moiety was located outside the concave bicyclo[3·3·0]octane framework, showed potent inhibition of PDE4. The LPDE4 inhibitory activity of **2a–b** was equipotent with that of Ariflo **1**, while that of **3** was 15-fold weaker than that of **1**. Compounds **4** and **5**, with the aromatic moiety located inside the concave framework, showed no LPDE4 inhibitory activity at a concentration of 300 nM.

The carboxylic acid group was preferably oriented in the *syn*-direction of the nitrile and located inside the concave molecule, as illustrated by the greater potency of **2a** over **3**. Thus, these results showed that the PDE4 enzyme might recognize the more accessible aromatic moiety first, while favoring more hindered carboxylic acid and nitrile groups.

Inhibition of LPS-induced TNF-α production in rats was evaluated using **2a–b** and **3**. The in vivo activity of **2a** was fourfold more potent than that of **1**, while both **2b** and **3** were less potent than **1**. Compound **2a** was nearly 10-fold more potent than **3** (based on ID_{50} values) for inhibition of LPS-

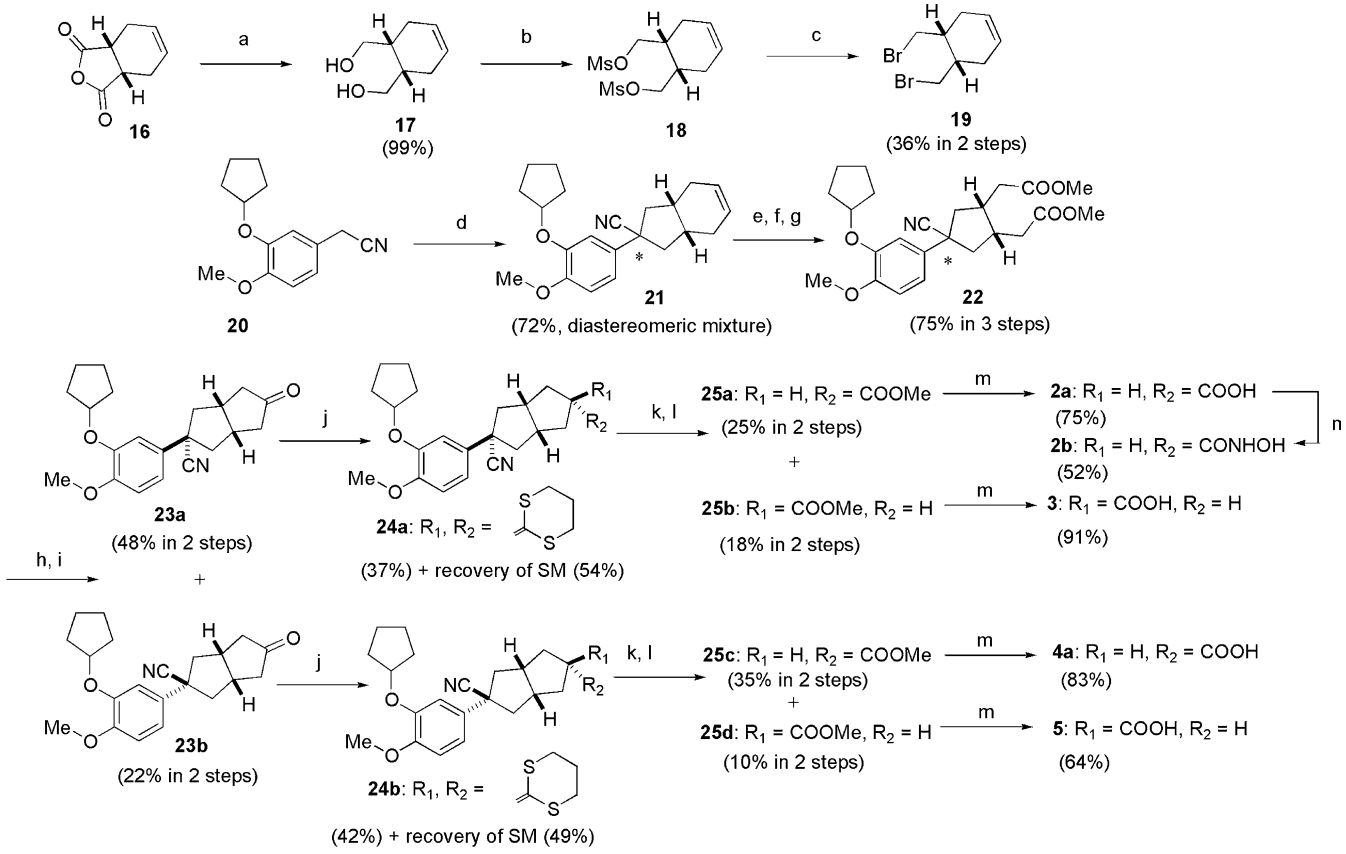


Fig. 4. Synthesis of compounds **2–5**. (a) $LiAlH_4$, THF, 0 °C; (b) MsCl, Et_3N, CH_2Cl_2, –78 °C; (c) LiBr, DMF, 100 °C; (d) **19**, NaHMDS, THF, –78 °C; (e) O_3, CH_2Cl_2 then PPh_3, –78 °C; (f) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2-methyl-2-butene; (g) CH_2N_2, Et_2O, 0 °C; (h) NaH, DME, reflux; (i) NaCl, DMSO, H_2O, 165 °C; (j) 2-trimethylsilyl-1, 3-dithiane, *n*-BuLi, THF, –78 °C; (k) TFA, 30% H_2O_2aq, CH_3CN, H_2O 80 °C, then 2 N NaOHaq 40 °C; (l) CH_2N_2, Et_2O 0 °C; (m) 2 N KOHaq, THF, MeOH; (n) $NH_2OC(CH_3)_2OMe$, EDC, HOBt, DMF then MeOH, 1 N HClaq.

induced TNF-α production in rats, as predicted from their in vitro SAR.

PDE4 is reported to be inhibited by various hydroxamic acid derivatives [13,14]. This led us to the synthesis of a series of hydroxamic acid analogs that were expected to show increased activity via strong metal-mediated interactions with the enzyme. Conversion of the carboxylic acid moiety of **2a** to a hydroxamic acid afforded **2b**, which retained PDE4 inhibitory activity, but the new hydroxamic acid moiety did not markedly increase the inhibition of PDE4 contrary to our expectations. In addition, the inhibition of LPS-induced TNF-α production in rats by **2b** was markedly decreased relative to the effect of **2a**, probably because of pharmacodynamic factors such as the low oral availability of the hydroxamic acid analog.

To assess the influence of the benzylic nitrile moiety on LPDE4 inhibitory activity, decyanated analogs **2c** and **4b** were also synthesized and evaluated. Removal of the benzylic nitrile of **2a** produced **2c**, with nearly 17-fold reduction of LPDE4 inhibitory activity, while **2c** showed 79% inhibition of LPS-induced TNF-α production at an oral dose of 3 mg/kg. Although **4a** showed less than 50% LPDE4 inhibitory activity at 300 nM, compound **4b** without the benzylic nitrile moiety had an IC_{50} value of 80 nM and an ID_{50} value of 2.2 mg/kg, po. Based on these findings, there seemed to be two different sets of SAR for the cyanated and decyanated analogs.

The benzylic nitrile moiety seemed to increase the LPDE4 inhibitory activity in conversion from **2c** to **2a**, but in the corresponding conversion from **4b** to **4a** it seemed to have a deleterious effect. More detailed study of SAR, including the role played by the benzylic nitrile moiety, is currently underway and will be reported in due course.

Further structural optimization of the cyclopentyl moiety of **2a**, which exhibited the most potent activity among the compounds listed in Table 1, was carried out as shown in Table 2. Replacement of the cyclopentyl moiety of **2a** with methyl, ethyl and isopropyl groups produced **6a, 7a**, and **8a**, respectively, which all showed weaker inhibition of LPDE4. Replacement of the cyclopentyl moiety of **2a** with cyclobutyl or cyclohexyl moieties afforded **9a** and **10a**, respectively, which had fourfold and ninefold less potent LPDE4 inhibitory activity. Introduction of ether oxygen into the cyclohexyl moiety of **10a** gave **11a**, which showed nearly threefold weaker LPDE4 inhibitory activity because of the presumed lower affinity of the hydrophilic moiety. Replacement of the cyclopentyl moiety of **2a** with an 2-isoindanyl group resulted in **12a**, which showed nearly sevenfold stronger LPDE4 inhibitory activity, but was almost fourfold less potent at inhibiting LPS-induced TNF-α production, presum-

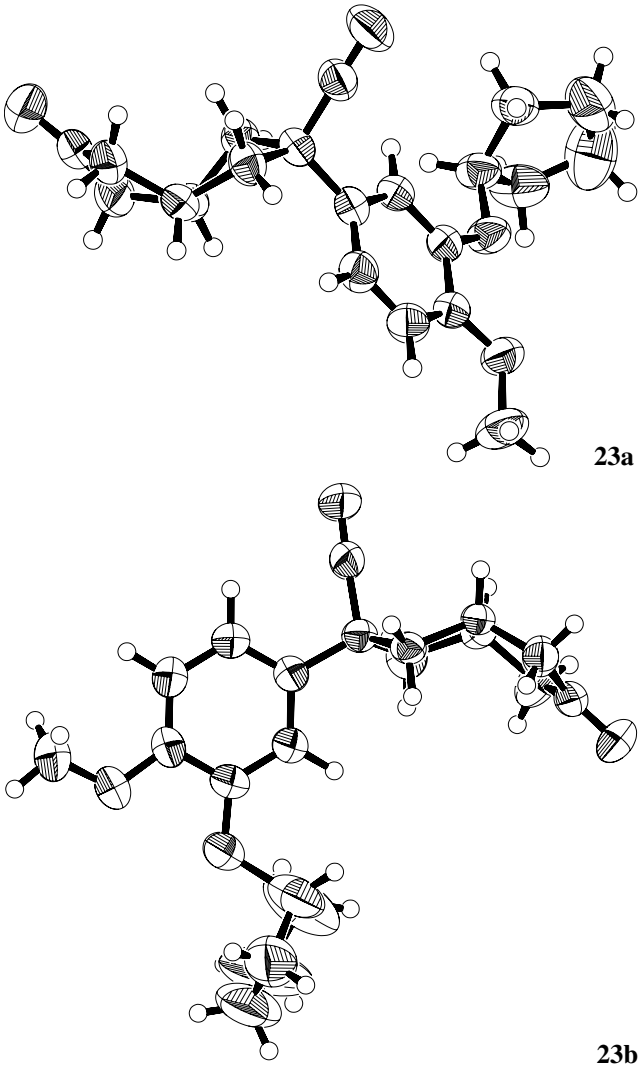


Fig. 5. X-ray crystallography of **23a, 23b**.

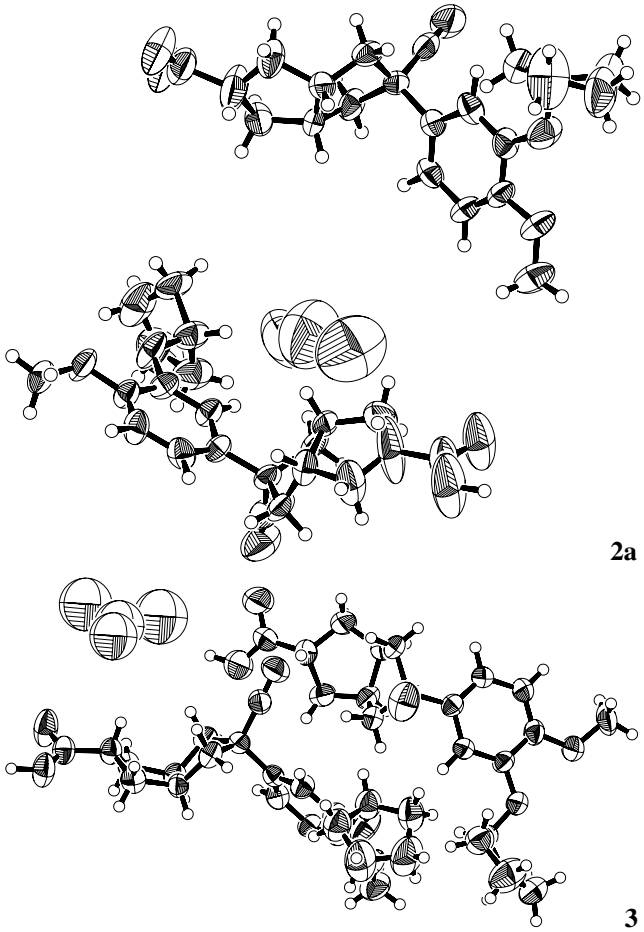


Fig. 6. X-ray crystallography of **2a** and **3**.
The asymmetric unit of crystal of **3** contained two molecules of **3** and a molecule of isopropanol, while the asymmetric unit of crystal of **2a** contained two molecules of **2a** and a molecule of methanol. The solvent molecules found in both of the units were refined with isotropic temperature parameters, but H atoms were not assigned.

ably because of pharmacodynamic problems. Replacement of the cyclopentyl moiety of **2a** with a cyclopropylmethyl group produced **13a**, which showed 11-fold weaker LPDE4 inhibitory activity. Replacement of the cyclopentyl moiety of **2a** with 4-methylthiobutyl or 3,3,3-trifluoropropyl moieties afforded **14a** and **15a**, respectively, which showed fourfold and eightfold lower LPDE4 inhibitory activity. Both of these analogs failed to inhibit LPS-induced TNF-α production in rats at an oral dose of 3 mg/kg. Based on the results of the LPS-induced TNF-α production assay, the most optimal structure was **2a** among all the tested carboxylic acid analogs. The corresponding hydroxamic acid analogs **6b–15b** were also synthesized and evaluated. Each of these compounds showed stronger LPDE4 inhibitory activity relative to their corresponding carboxylic acid analogs. However, the in vivo potency of **12b** with the strongest LPDE4 inhibitory activity did not increase as expected, presumably because of pharmacodynamic problems. This was also true for the in vivo potencies of **8b** and **14b**.

Further evaluation of compounds **2a, 7a–b** and **12a**, which were selected on the basis of inhibition of in vivo TNF-α production, was carried out as shown in Table 3. These compounds were evaluated for inhibition of slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction [15,16] (a desirable effect) and for inhibition of gastric emptying [11] (an adverse effect) in rats. The results were expressed as ID_{50} values, i.e., the dose that caused 50% inhibition relative to the vehicle. These compounds were also evaluated for inhibition of LPS-induced TNF-α production in human whole blood (HWB) [17] to predict their clinical potential. Results were expressed as IC_{50} values, i.e., the test compound concentration that caused 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 activity against LPS-induced TNF-α production in rats, probably because of differences in pharmacokinetics, the disease models, to the species studied. Compounds **1, 2a** and **7b** were evaluated for their ability to inhibit SRS-A-mediated bronchoconstriction in guinea pigs challenged by intravenous administration of OVA (0.15 mg/kg). Compound **2a** was nearly twofold less

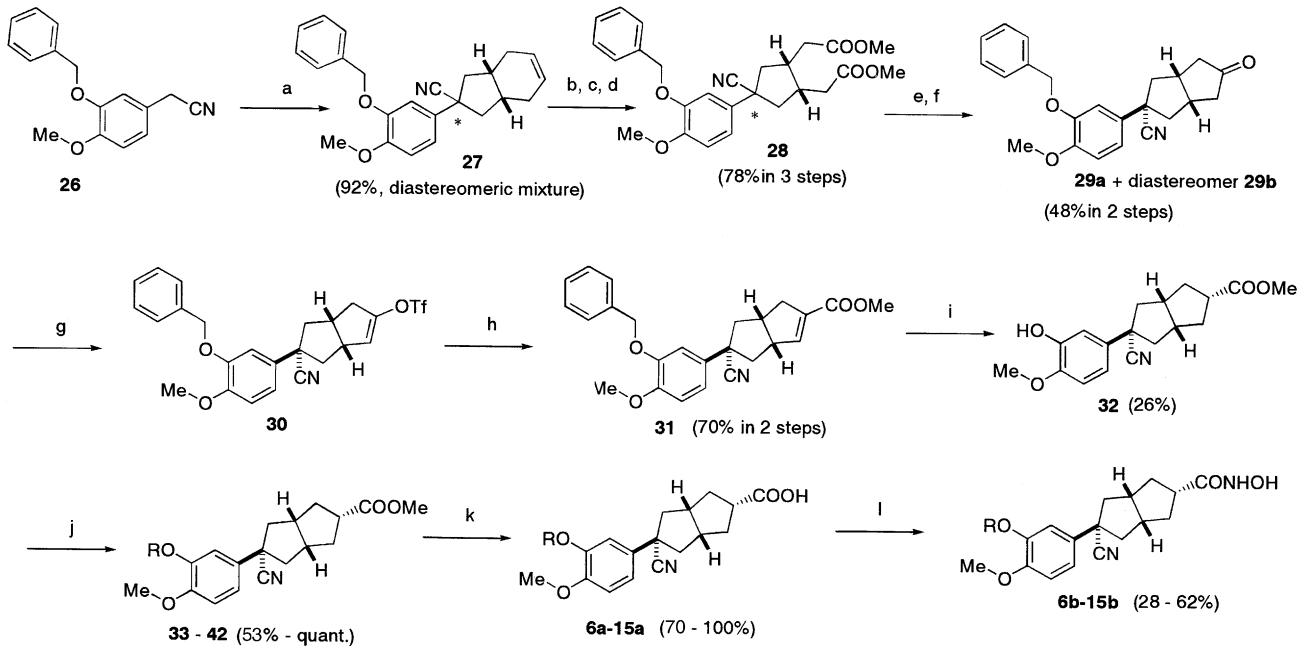


Fig. 7. Synthesis of compounds **6–15**. (a) NaHMDS, THF, –78 °C; (b) O_3, CH_2Cl_2 then PPh_3, –78 °C; (c) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2-methyl-2-butene; (d) CH_2N_2, Et_2O, 0 °C; (e) NaH, DME, reflux; (f) NaCl, DMSO, H_2O, 165 °C; (g) LiHMDS, $PhNTf_2$, THF, –78 °C – rt; (h) $Pd(OAc)_2$, Et_3N, PPh_3, CO, MeOH, DMF; (i) H_2, Pd/C, MeOH; (j) RX, K_2CO_3, DMF or ROH, RCON=NCOR, PPh_3, CH_2Cl_2; (k) 1 N NaOHaq, MeOH, THF; (l) EDC, HOBt, Et_3N, $NH_2OC(CH_3)_2OMe$, DMF then 1 N HClaq., MeOH.

potent at inhibiting this type of SRS-A-mediated bronchoconstriction in guinea pigs than Ariflo **1**, although **2a** was fourfold stronger for inhibition of LPS-induced TNF-α production in rats. Compound **7b** showed nearly the same potency as Ariflo **1** in both animal models. Compounds **7a, 7b** and **12a** were also evaluated for their ability to inhibit SRS-A-mediated bronchoconstriction after challenge by a higher dose of OVA (0.5 mg/kg, iv). Compound **7a** failed to inhibit bronchoconstriction despite a high oral dose of 30 mg/kg while **7b** achieved 49% inhibition at an oral dose of 10 mg/kg. Compound **12a** showed 50% inhibition of this type of SRS-A-mediated bronchoconstriction at an oral dose of 5.9 mg/kg. Thus, the relative potency of these compounds against SRS-A-mediated bronchoconstriction in OVA-sensitized guinea pigs was **12a > 7b, 1 > 2a, 7a**.

To assess safety within a single species, inhibition of gastric emptying by **2a, 7a–b** and **12a** was evaluated. After oral dosing, compound **2a** caused 73% inhibition of gastric emptying at 10 mg/kg, while **7a** and **7b** had ID_{50} values of 17 and 12 mg/kg, respectively. The oral doses of **7b** and **12a** that inhibited gastric emptying were higher than those having a desirable effect (inhibition of LPS-induced TNF-α production in rats). Based on their greater potency than that of Ariflo **1** for blocking LPS-induced TNF-α production in HWB, **7a–b** and **12a** were estimated to have a greater therapeutic potential and an improved side effect profile.

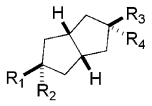
Compound **2a** was nearly fourfold more potent for inhibition of LPS-induced TNF-α production in rats, while it was nearly twofold less potent and slightly less potent for inhibition of bronchoconstriction in guinea pigs and inhibition of TNF-α production in HWB, respectively. Thus, the therapeutic potential of **2a** and Ariflo **1** seemed to be similar. To evaluate the side effect profile by cross-species comparison, compounds **2a, 7a–b** and **12a** were also tested in a ferret emesis model. All of these compounds did not cause emesis at oral doses of up to 3 mg/kg, while most caused emesis at an oral dose of 10 mg/kg.

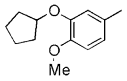
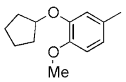
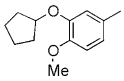
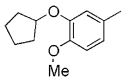
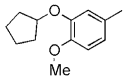
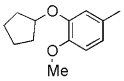
Pharmacokinetic data for compounds **2a** and **7b** obtained after single-dose administration to rats are presented as typical examples of these derivatives in Table 4. Intravenous administration of compounds **2a** and **7b** to rats (3 mg/kg, $n = 3$) resulted in increased and decreased $T_{1/2}$ values of 5.6 and 0.47 h, respectively. The volume of distribution at steady state (V_{ss}) was calculated to be 1190 and 647 ml/kg, respectively. Systemic clearance (CL) was 216 and 1953 ml/h/kg, respectively. The area under the concentration vs. time curve (AUC) was 14.6 and 1.55 μg h/ml, respectively. Oral administration of **2a** and **7b** to rats (10 mg/kg, $n = 3$) yielded a longer and shorter $T_{1/2}$ of 8.1 and 4.2 h, respectively. T_{max} was 2.8 and 0.50 h, respectively, while the AUC was 48.4 and 0.296 μg h/ml, respectively. The bioavailability (BA) of these compounds was calculated to be 99.3% and 5.7%, respectively. Despite its much lower BA (5.7%), **7b** demonstrated a relatively higher efficacy in both of the in vivo models. A contribution of **7a**, which could be produced by in vivo hydrolysis, was presumed to be involved in this improved efficacy.

4. Conclusion

Based on the hypothesis that more rigid spatial fixing of the three pharmacophores (the carboxylic acid, nitrile, and

Table 1



Compd	R_1	R_2	R_3	R_4	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[b] ID_{50} (mg/kg, po)
1 (Ariflo)					10	1.7
2a		CN	H	COOH	9.0	0.4
2b				CONHOH	9.7	(52%)[c]
3		CN	COOH	H	150	3.8
4a	CN		H	COOH	>300	NT[d]
5	CN		COOH	H	>300	NT[d]
2c		H	H	COOH	150	(79%)[c]
4b	H		H	COOH	80	2.2

[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] Not tested.

aromatic moieties) of Ariflo **1** within their optimized stereochemistry using another template could lead to the discovery of a new inhibitor, the design, synthesis, and evaluation of bicyclo[3·3·0]octane derivatives showing more stereochemical diversity than the cyclohexane derivatives was carried out. Among the compounds tested, compounds **2a, 7a–b**, and **12a** were estimated to have improved therapeutic potential with few side effects based on biological data obtained by both cross-species and same-species comparison. Single-dose rat pharmacokinetic data for **2a** and **7b** were also determined. Synthesis and more detailed SAR will be reported elsewhere.

5. Experimental

5.1. Chemistry

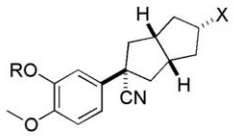
5.1.1. General procedure

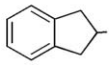
Analytical samples were homogeneous as confirmed by thin-layer chromatography (TLC), and yielded spectroscopic data consistent with the assigned structures. All ^{1}H NMR spectra were obtained with a Varian Gemini-200 or MERCURY-300 spectrometer. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionisation (EI) mass spectra were obtained with a JEOL JMS-DX303HF or JMS-700 spectrometer. Atmospheric pressure chemical ionisation (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}).

*5.1.2. Preparation of compounds **23a** and **23b***

*5.1.2.1. (1*R*,2*S*)-Cyclohex-4-ene-1,2-diyldimethanol (**17**).* To a stirred suspension of $LiAlH_4$ (37 g, 990 mmol) in THF (1.0 l) was added *cis*-1,2,3,6-tetrahydrophthalic anhydride **16** (60.0 g, 394 mmol) at 0 °C. After being stirred for 2.5 h,

Table 2



Compd	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α production in rats[a] ID_{50} (mg/kg, po)
6a (X = COOH)	Me-	160	NT[c]
6b (X = CONHOH)		62	(55%)[d]
7a (X = COOH)	Et-	26	2.9
7b (X = CONHOH)		(56%)[b]	1.8
8a (X = COOH)	*i*-Pr-	62	(57%)[d]
8b (X = CONHOH)		12	(47%)[d]
9a (X = COOH)	*c*-Butyl-	38	(58%)[d]
9b (X = CONHOH)		(53%)[b]	NE[e]
10a (X = COOH)	*c*-hexyl	85	NT[c]
10b (X = CONHOH)		34	NE[e]
11a (X = COOH)		250	NT[c]
11b (X = CONHOH)		250	NT[c]
12a (X = COOH)		1.3	1.7
12b (X = CONHOH)		0.85	NE[e]
13a (X = COOH)		100	NT[c]
13b (X = CONHOH)		29	3.0
14a (X = COOH)	MeS-$(CH_2)_4$-	34	NE[e]
14b (X = CONHOH)		8.5	NE[e]
15a (X = COOH)	CF_3-$(CH_2)_2$-	71	NE[e]
15b (X = CONHOH)		58	NT[c]

[a] See corresponding footnotes from Table 1. [b] Inhibition % at 10 nM. [c] Not tested. d Inhibition % at 3 mg/kg, po. [e] Not effective at 3 mg/kg, po.

the reaction mixture was quenched with MeOH and poured into saturated aq Na_2SO_4. The resulting solid was removed by filtration and washed with EtOAc. The filtrate was diluted with brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give **17** (55.4 g, 390 mmol, 99%) as a yellow oil: TLC R_f = 0.36 (EtOAc); MS (APCI, Pos. 20 V) *m/z* = 143 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.62 (brt, 2H), 3.74 (dd, *J* = 10.8 and 6.6 Hz, 2H), 3.61 (dd, *J* = 10.8 and 3.0 Hz, 2H), 2.40–2.00 (m, 8H).

*5.1.2.2. ((1*R*,6*S*)-6-{[(Methylsulfonyl)oxy]methyl}cyclohex-3-en-1-yl)methyl methanesulfonate (**18**).* To a stirred solution of **17** (8.98 g, 63.2 mmol) and Et_3N (26 ml, 19 mmol) in CH_2Cl_2 (100 ml) was added MsCl (15 ml, 190 mmol) at –78 °C. After being stirred for 1 h, the reaction mixture was diluted with brine, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **18** (18.9 g, quant) as a pale yellow oil: TLC R_f = 0.18 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.67 (t, *J* = 1.5 Hz, 2H), 4.29 (dd, *J* = 10.2 and 7.2 Hz, 2H), 4.17 (dd, *J* = 10.2 and 7.2 Hz, 2H), 3.04 (s, 6H), 2.50–2.35 (m, 2H), 2.35–2.15 (m, 2H), 2.10–1.90 (m, 2H).

*5.1.2.3. (4*R*,5*S*)-4,5-Bis(bromomethyl)cyclohexene (**19**).* The following reaction was carried out under argon atmosphere. To a stirred solution of **18** (18.9 g) in DMF (150 ml) was added LiBr (17 g, 190 mmol). After being stirred at 100 °C for 2.5 h, the reaction mixture was diluted with H_2O/Et_2O. The resulting solid was removed by filtration through a pad of celite, and washed with Et_2O. The filtrate was washed with H_2O and brine, dried over anhydrous

Table 3
Biological profile of **1, 2a, 7a, 7b** and **12a**

Compd	Inhibition of bronchoconstriction[a] ID_{50} (mg/kg, po) OVA challenge (mg/kg, iv) 0.15	0.5	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) 1 (mg/kg, po)	3	10
1 (Ariflo)	4.5	NT[f]	1.7	5.7	18	NT[f]	NT[f]	NT[f]
2a	9.6	NT[f]	0.4	(73%)[g]	21	NT[f]	0/3	0/3
7a	NT[f]	NE[h]	2.9	17	5.8	NT[f]	0/4	1/5
7b	5.0	(49%)[g]	1.8	12	0.80	0/2	0/4	1/4
12a	NT[f]	5.9	1.7	13	2.2	NT[f]	0/5	2/5

[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] Not tested. [g] Inhibition % at 10 mg/kg, po. [h] Not effective at 30 mg/kg, po.

Table 4
Single-dose pharmacokinetic data for compounds **2a** and **7b**

parameter	**2a** iv (3 mg/mL/kg)	**2a** po (10 mg/5mL/kg)	**7b** iv (3 mg/mL/kg)	**7b** po (10 mg/5mL/kg)
C_{max} (μg/mL)		4.22 ± 2.26		0.0908 ± 0.0166
$T_{1/2}$ (h)	5.6 ± 2.7	8.1 ± 3.1	0.47 ± 0.19	4.2 ± 2.4
T_{max} (h)		2.8 ± 2.8		0.50 ± 0.00
AUC (μg·h/mL)	14.6 ± 3.8	48.4 ± 29.3	1.55 ± 0.14	0.296 ± 0.093
V_{ss} (mL/kg)	1190 ± 345		647 ± 87	
CL_{total} (mL/h/kg)	216 ± 63		1953 ± 178	
% bioavail		99.3		5.7

$MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane) to give **19** (6.09 g, 22.9 mmol, 36% in two steps) as a pale yellow oil: TLC R_f = 0.76 (*n*-hexane/EtOAc, 8/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.63 (t, J = 1.2 Hz, 2H), 3.41 (dd, J = 10.2 and 5.4 Hz, 2H), 3.32 (dd, J = 10.2 and 8.4 Hz, 2H), 2.45–2.30 (m, 2H), 2.35–2.20 (m, 2H), 2.15–2.00 (m, 2H).

*5.1.2.4. A mixture (**21**) of (2r,3a*R*,7a*S*)-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-2,3,3a,4,7,7a-hexahydro-1*H*-indene-2-carbonitrile and (2s,3a*R*,7a*S*)-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-2,3,3a,4,7,7a-hexahydro-1*H*-indene-2-carbonitrile.* The following reaction was carried out under argon atmosphere. To a stirred solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)acetonitrile **20** (1.3 g, 5.4 mmol) in THF

(20 ml) was added NaHMDS (1.0 M solution in THF, 12 ml, 12 mmol) at –78 °C. The reaction mixture was stirred at –78 °C for 20 min, and a solution of **19** (799 mg, 3.00 mmol) in THF (10 ml) was added dropwise. After being stirred at room temperature for 2 h, the resulting 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. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 10/1) to give an isomeric mixture **21** (725 mg, 2.15 mmol, 72%; major:minor = 3:1) as a pale yellow oil: TLC R_f = 0.64 (*n*-hexane/EtOAc, 4/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.90 (m, 2H), 6.85–6.80 (m, 1H), 5.83 & 5.75 (m, 2H), 4.81 (m, 1H), 3.84 & 3.84 (s, 3H), 2.70–2.20 (m, 8H), 2.20–2.00 (m, 2H), 2.00–1.70 (m, 6H), 1.70–1.50 (m, 2H).

*5.1.2.5. An isomeric mixture (**22**) of dimethyl 2,2′-{(1*R*, 2*S*,4s)-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl] cyclopentane-1,2-diyl}diacetate and dimethyl 2,2′-{(1*R*, 2*S*,4r)-4-cyano-4-[3-(cyclopentyloxy)-4-methoxy-phenyl] cyclopentane-1,2-diyl}diacetate.* To a stirred solution of **21** (2.78 g, 8.25 mmol) in CH_2Cl_2 (50 ml) was bubbled ozone (O_2 containing ca 3% O_3) at –78 °C for 1 h. To the resulting mixture was added Ph_3P (3.2 g, 12 mmol), and stirring was continued at –78 °C for 30 min. After being stirred at room temperature for 1 h, the resulting mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. TLC R_f = 0.50 (*n*-hexane/EtOAc, 1/2).

To a stirred solution of the above-described residue in *t*-BuOH (50 ml)/2-methyl-2-butene (5.2 ml)/H_2O (10 ml) were added $NaClO_2$ (3.0 g, 33 mmol) and NaH_2PO_4 (2.4 g, 20 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and extracted with 2 N aq NaOH. The aqueous layer was acidified with 2 N aq HCl, and then extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was used for the next reaction without further purification: TLC R_f = 0.39 (EtOAc).

A solution of the above-described residue in Et_2O (60 ml) was treated with CH_2N_2/Et_2O at 0 °C until the evolution of gas subsided. The resulting solution was quenched with AcOH and then evaporated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2/1) to give an isomeric mixture **22** (2.66 g, 6.20 mmol, 75% in three steps; mixture rate was not clear.) as a pale yellow oil: TLC R_f = 0.35 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20V) *m/z* = 430 (M + H)+, 362, 330; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.90 (m, 2H), 6.85–6.80 (m, 1H), 4.81 (m, 1H), 3.84 (s, 3H), 3.70 & 3.70 (s, 6H), 3.10–2.60 (m, 2H), 2.70–2.20 (m, 8H), 2.00–1.75 (m, 6H), 1.80–1.50 (m, 2H).

*5.1.2.6. (2s,3a*R*,6a*S*)-2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-5-oxooctahydropentalene-2-carbonitrile (**23a**) and (2r,3a*R*,6a*S*)-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-5-oxooctahydropentalene-2-carbonitrile (**23b**).* The following reaction was carried out under argon atmosphere. To a stirred solution of **22** (2.66 g, 6.20 mmol) in DME (50 ml) was added NaH (60% in mineral oil, 1.5 g, 37 mmol). After heating under reflux for 1.5 h, the reaction mixture was neutralized with 2 N aq HCl (19 ml), diluted with H_2O, and extracted with Et_2O. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was used for the next reaction without further purification: TLC R_f = 0.61 and 0.53 (*n*-hexane/EtOAc, 2/1).

To a stirred solution of the above-described residue in DMSO (50 ml) and H_2O (5.0 ml) was added NaCl (3.0 g). After being stirred at 165 °C for 1 h, the reaction mixture was diluted with H_2O, and extracted with *n*-hexane/EtOAc. The organic layer was dried over anhydrous $MgSO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4/1) to give **23a** (1.00 g, 2.95 mmol, 48% in two steps) and **23b** (469 mg, 1.38 mmol, 22% in two steps). Compound **23a** was obtained as a pale yellow powder: TLC R_f = 0.29 (*n*-hexane/EtOAc, 2/1); MS (EI, Pos.) *m/z* = 339 (M)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, *J* = 2.1 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.81 (m, 1H), 3.85 (s, 3H), 3.00–2.85 (m, 2H), 2.75–2.55 (m, 4H), 2.40–2.20 (m, 4H), 2.00–1.75 (m, 6H), 1.75–1.55 (m, 2H). Compound **23b** was obtained as a pale yellow powder: TLC R_f = 0.36 (*n*-hexane/EtOAc, 2/1); MS (EI, Pos.) *m/z* = 339 (M)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.90 (m, 2H), 6.84 (d, *J* = 9.0 Hz, 1H), 4.79 (m, 1H), 3.85 (s, 3H), 3.30–3.15 (m, 2H), 2.85 (dd, *J* = 13.2, 7.5 Hz, 2H), 2.68 (dd, *J* = 19.5, 9.9 Hz, 2H), 2.17 (dd, *J* = 19.5, 3.9 Hz, 2H), 2.00–1.80 (m, 8H), 1.70–1.55 (m, 2H).

*5.1.3. Preparation of compounds **25a–25d***

*5.1.3.1. (2s,3a*R*,6a*S*)-2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-5-(1,3-dithian-2-ylidene)octahydropentalene-2-carbonitrile (**24a**).* The following reaction was carried out under argon atmosphere. To a stirred solution of 2-trimethylsilyl-1, 3-dithiane (2.2 g, 12 mmol) in THF (10 ml) was added *n*-BuLi (1.53 M solution in *n*-hexane, 7.5 ml, 12 mmol) at 0 °C, and then the reaction mixture was stirred at 0 °C for 10 min. To this solution was added a solution of **23a** (1.30 g, 3.83 mmol) in THF (5.0 ml) at –78 °C. After being stirred at 0 °C for 30 min, 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, 8/1–6/1) to give **24a** (633 mg, 1.44 mmol, 37%) as a pale yellow amorphous solid and to recover **23a** (700 mg, 2.06 mmol, 54%): TLC R_f = 0.54 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 442 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.82 (d, *J* = 8.0 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.00–2.85 (m, 2H), 2.90–2.75 (m, 2H), 2.80–2.40 (m, 6H), 2.40–2.00 (m, 4H), 2.00–1.70 (m, 8H), 1.80–1.50 (m, 2H).

*5.1.3.2. (2r,3a*R*,6a*S*)-2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-5-(1,3-dithian-2-ylidene)octahydropentalene-2-carbo-*

*nitrile (**24b**).* Compound **24b** was prepared from **23b** in 42% yield according to the same procedure as described for the preparation of **24a** from **23a**. Pale yellow amorphous solid: TLC R_f = 0.69 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 442 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.90 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.10–2.95 (m, 2H), 2.95–2.75 (m, 4H), 2.75–2.55 (m, 4H), 2.47 (dd, *J* = 18.3 and 2.1 Hz, 2H), 2.20–2.10 (m, 2H), 2.00–1.80 (m, 6H), 1.80–1.65 (m, 2H), 1.70–1.55 (m, 2H).

*5.1.3.3. Methyl (2r,3a*R*,5r,6a*S*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**25b**) and methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**25a**).* To a stirred solution of **24a** (548 mg, 1.24 mmol) in MeCN/H_2O (4/1, 10 ml) were added TFA (0.38 ml) and 30% aq H_2O_2 (2.0 ml). After being stirred at 80 °C for 30 min, the reaction mixture was cooled to 40 °C. To this solution was added 2 N aq NaOH (19 ml). After being stirred at 40 °C for 20 min, the reaction mixture was neutralized with 2 N aq HCl (16 ml), and then extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 1/0–20/1) to give a mixture of **2a** and **3**: TLC R_f = 0.52 ($CHCl_3$/MeOH, 10/1).

A solution of a mixture of **2a** and **3** in Et_2O/THF (5/2, 7.0 ml) was treated with CH_2N_2/Et_2O at 0 °C until the evolution of gas subsided. The resulting solution was quenched with AcOH, and then concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 6/1–4/1) to give **25b** (80.4 mg, 0.210 mmol, 18% in two steps) and **25a** (108 mg, 0.282 mmol, 25% in two steps). Compound **25b** was obtained as a pale yellow oil: TLC R_f = 0.65 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 40 V) *m/z* = 384 (M + H)+, 352, 316; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (d, *J* = 1.8 Hz, 1H), 6.90 (dd, *J* = 7.5, 1.8 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.69 (s, 3H), 2.79 (m, 1H), 2.80–2.55 (m, 4H), 2.05–1.80 (m, 12H), 1.70–1.55 (m, 2H). Compound **25a** was obtained as a pale yellow oil: TLC R_f = 0.57 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 40 V) *m/z* = 384 (M + H)+, 352, 316, 284; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (d, *J* = 1.8 Hz, 1H), 6.91 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 2.80 (m, 1H), 2.80–2.55 (m, 2H), 2.60–2.40 (m, 2H), 2.35–2.20 (m, 4H), 2.00–1.75(m, 8H), 1.70–1.50 (m, 2H).

*5.1.3.4. Methyl (2s,3a*R*,5s,6a*S*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**25c**) and methyl (2r,3a*R*,5s,6a*S*)-5-cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**25d**).* Compounds **25c** and **25d** were prepared from **24** according to the same procedures as described for the preparation of **25a** and **25b** from **24a**. Compound **25d** was obtained (10% in two steps) as a white powder: TLC R_f = 0.61 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 384 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.90 (m, 2H), 6.84 (d, *J* = 9.0 Hz, 1H), 4.81 (m, 1H), 3.84 (s, 3H), 3.68 (s, 3H), 3.05–2.90 (m, 3H), 2.70–2.55 (m, 2H), 2.30–2.15 (m, 2H), 2.05–1.60 (m, 12H). Compound **25c** was obtained (35% in two steps) as a pale yellow oil: TLC R_f = 0.58 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 384 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.69 (s, 3H), 3.15–3.00 (m, 2H), 2.81 (m, 1H), 2.70–2.60 (m, 2H), 2.05–1.75 (m, 12H), 1.70–1.55 (m, 2H).

*5.1.4. General procedure for the preparation of compounds **2a, 3, 4a** and **5***

The following compounds **2a, 3, 4a** and **5** were prepared from the corresponding ester derivatives according to the same procedures as described for the preparation of **2a** from **25a**.

*5.1.4.1. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**2a**).* To a stirred solution of **25a** (96.7 mg, 0.252 mmol) in MeOH/THF (2/1, 1.5 ml) was added 1 N aq KOH (0.50 ml). After being stirred at room temperature for 4 h, the reaction mixture was extracted with EtOAc. The aqueous layer was acidified with 2 N aq HCl (0.50 ml), and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual solid was triturated with a mixture of *n*-hexane/Et_2O (9/1) to give **2a** (69.8 mg, 0.189 mmol, 75%) as a white powder: TLC R_f = 0.39 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 40.V) *m/z* = 368 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.82 (d, *J* = 7.8 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 2.84 (m, 1H), 2.90–2.60 (m, 2H), 2.55–2.40 (m, 2H), 2.40–2.20 (m, 4H), 2.10–1.75 (m, 9H), 1.80–1.55 (m, 2H); IR (KBr) 2960, 2872, 2232, 1701, 1591, 1517, 1468, 1443, 1415, 1343, 1252, 1150, 1026, 992; Anal. Found: $C_{22}H_{27}NO_4$ (C, H, N).

*5.1.4.2. (2r,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**3**).* Compound **3** was obtained (91%) as a white powder: TLC R_f = 0.39 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 40 V) *m/z* = 368 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.85 (m, 2H), 6.82 (d, *J* = 8.1 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 3.03 (m, 1H), 2.80–2.65 (m, 2H), 2.70–2.55 (m, 2H), 2.10–1.90 (m, 4H), 1.95–1.80 (m, 9H), 1.70–1.55 (m, 2H); IR (KBr) 2959, 2872, 2232, 1736, 1701, 1590, 1517, 1467, 1445, 1415, 1343, 1292, 1249, 1233, 1169, 1151, 1138, 1027, 990; Anal. Found: $C_{22}H_{27}NO_4 \cdot 0.25H_2O$ (C, H, N).

*5.1.4.3. (2s,3a*R*,5s,6a*S*)-5-Cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**4a**).* Compound **4a** was obtained (83%) as a white powder: TLC R_f = 0.56 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 40 V) *m/z* = 368 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (d, *J* = 2.1 Hz, 1H), 6.91 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.83 (d,

J = 8.1 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.20–3.00 (m, 2H), 2.85 (m, 1H), 2.75–2.60 (m, 2H), 2.05–1.75 (m, 12H), 1.70–1.55 (m, 3H); IR (KBr) 2960, 2871, 2228, 1131, 1702, 1590, 1517, 1464, 1442, 1418, 1265, 1166, 1145, 1027, 855; Anal. Found $C_{22}H_{27}NO_4$ (C, H, N).

*5.1.4.4. (2r,3a*R*,5s,6a*S*)-5-Cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**5**).* Compound **5** was obtained (64%) as a white powder: TLC R_f = 0.56 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 60 V) *m/z* = 368 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.90 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.10–2.95 (m, 3H), 2.70–2.60 (m, 2H), 2.40–2.20 (m, 2H), 2.00–1.60 (m, 10H), 1.70–1.55 (m, 3H); IR (KBr) 2960, 2871, 2228, 1698, 1602, 1589, 1517, 1462, 1442, 1418, 1266, 1166, 1145, 855; Anal. Found: $C_{22}H_{27}NO_4$ (C, H, N).

*5.1.5. Preparation of compound **2b***

*5.1.5.1. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclopentyloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (**2b**).* To a stirred solution of **2a** (490 mg, 1.33 mmol) in DMF (10 ml) were added EDC·HCl (380 mg, 2.0 mmol), HOBt (270 mg, 2.0 mmol) and (1-methoxy-1-methyethyl)oxyamine (700 mg, 6.7 mmol). After being stirred at room temperature for 2 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 short column chromatography on silica gel (*n*-hexane/EtOAc, 2/3-0/1) to give an amide (453 mg) as a white amorphous solid: TLC R_f = 0.55 ($CHCl_3$/MeOH, 10/1).

To a stirred solution of an amide (453 mg) in MeOH (8.0 ml) was added 2 N aq HCl (49 μl, 0.098 mmol, 0.1 eq). After being stirred at room temperature for 2 h, the reaction mixture was concentrated in vacuo. The residue was triturated with *i*-Pr_2O/MeOH to give **2b** (258 mg, 0.673 mmol, two steps 52%) as a white powder: TLC R_f = 0.50 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 40 V) *m/z* = 383 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.40–8.50 (br, 2H), 6.95–6.85 (m, 2H), 6.82 (d, *J* = 8.7 Hz, 1H), 4.79 (m, 1H), 3.84 (s, 3H), 2.80–2.50 (m, 3H), 2.50–2.10 (m, 6H), 2.05–1.70 (m, 8H), 1.70–1.50 (m, 2H); IR (KBr) 3344, 2952, 2870, 2221, 1670, 1590, 1513, 1441, 1417, 1358, 1321, 1301, 1260, 1163, 1140, 1028, 989; Anal. Found: $C_{22}H_{28}N_2O_4 \cdot 0.25H_2O$ (C, H, N).

*5.1.6. Preparation of compound **32***

*5.1.6.1. An isomeric mixture (**27**) of (2r,3a*R*,7a*S*)-2-[3-(benzyloxy)-4-methoxyphenyl]-2,3,3a,4,7,7a-hexahydro-1*H*-indene-2-carbonitrile and (2s,3a*R*,7a*S*)-2-[3-(benzyloxy)-4-methoxyphenyl]-2,3,3a,4,7,7a-hexahydro-1*H*-indene-2-carbonitrile.* An isomeric mixture **27** (major:minor = 3:1) was prepared from **26** in 92% yield according to the same procedures as described for the preparation of **21** from **20**. Pale yellow oil: TLC R_f = 0.61 (*n*-hexane/EtOAc, 3/1); MS (APCI, Pos. 20 V) *m/z* = 360 (M + H)+. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48–7.26 (m, 5H), 7.04 & 7.00 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.18 & 5.16 (s, 2H), 3.89 & 3.88 (s, 3H), 2.70–2.45 (m, 1H), 2.45–1.93 (m, 8H), 1.93–1.70 (m, 1H).

*5.1.6.2. An isomeric mixture (**28**) of dimethyl 2,2′-{(1*R*,2*S*,4s)-4-[3-(benzyloxy)-4-methoxyphenyl]-4-cyanocyclopentane-1,2-diyl}diacetate and dimethyl 2,2′-{(1*R*,2*S*,4r)-4-[3-(benzyloxy)-4-methoxyphenyl]-4-cyanocyclopentane-1,2-diyl}diacetate.* An isomeric mixture **28** was prepared from **27** in 78% yield in three steps according to the same procedures as described for the preparation of **22** from **21**. Pale yellow oil: TLC R_f = 0.38 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 452 (M + H)+; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49–7.26 (m, 5H), 7.00 (m, 1H), 6.93 (d, *J* = 2.2 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 5.18 & 5.16 (s, 2H), 3.89 (s, 3H), 3.70 & 3.70 (s, 6H), 3.10–2.58 (m, 2H), 2.80–2.14 & 1.84–1.72 (m, 8H).

*5.1.6.3. (2s,3a*R*,6a*S*)-2-[3-(Benzyloxy)-4-methoxyphenyl]-5-oxooctahydropentalene-2-carbonitrile (**29a**) and (2r,3a*R*,6a*S*)-2-[3-(benzyloxy)-4-methoxyphenyl]-5-oxooctahydropentalene-2-carbonitrile (**29b**).* An isomeric mixture consisting of **29a** and **29b** was prepared from **28** according to the same procedures as described for the preparation of an isomeric mixture consisting of **23a–b** from **22** and purified by column chromatography on silica gel to afford **29a** and **29b**. Compound **29a** was obtained (48% in two steps) as a white powder: TLC R_f = 0.44 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m/z* = 394 (M + MeOH + H)+, 362 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.28 (m, 5H), 6.98 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 2.1 Hz, 1H), 5.19 (s, 2H), 3.90 (s, 3H), 2.80–2.65 (m, 2H), 2.62–2.48 (m, 4H), 2.30–2.18 (m, 4H). Compound **29b** was obtained as a white powder: TLC R_f = 0.53 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.29 (m, 5H), 6.98 (dd, *J* = 8.5 and 2.3 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.87 (d, *J* = 2.3 Hz, 1H), 5.20 (s, 2H), 3.90 (s, 3H), 2.78–2.64 (m, 2H), 2.62–2.48 (m, 4H), 2.30–2.18 (m, 4H).

*5.1.6.4. An enantiomeric mixture (**30**) of ((3a*S*,5*S*,6a*S*)-5-[3-(benzyloxy)-4-methoxyphenyl]-5-cyano-1,3a,4,5,6,6a-hexahydropentalen-2-yl trifluoromethanesulfonate and (3a*R*,5*R*,6a*R*)-5-[3-(benzyloxy)-4-methoxyphenyl]-5-cyano-1,3a,4,5,6,6a-hexahydropentalen-2-yl trifluoromethanesulfonate.* The following reaction was carried out under argon atmosphere. To a stirred solution of **29a** (11.4 g, 31.4 mmol) in THF (300 ml) was added LiHMDS (1.0 M solution in THF, 38 ml, 38 mmol) at –78 °C. After being stirred at –78 °C for 45 min, Tf_2NPh (12 g, 35 mmol) was added. After being stirred at 0 °C for additional 1 h, 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 a mixture **30** (29.1 g) as a yellow oil: TLC R_f = 0.53 (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.20 (m, 5H), 6.94 (dd, J = 8.3, 2.3 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.85 (d, J = 2.3 Hz, 1H), 5.62 (m, 1H), 5.18 (s, 2H), 3.89 (s, 3H), 3.27–3.15 (m, 1H), 2.90–2.79 (m, 1H), 2.74–2.56 (m, 1H), 2.56–2.40 (m, 2H), 2.30–2.10 (m, 3H).

*5.1.6.5. An enantiomeric mixture (**31**) of methyl (3a*S*,5*S*,6a*S*)-5-[3-(benzyloxy)-4-methoxyphenyl]-5-cyano-1,3a,4,5,6,6a-hexahydropentalene-2-carboxylate and methyl (3a*R*,5*R*,6a*R*)-5-[3-(benzyloxy)-4-methoxyphenyl]-5-cyano-1,3a,4,5,6,6a-hexahydropentalene-2-carboxylate.* To a stirred solution of **30** (29.1 g) in DMF/MeOH (6/1, 350 ml) were added PPh_3 (820 mg, 3.1 mmol), $Pd(OAc)_2$ (350 mg, 1.6 mmol) and Et_3N (8.8 ml, 63 mmol). After being stirred at room temperature overnight 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, 4/1) to give an enantiomeric mixture **31** (8.82 g, 21.9 mmol, 70% in two steps) as a yellow oil: TLC R_f = 0.46 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 404 (M + H)+; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.47–7.26 (m, 5H), 6.96 (dd, J = 8.4 and 2.2 Hz, 1H), 6.88 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.64 (m, 1H), 5.18 (s, 2H), 3.89 (s, 3H), 3.75 (s, 3H), 3.44–3.25 (m, 1H), 2.90–2.35 (m, 5H), 2.27–2.09 (m, 2H).

*5.1.6.6. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-(3-hydroxy-4-methoxyphenyl)octahydropentalene-2-carboxylate (**32**).* A solution of **31** (9.36 g, 23.2 mmol) in MeOH (230 ml) was hydrogenated under atmospheric pressure of H_2 gas in the presence of 10% Pd/C (1.0 g) for 3 days. The catalyst was removed by filtration through a pad of celite, and washed with MeOH. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 3/1) to give **32** (1.93 g, 6.12 mmol, 26%) as a white powder: TLC R_f = 0.48 (*n*-hexane/EtOAc, 1/1); MS (APCI, Neg. 20 V) *m/z* = 314 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98–6.80 (m, 3H), 5.63 (s, 1H), 3.90 (s, 3H), 3.69 (s, 3H), 2.89–2.58 (m, 3H), 2.52–2.40 (m, 2H), 2.32–2.17 (m, 4H), 1.97–1.79 (m, 2H).

*5.1.7. Preparation of compounds **33** and **42***

*5.1.7.1. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-(3,4-dimethoxyphenyl)octahydropentalene-2-carboxylate (**33**).* To a stirred solution of **32** (0.10 g, 0.32 mmol) in DMF (4 ml) were added K_2CO_3 (0.13 g, 0.95 mmol, 3.0 eq) and MeI (60 μl, 0.95 mmol). After being stirred at room temperature for 2 h, the reaction mixture was poured into H_2O, and diluted with EtOAc. The organic layer was washed with H_2O, brine, and dried over anhydrous $MgSO_4$. The resulting solution was concentrated in vacuo to give **33** (0.11 g, quant) as a white powder: TLC R_f = 0.29 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos.20V) *m/z* = 330 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.90 (m, 2H), 6.85–6.81 (m, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.70 (s, 3H), 2.87–2.74 (m, 1H), 2.74–2.62 (m, 2H), 2.52–2.44 (m, 2H), 2.34–2.20 (m, 4H), 1.96–1.84 (m, 2H).

*5.1.7.2. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-(3-isopropoxy-4-methoxyphenyl)octahydropentalene-2-carboxylate (**35**).* To a stirred solution of **32** (0.10 g, 0.32 mmol) in CH_2Cl_2 (4.0 ml) were added *i*-PrOH (30 μl, 0.38 mmol), PPh_3 (0.12 g, 0.48 mmol) and ADDP (0.12 g, 0.48 mmol). After being stirred at room temperature overnight, the reaction mixture was poured into *n*-hexane and the precipitates were removed by filtration. The filtrates and washings were combined and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 3/1) to give **35** (0.11 g, 0.31 mmol, 98%) as a pale yellow oil: TLC R_f = 0.38 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 358 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96–6.92 (m, 2H), 6.85–6.82 (m, 1H), 4.54 (septet, J = 6.0 Hz, 1H), 3.85 (s, 3H), 3.70 (s, 3H), 2.87–2.74 (m, 1H), 2.74–2.60 (m, 2H), 2.51–2.42 (m, 2H), 2.32–2.20 (m, 4H), 1.95–1.83 (m, 2H), 1.37 (d, J = 6.0 Hz, 6H).

*5.1.7.3. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[3-(2,3-dihydro-1*H*-inden-2-yloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**39**).* To a stirred solution of **32** (0.10 g, 0.32 mmol) in THF (3.0 ml) were added indan-2-ol (64 mg, 0.48 mmol), PPh_3 (125 mg, 0.48 mmol) and DIPAD (0.10 ml, 0.51 mmol). After being stirred at room temperature overnight, the reaction mixture was concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2/1) to give **39** (192 mg, quant) as a colorless oil: TLC R_f = 0.65 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m/z* = 432 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.16 (m, 4H), 6.99–6.95 (m, 2H), 6.87–6.83 (m, 1H), 5.25–5.17 (m, 1H), 3.81 (s, 3H), 3.70 (s, 3H), 3.38 (dd, J = 16.7, 6.6 Hz, 2H), 3.23 (dd, J = 16.7, 3.9 Hz, 2H), 2.87–2.61 (m, 3H), 2.51–2.43 (m, 2H), 2.34–2.21 (m, 4H), 1.96–1.84 (m, 2H).

*5.1.7.4. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-(3-ethoxy-4-methoxy-phenyl)octahydropentalene-2-carboxylate (**34**).* Compound **34** was prepared from **32** in quantitative yield according to the same procedures as described for the preparation of **33** from **32**. White powder: TLC R_f = 0.40 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 344 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.89 (m, 2H), 6.85–6.81 (m, 1H), 4.12 (q, J = 7.0 Hz, 2H), 3.87 (s, 3H), 3.70 (s, 3H), 2.86–2.74 (m, 1H), 2.74–2.61 (m, 2H), 2.51–2.43 (m, 2H), 2.32–2.20 (m, 4H), 1.95–1.83 (m, 2H), 1.47 (t, J = 7.0 Hz, 3H).

*5.1.7.5. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[3-(cyclobutyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**36**).* Compound **36** was prepared from **32** in 53% yield according to the same procedures as described for the prepa-

ration of **35** from **32**. Colorless oil: TLC R_f = 0.40 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 370 (M + H)+; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.91 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 4.68 (quintet, *J* = 7.0 Hz, 1H), 3.86 (s, 3H), 3.70 (s, 3H), 2.90–2.60 (m, 3H), 2.56–2.36 (m, 4H), 2.36–2.18 (m, 6H), 1.98–1.66 (m, 4H).

*5.1.7.6. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[3-(cyclohexyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**37**).* Compound **37** was prepared from **32** in 60% yield according to the same procedures as described for the preparation of **35** from **32**. Colorless oil: TLC R_f = 0.54 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 398 (M + H)+; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.97–6.91 (m, 2H), 6.86–6.81 (m, 1H), 4.26–4.10 (m, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 2.90–2.56 (m, 3H), 2.53–2.40 (m, 2H), 2.33–2.18 (m, 4H), 2.08–1.68 (m, 6H), 1.68–1.42 (m, 2H), 1.42–1.22 (m, 4H).

*5.1.7.7. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[4-methoxy-3-(tetrahydro-2H-pyran-4-yloxy)phenyl]octahydropentalene-2-carboxylate (**38**).* Compound **38** was prepared from **32** in 79% yield according to the same procedures as described for the preparation of **35** from **32**. Colorless oil: TLC R_f = 0.39 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m/z* = 400 (M + H)+; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03–6.96 (m, 2H), 6.89–6.83 (m, 1H), 4.50–4.34 (m, 1H), 4.08–3.95 (m, 2H), 3.85 (s, 3H), 3.70 (s, 3H), 3.60–3.48 (m, 2H), 2.89–2.58 (m, 3H), 2.51–2.39 (m, 2H), 2.33–2.18 (m, 4H), 2.08–1.76 (m, 6H).

*5.1.7.8. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[3-(cyclopropylmethoxy)-4-methoxyphenyl]octahydropentalene-2-carboxylate (**40**).* Compound **40** was prepared from **32** in quantitative yield according to the same procedures as described for the preparation of **33** from **32**. White powder: TLC R_f = 0.70 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m/z* = 370 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.90 (m, 2H), 6.85–6.80 (m, 1H), 3.88–3.84 (m, 5H), 3.70 (s, 3H), 2.85–2.74 (m, 1H), 2.74–2.60 (m, 2H), 2.55–2.45 (m, 2H), 2.36–2.19 (m, 4H), 1.96–1.82 (m, 2H), 1.40–1.22 (m, 1H), 0.70–0.60 (m, 2H), 0.41–0.35 (m, 2H).

*5.1.7.9. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-{4-methoxy-3-[4-(methylsulfanyl)butoxy]phenyl}octahydropentalene-2-carboxylate (**41**).* Compound **41** was prepared from **32** in quantitative yield according to the same procedures as described for the preparation of **35** from **32**. Colorless oil: TLC R_f = 0.61 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos. 20 V) *m/z* = 418 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.90 (m, 2H), 6.85–6.81 (m, 1H), 4.05 (t, *J* = 6.3 Hz, 2H), 3.85 (s, 3H), 3.70 (s, 3H), 2.86–2.74 (m, 1H), 2.74–2.62 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.51–2.43 (m, 2H), 2.32–2.20 (m, 4H), 2.11 (s, 3H), 2.01–1.75 (m, 4H), 1.73–1.66 (m, 2H).

*5.1.7.10. Methyl (2s,3a*R*,5r,6a*S*)-5-cyano-5-[4-methoxy-3-(3,3,3-trifluoropropoxy)phenyl]octahydropentalene-2-carboxylate (**42**).* Compound **42** was prepared from **32** in 58% yield according to the same procedures as described for the preparation of **35** from **32**. Colorless oil: TLC R_f = 0.46 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos. 20 V) *m/z* = 412 (M + H)+; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.25 (t, *J* = 6.8 Hz, 2H), 3.86 (s, 3H), 3.70 (s, 3H), 2.87–2.60 (m, 5H), 2.50–2.42 (m, 2H), 2.33–2.20 (m, 4H), 1.96–1.84 (m, 2H).

*5.1.8. General procedure for the preparation of compounds **6a–15a***

The following compounds **6a–15a** were prepared from the corresponding ester derivatives **33–42**, respectively, according to the same procedures as described for the preparation of **6a** from **33**.

*5.1.8.1. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-(3,4-dimethoxyphenyl) octahydropentalene-2-carboxylic acid (**6a**).* To a stirred solution of **33** (0.11 g, 0.32 mmol) in MeOH/THF (1/1, 4.0 ml) was added 1 N aq NaOH (1.6 ml). After being stirred at room temperature for 1 h, the reaction mixture was acidified with 1 N aq HCl, and extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residual solid was triturated with *n*-hexane/EtOAc to give **6a** (0.10 g, 0.32 mmol, 99%) as a white powder: TLC R_f = 0.53 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 314 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96–6.91 (m, 2H), 6.85–6.81 (m, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 2.91–2.78 (m, 1H), 2.78–2.65 (m, 2H), 2.52–2.44 (m, 2H), 2.36–2.25 (m, 4H), 2.02–1.90 (m, 2H), 1.60 (br, 1H); IR (KBr) 3449, 2941, 2879, 2838, 2227, 1690, 1589, 1521, 1465, 1414, 1291, 1256, 1214, 1148, 1024, 873; Anal. Found: $C_{18}H_{21}NO_4$ (C, H, N).

*5.1.8.2. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-(3-ethoxy-4-methoxyphenyl)octahydropentalene-2-carboxylic acid (**7a**).* Compound **7a** was obtained (quant) from **34** as a white powder: TLC R_f = 0.53 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 328 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.90 (m, 2H), 6.85–6.81 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 3H), 2.90–2.78 (m, 1H), 2.78–2.64 (m, 2H), 2.51–2.43 (m, 2H), 2.35–2.25 (m, 4H), 2.00–1.89 (m, 2H), 1.55 (br, 1H), 1.48 (t, *J* = 7.1 Hz, 3H); IR (KBr) 3450, 2927, 2216, 1690, 1590, 1522, 1466, 1445, 1420, 1326, 1296, 1251, 1215, 1165, 1147, 1040, 929; Anal. Found: $C_{19}H_{23}NO_4$ (C, H, N).

*5.1.8.3. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-(3-isopropoxy-4-methoxy-phenyl)octahydropentalene-2-carboxylic acid (**8a**).* Compound **8a** was obtained (98%) from **35** as a white powder: TLC R_f = 0.53 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 342 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96–6.92 (m, 2H), 6.86–6.82 (m, 1H), 4.54 (septet,

J = 6.0 Hz, 1H), 3.85 (s, 3H), 2.90–2.78 (m, 1H), 2.77–2.64 (m, 2H), 2.50–2.42 (m, 2H), 2.34–2.24 (m, 4H), 2.00–1.88 (m, 2H), 1.60 (br, 1H), 1.3 7 (d, *J* = 6.0 Hz, 6H); IR (KBr) 2973, 2220, 1705, 1590, 1518, 1466, 1442, 1417, 1384, 1372, 1330, 1292, 1257, 1218, 1162, 1146, 1112, 1031, 947; Anal. Found: $C_{20}H_{25}NO_4$ (C, H, N).

*5.1.8.4. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclobutyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**9a**).* Compound **9a** was obtained (97%) from **36** as a white powder: TLC R_f = 0.57 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 354 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 4.68 (quintet, *J* = 7.5 Hz, 1H), 3.86 (s, 3H), 2.91–2.78 (m, 1H), 2.78–2.64 (m, 2H), 2.54–2.41 (m, 4H), 2.36–2.19 (m, 6H), 2.00–1.81 (m, 3H), 1.78–1.62 (m, 2H); IR (KBr) 3325, 2963, 2876, 2230, 1733, 1592, 1519, 1466, 1446, 1421, 1400, 1357, 1308, 1260, 1244, 1194, 1148, 1073, 1019, 945; Anal. Found: $C_{21}H_{25}NO_4$ (C, H, N).

*5.1.8.5. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclohexyloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**10a**).* Compound **10a** was obtained (90%) from **37** as a white powder: TLC R_f = 0.68 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 382 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97–6.92 (m, 2H), 6.86–6.82 (m, 1H), 4.24–4.14 (m, 1H), 3.84 (s, 3H), 2.90–2.78 (m, 1H), 2.76–2.64 (m, 2H), 2.50–2.42 (m, 2H), 2.34–2.24 (m, 4H), 2.05–1.88 (m, 4H), 1.88–1.78 (m, 2H), 1.63–1.49 (m, 3H), 1.42–1.25 (m, 4H); IR (KBr) 3431, 2936, 2858, 2225, 1701, 1604, 1586, 1517, 1438, 1420, 1297, 1254, 1219, 1145, 1043, 1022, 959; Anal. Found: $C_{23}H_{29}NO_4 \cdot 0.25H_2O$ (C, H, N).

*5.1.8.6. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[4-methoxy-3-(tetrahydro-2H-pyran-4-yloxy)phenyl]octahydropentalene-2-carboxylic acid (**11a**).* Compound **11a** was obtained (94%) from **38** as a white powder: TLC R_f = 0.49 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 384 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02–6.97 (m, 2H), 6.88–6.84 (m, 1H), 4.47–4.38 (m, 1H), 4.06–3.98 (m, 2H), 3.85 (s, 3H), 3.59–3.50 (m, 2H), 2.90–2.78 (m, 1H), 2.78–2.64 (m, 2H), 2.49–2.40 (m, 2H), 2.34–2.25 (m, 4H), 2.05–1.91 (m, 4H), 1.91–1.77 (m, 2H), 1.56 (br, 1H); IR (KBr) 3435, 2958, 2217, 1732, 1708, 1590, 1519, 1417, 1303, 1259, 1146, 1067, 1051, 1028, 853; Anal. Found: $C_{22}H_{27}NO_5 \cdot 0.25H_2O$ (C, H, N).

*5.1.8.7. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(2,3-dihydro-1*H*-inden-2-yloxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**12a**).* Compound **12a** was obtained (90%) from **39** as a white powder: TLC R_f = 0.52 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 416 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.16 (m, 4H), 6.99–6.95 (m, 2H), 6.87–6.83 (m, 1H), 5.25–5.17 (m, 1H), 3.81 (s, 3H), 3.38 (dd, *J* = 16.5, 6.5 Hz, 2H), 3.21 (dd, *J* = 16.5, 3.9 Hz, 2H), 2.91–2.78 (m, 1H), 2.78–2.65 (m, 2H), 2.51–2.43 (m, 2H), 2.36–2.25 (m, 4H), 2.01–1.89 (m, 2H), 1.80–1.30 (br, 1H); IR (KBr) 3271, 3071, 3042, 3000, 2958, 2906, 2875, 2836, 2231, 1740, 1705, 1594, 1515, 1465, 1441, 1417, 1402, 1352, 1332, 1254, 1207, 1173, 1152, 1081, 1020, 953; Anal. Found: $C_{26}H_{27}NO_4$ (C, H, N).

*5.1.8.8. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclopropylmethoxy)-4-methoxyphenyl]octahydropentalene-2-carboxylic acid (**13a**).* Compound **13a** was obtained (70%) from **40** as a white powder: TLC R_f = 0.40 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 354 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.90 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 3.87 (s, 3H), 3.87 (d, *J* = 6.9 Hz, 2H), 2.90–2.76 (m, 1H), 2.76–2.64 (m, 2H), 2.52–2.40 (m, 2H), 2.36–2.24 (m, 4H), 2.00–1.85 (m, 2H), 1.62 (brs, 1H), 1.42–1.22 (m, 1H), 0.70–0.62 (m, 2H), 0.42–0.34 (m, 2H); IR (KBr) 3225, 3000, 2969, 2898, 2841, 2244, 2034, 1738, 1591, 1519, 1470, 1448, 1407, 1323, 1285, 1246, 1203, 1188, 1170; Anal. Found: $C_{21}H_{25}NO_4 \cdot 0.5H_2O$ (C, H, N).

*5.1.8.9. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-{4-methoxy-3-[4-(methylsulfanyl)butoxy]phenyl}octahydropentalene-2-carboxylic acid (**14a**).* Compound **14a** was obtained (86%) from **41** as a white powder: TLC R_f = 0.48 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 402 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.91 (m, 2H), 6.85–6.81 (m, 1H), 4.06 (t, *J* = 6.3 Hz, 2H), 3.86 (s, 3H), 2.91–2.78 (m, 1H), 2.78–2.65 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.51–2.43 (m, 2H), 2.35–2.25 (m, 4H), 2.12 (s, 3H), 2.01–1.88 (m, 4H), 1.88–1.75 (m, 2H), 1.61 (br, 1H); IR (KBr) 3436, 2963, 2838, 2218, 1695, 1637, 1589, 1519, 1465, 1420, 1331, 1295, 1260, 1212, 1163, 1146, 1060, 1022, 936; Anal. Found: $C_{22}H_{29}NO_4S$ (C, H, N, S).

*5.1.8.10. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[4-methoxy-3-(3,3,3-trifluoropropoxy)phenyl]octahydropentalene-2-carboxylic acid (**15a**).* Compound **15a** was obtained (79%) from **42** as a white powder: TLC R_f = 0.68 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 369 (M – H)–; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.95 (d, *J* = 2.1 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 4.26 (t, *J* = 6.9 Hz, 2H), 3.86 (s, 3H), 2.91–2.78 (m, 1H), 2.78–2.60 (m, 4H), 2.50–2.41 (m, 2H), 2.36–2.25 (m, 4 H), 2.01–1.90 (m, 2H), 1.90–1.40 (br, 1H); IR (KBr) 2964, 2887, 2226, 1698, 1603, 1588, 1521, 1462, 1441, 1414, 1349, 1327, 1290, 1246, 1219, 1196, 1156, 1143, 1042, 1024, 1004, 905; Anal. Found: $C_{20}H_{22}F_3NO_4$ (C, H, N).

*5.1.9. General procedure for the preparation of compounds **6b** and **15b***

The following compounds **6b–15b** were prepared from the corresponding carboxylic acid derivatives **6a–15a**, respectively, according to the same procedures as described for the preparation of **2b** from **2a**.

*5.1.9.1. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-(3,4-dimethoxyphenyl)-N-hydroxyoctahydropentalene-2-carboxamide (**6b**).* Compound **6b** was obtained (49%) from **6a** as a white powder:

TLC R_f = 0.50 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) *m/z* = 331 (M + H)+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 & 9.93 (br, 1H), 9.00 & 8.68 (br, 1H), 7.00–6.91 (m, 3H), 3.77 (s, 3H), 3.74 (s, 3H), 2.64–2.37 (m, 5H), 2.28–2.18 (m, 2H), 2.02–1.92 (m, 2H), 1.77–1.66 (m, 2H); IR (KBr) 3403, 3222, 3019, 2943, 2835, 2220, 1683, 1653, 1592, 1518, 1457, 1412, 1329, 1257, 1146, 1062, 1023, 859; HRMS (FAB) calcd for $C_{18}H_{23}N_2O_4$ (M + H)+ *m/z* = 331.1658, found 331.1636.

*5.1.9.2. 2s,3a*R*,5r,6a*S*)-5-Cyano-5-(3-ethoxy-4-methoxyphenyl)-N-hydroxyoctahydropentalene-2-carboxamide (**7b**).* Compound **7b** was obtained (30%) from **7a** as a white powder: TLC R_f = 0.43 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) *m/z* = 345 (M + H)+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 & 9.98 (br, 1H), 10.17 & 9.75 (br, 1H), 7.00–6.91 (m, 3H), 4.03 (q, *J* = 6.9 Hz, 2H), 3.74 (s, 3H), 2.68–2.36 (m, 5H), 2.26–2.17 (m, 2H), 2.02–1.92 (m, 2H), 1.77–1.65 (m, 2H), 1.31 (t, *J* = 6.9 Hz, 3H); IR (KBr) 3192, 2976, 2220, 1655, 1523, 1465, 1446, 1419, 1391, 1329, 1258, 1146, 1041, 1025, 930; Anal. Found: $C_{19}H_{24}N_2O_4$ (C, H, N).

*5.1.9.3. (2s,3a*R*,5r,6a*S*)-5-Cyano-*N*-hydroxy-5-(3-isopropoxy-4-methoxyphenyl)octahydropentalene-2-carboxamide (**8b**).* Compound **8b** was obtained (43%) from **8a** as a white powder: TLC R_f = 0.45 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 357 (M – H)–; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 & 9.75 (br, 1H), 8.99 & 8.68 (br, 1H), 7.00–6.92 (m, 3H), 4.57 (septet, *J* = 6.0 Hz, 1H), 3.73 (s, 3H), 2.64–2.37 (m, 5H), 2.24–2.17 (m, 2H), 2.02–1.92 (m, 2H), 1.77–1.65 (m, 2H), 1.23 (d, *J* = 6.0 Hz, 6H); IR (KBr) 3434, 3185, 2973, 2233, 1627, 1520, 1466, 1418, 1376, 1256, 1223, 1176, 1148, 1110, 1020, 989; Anal. Found: $C_{20}H_{26}N_2O_4{\cdot}0.5H_2O$ (C, H, N).

*5.1.9.4. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclobutyloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (**9b**).* Compound **9b** was obtained (57%) from **9a** as a white powder: TLC R_f = 0.27 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 369 (M – H)–; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (d, *J* = 1.5 Hz, 1H), 8.68 (d, *J* = 1.5 Hz, 1H), 6.98–6.91 (m, 2H), 6.83–6.79 (m, 1H), 4.70 (quintet, *J* = 7.7 Hz, 1H), 3.74 (s, 3H), 2.62–2.33 (m, 7H), 2.24–2.18 (m, 2H), 2.10–1.92 (m, 4H), 1.82–1.54 (m, 4H); IR (KBr) 3234, 2939, 2231, 1627, 1519, 1465, 1443, 1417, 1259, 1147, 1077, 1023, 959; HRMS (FAB) calcd for $C_{21}H_{27}N_2O_4$ (M + H)+ *m/z* = 371.1971, found 371.1988.

*5.1.9.5. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclohexyloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (**10b**).* Compound **10b** was obtained (76%) from **10a** as a white powder: TLC R_f = 0.38 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 397 (M – H)–; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 & 9.75 (s, 1H), 8.99 & 8.68 (s, 1H), 7.00–6.92 (m, 3H), 4.35–4.24 (m, 1H), 3.74 (s, 3H), 2.64–2.36 (m, 5H), 2.25–2.16 (m, 2H), 2.03–1.92 (m, 2H), 1.90–1.83 (m, 2H), 1.77–1.64 (m, 4H), 1.55–1 .22 (m, 6H); IR (KBr) 3423, 2937, 2860, 2232, 1655, 1509, 1446, 1415, 1259, 1145, 1020, 855; Anal. Found: $C_{23}H_{30}N_2O_4{\cdot}0.25H_2O$ (C, H, N).

*5.1.9.6. (2s,3a*R*,5r,6a*S*)-5-Cyano-*N*-hydroxy-5-[4-methoxy-3-(tetrahydro-2*H*-pyran-4-yloxy)phenyl]octahydropentalene-2-carboxamide (**11b**).* Compound **11b** was obtained (63%) from **11a** as a white powder: TLC R_f = 0.30 ($CHCl_3$/MeOH, 10/1); MS (APCI, Neg. 20 V) *m/z* = 399 (M – H)-; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 & 9.75 (s, 1H), 8.99 & 8.67 (s, 1H), 7.06–6.95 (m, 3H), 4.57–4.46 (m, 1H), 3.88–3.80 (m, 2H), 3.75 (s, 3H), 3.49–3.40 (m, 2H), 2.57–2.37 (m, 5H), 2.30–2.18 (m, 2H), 2.02–1.85 (m, 4H), 1.77–1 .66 (m, 2H), 1.64–1.50 (m, 2H); IR (KBr) 3250, 2956, 2231, 1659, 1515, 1467, 1416, 1362, 1258, 1184, 1146, 1088, 1031, 989; HRMS (FAB) calcd for $C_{22}H_{29}N_2O_5$ (M + H)+ *m/z* = 401.2076, found 401.2101.

*5.1.9.7. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(2,3-dihydro-1*H*-inden-2-yloxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (**12b**).* Compound **12b** was obtained (34%) from **12a** as a white powder: TLC R_f = 0.31 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) *m/z* = 433 (M + H)+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.67 (s, 1H), 7.28–7.24 (m, 2H), 7.19–7.14 (m, 2H), 7.04–6.98 (m, 2H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.30–5.22 (m, 1H), 3.69 (s, 3H), 3.38–3.30 (m, 2H), 3.01 (dd, *J* = 17.1, 2.1 Hz, 2H), 2.65–2.36 (m, 5H), 2.28–2.21 (m, 2H), 2.04–1.94 (m, 2H), 1.79–1.67 (m, 2H); IR (KBr) 3390, 3239, 2960, 2904, 2836, 2218, 1655, 1591, 1515, 1460, 1442, 1416, 1355, 1330, 1251, 1208, 1150, 1119, 1020, 952; Anal. Found: $C_{26}H_{28}N_2O_4{\cdot}0.25H_2O$ (C, H, N).

*5.1.9.8. (2s,3a*R*,5r,6a*S*)-5-Cyano-5-[3-(cyclopropylmethoxy)-4-methoxyphenyl]-*N*-hydroxyoctahydropentalene-2-carboxamide (**13b**).* Compound **13b** was obtained (28%) from **13a** as a white powder: TLC R_f = 0.40 ($CHCl_3$/MeOH, 9/1); MS (APCI, Neg. 20 V) *m/z* = 369 (M – H)–; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.68 (s, 1H), 7.02–6.94 (m, 3H), 3.81 (d, *J* = 6.9 Hz, 2H), 3.75 (s, 3H), 2.62–2.38 (m, 5H), 2.28–2.14 (m, 2H), 2.02–1.90 (m, 2H), 1.78–1.62 (m, 2H), 1.28–1.12 (m, 1H), 0.60–0.52 (m, 2H), 0.36–0.26 (m, 2H); IR (KBr) 3213, 2935, 2359, 2232, 1619, 1519, 1465, 1407, 1258, 1145, 1021; Anal. Found: $C_{21}H_{26}N_2O_4{\cdot}0.25H_2O$ (C, H, N).

*5.1.9.9. (2s,3a*R*,5r,6a*S*)-5-Cyano-*N*-hydroxy-5-{4-methoxy-3-[4-(methylsulfanyl)butoxy]phenyl}octahydropentalene-2-carboxamide (**14b**).* Compound **14b** was obtained (55%) from **14a** as a white powder: TLC R_f = 0.38 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) *m/z* = 419 (M + H)+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.68 (s, 1H), 6.99–6.91 (m, 3H), 3.99 (t, *J* = 6.2 Hz, 2H), 3.74 (s, 3H), 2.60–2.38 (m, 7H), 2.24–2.18 (m, 2H), 2.03 (s, 3H), 2.05–1.92 (m, 2H), 1.83–1.63 (m, 6H); IR (KBr) 3435, 3188, 3013, 2955, 1836,

2233, 1626, 1520, 1467, 1421, 1388, 1257, 1224, 1149, 1022, 989; Anal. Found: $C_{22}H_{30}N_2O_4S$ (C, H, N, S).

*5.1.9.10. (2s,3a*R*,5r,6a*S*)-5-Cyano-*N*-hydroxy-5-[4-methoxy-3-(3,3,3-trifluoropropoxy)phenyl]octahydropentalene-2-carboxamide (**15b**).* Compound **15b** was obtained (44%) from **15a** as a white powder: TLC R_f = 0.35 ($CHCl_3$/MeOH, 10/1); MS (FAB, Pos.) *m/z* = 413 (M + H)+; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.66 (s, 1H), 7.05–7.01 (m, 2H), 6.99–6.95 (m, 1H), 4.21 (t, *J* = 6.2 Hz, 2H), 3.75 (s, 3H), 2.85–2.69 (m, 2H), 2.64–2.38 (m, 5H), 2.28–2.18 (m, 2H), 2.02–1.93 (m, 2H), 1.78–1.66 (m, 2H); IR (KBr) 3193, 2961, 2234, 1627, 1521, 1467, 1420, 1395, 1258, 1223, 1150, 1071, 1022, 854; HRMS (FAB) calcd for $C_{20}H_{24}F_3N_2O_4$ (M + H)+ *m/z* = 413.1688, found 413.1678.

5.2. X-ray crystallography

All diffraction data were obtained using a Rigaku AFC5R 4-circle diffractometer with graphite monochromate Cu-Kα (λ = 1.54187 Å) radiation and an RU-200 X-ray generator. Data collection was carried out at room temperature. Correction was done for Lorentz polarization absorption and decay. The software package teXsan [18] was used for analysis and for drawing figures. The positions of non-H atoms were easily determined using the program SHELXS86 [19], while the positions of the H atoms were deduced from the non-H atom coordinations and confirmed by Fourier synthesis. Then the non-H atoms were refined with anisotropic temperature parameters, and H atoms were refined w ith isotropic parameters.

5.2.1. Summary of the X-ray crystallographic studies

Compounds	**23b**	**23a**	**3**	**2a**
Lattice parameter	*a*(Å) 10.357(4)	8.248(6)	11.14(2)	11.135(6)
	b(Å) 15.537(3)	22.814(6)	7.561(1)	21.07(1)
	c(Å) 6.248(2)	10.107(7)	9.98(1)	9.962(4)
	α(°) 95.52(2)		92.9(1)	92.05(4)
	β(°) 104.46(2)	105.53(6)	114.8(1)	14.95(3)
	γ(°) 106.76(2)		86.8(1)	87.20(5)
Volume (Å^3)	916.7(5)	1832(2)	2108(5)	2116(1)
Space group	P-1	P21/a	P-1	P-1
No. observed reflections (I>3.00σ(I))	1758	2314	2309	2091
No. variables	226	226	509	511
R-factor	0.063	0.055	0.066	0.099
Rw	0.049	0.085	0.076	0.105

$R = \Sigma(|F_{obs}| - |F_{calc}||)/\Sigma|F_{obs}|$

$Rw = [(\Sigma\, w(|F_{obs}| - |F_{calc}|)^2/\Sigma\, w\, F_{obs}{}^2)]^{1/2}$

5.3. Pharmacology

5.3.1. Assay of human PDE4 activity

The method of Reeves et al. [20] 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-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 *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 3–4 different concentrations of each test compound, and the IC_{50} values were determined.

5.3.2. 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 i.v.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:

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

where C is the plasma TNF-α concentration in LPS-treated animals pretreated with a test compound, L is the plasma TNF-α concentration in LPS-treated animals pretreated with saline, S is the plasma TNF-α concentration in saline-treated animals pretreated with saline.

5.3.3. 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 i.v. 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).

5.3.4. Gastric emptying in rats

Male Sprague–Dawley rats were fasted overnight and were orally administered test compounds or 0.5w/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:

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%.

5.3.5. Inhibitory activity on LPS-induced TNF-α production in HWB

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 3–4 different concentrations of each test compound, and the IC_{50} values were determined.

5.3.6. 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.

5.3.7. Pharmacokinetic study

Male Sprague–Dawley (SD) rats weighing 280 ± 20 g were fasted for 20 h before and during the experiment. Test compounds suspended in 0.5% methylcellulose were administered orally at a dose of 10 mg/5 ml/kg (compound **2a** and **7b**). Blood samples were taken from the jugular vein into a heparinized syringe at 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 h after oral administration.

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