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Synthesis and biological evaluation of novel phthalazinone derivatives as topically active phosphodiesterase 4 inhibitors

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ABSTRACT

Inhibitors of phosphodiesterase 4 (PDE4) are an important class of anti-inflammatory drug that act by inhibiting the production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α). We have synthesized and evaluated a series of 2-substituted phthalazinone derivatives as PDE4 inhibitors. Structure–activity relationship studies led to the identification of benzylamine-substituted phthalazinones as potent PDE4 inhibitors that also suppressed TNF-α production by whole rat blood cells. The most potent of these, when topically administered, were effective in a mouse model of dermatitis.

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs), which catalyze the hydrolysis of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to the corresponding nucleoside 5′-monophosphates, are important regulators of intracellular cyclic nucleotide signaling. To date, eleven families of PDE isoenzyme have been identified in mammals on the basis of their amino acid sequence, substrate specificity (cAMP or cGMP), tissue distribution, and other characteristics.[1] Among these isoenzymes, PDE4 is a high-affinity cAMP-specific phosphodiesterase that is expressed in immune and inflammatory cells such as eosinophils, macrophages, T-lymphocytes and neutrophils.[2,3] Elevation of cAMP through inhibition of PDE4 results in a wide range of anti-inflammatory effects in leukocytes.[1] Therefore PDE4 inhibitors are expected to be effective in the treatment of inflammatory diseases such as asthma,[4,5] chronic obstructive pulmonary disease[6,7] and atopic dermatitis.[8] Overproduction of tumor necrosis factor-α (TNF-α) is associated with a number of autoimmune and inflammatory diseases. PDE4 inhibition leads to suppression of TNF-α production through the activation of inducible cAMP early repressor (ICER),[9,10] an effect that plays a key role in the anti-inflammatory activity of PDE4 inhibitors.[11]

The first-generation PDE4 inhibitor rolipram (Fig. 1) has been the starting point for many medicinal chemistry studies.[12] However, the therapeutic usefulness of first-generation PDE4 inhibitors, which are orally taken, is limited by their side effects, including gastrointestinal side effects such as nausea and vomiting.[13] It has been hypothesized that these side effects could be due either to binding at a high-affinity allosteric binding site of PDE4 (the so-called high-affinity rolipram binding site)[14–16] or to the activation of emetic centers within the central nervous system (CNS).[13] Although a few PDE4 inhibitors such as roflumilast (Fig. 1) have been evaluated in clinical trials, most have gastrointestinal side effects.[17,18]

As an alternative to oral administration, topical application of PDE4 inhibitors is a possible way to maximize their efficacy in the treatment of inflammatory disease while minimizing their side

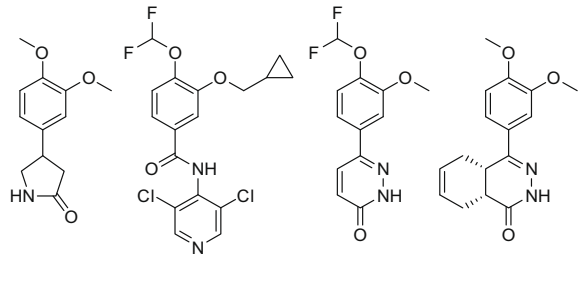

Figure 1. Chemical structures of PDE4 inhibitors.

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effects. Since topical administration minimizes systemic exposure, we would not be particularly concerned about binding at the high-affinity rolipram binding site or penetration into the CNS.[19–22]

The aim of the present study was to develop potent, topically active PDE4 inhibitors with minimal gastrointestinal side effects. We first performed a 3D docking study to identify a lead compound and to obtain guidance for chemical-modification studies. We then carried out structure–activity relationship studies on derivatives of phthalazinone **1**[23] and identified several highly potent PDE4 inhibitors that were topically active in a mouse model of dermatitis.

2. Results and discussion

2.1. Design and strategy

We made extensive use of the publicly available X-ray structures of the complexes of PDE4 with zardaverine (Fig. 1; Protein Data Bank (PDB) entry 1MKD[24]) and PDE4D2 with rolipram (PDB entry 1OYN[25]). We first computed the shape of the active-site pocket of PDE4 by using the 3D atomic coordinates of the enzyme, and then we calculated the surface properties of the active-site pocket with the molecular modeling suite Molecular Operating Environment (MOE). In the resulting model (Fig. 2), the white and red spheres indicate the predicted locations of an inhibitor's atoms, with zardaverine shown for reference. The spheres are classified as either hydrophilic (red) or hydrophobic (white) depending on whether or not they are in good hydrogen-bonding locations. Regions of the surface of the active-site pocket are colored according to whether nearby interior atoms are hydrophobic (green regions) or hydrophilic (blue regions), while purple indicates regions where hydrogen bonds are likely to form.

The major part of the surface is shown in green, indicating the hydrophobic nature of the active-site pocket (Fig. 2). The pocket is seen to be large, and zardaverine occupies only a part of it. Considerable hydrophobic space is available near zardaverine, as indicated by the white spheres, so extensive chemical modification should be possible in this region. To identify a promising lead compound, we manually docked known PDE4 inhibitors into the active-site pocket by reference to the available cocrystal X-ray structures 1MKD, 1OYN, 1PTW,[26] and 1Q9 M.[25] We selected the racemic phthalazinone **1**[23] for further study because (1) the cyclohexene ring of one isomer filled the active-site pocket much better than zardaverine did, and (2) the presence of the nitrogen atom at the 2-position of the phthalazinone ring permitted extensive chemical modification at that position (Fig. 3). We chose the *cis*-isomer of phthalazinone because, according to a previous study,[23] it was likely to have higher activity than the *trans*-isomer.

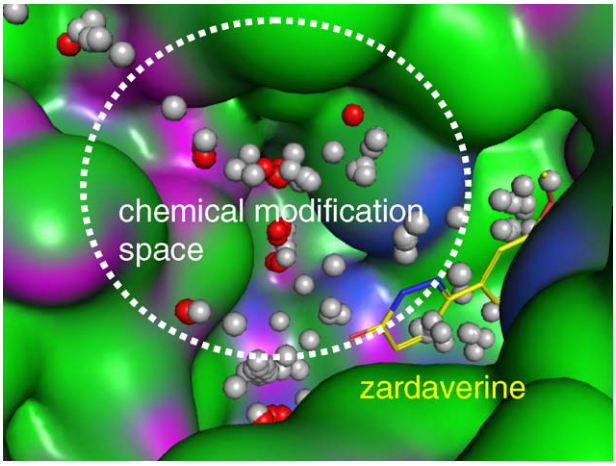


Figure 2. Structural properties of the PDE4 active-site pocket. Spheres indicate the probable locations of ligand atoms and are classified as either hydrophilic (red) or hydrophobic (white), depending on whether or not they are in good hydrogen-bonding locations. The green regions on the surface are hydrophobic and the blue regions are hydrophilic, while purple indicates regions in which hydrogen bonds are likely to form.

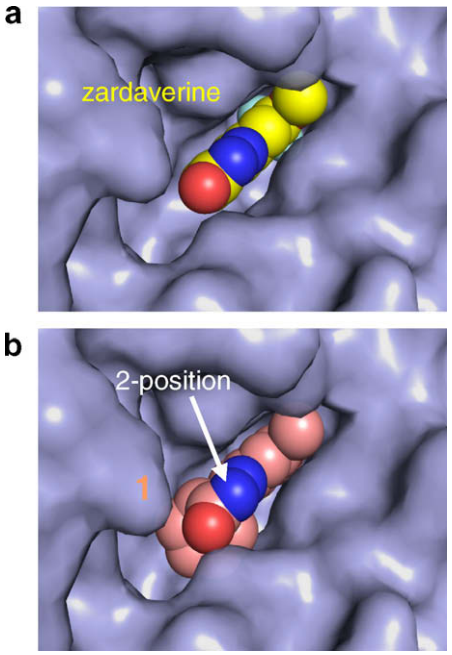
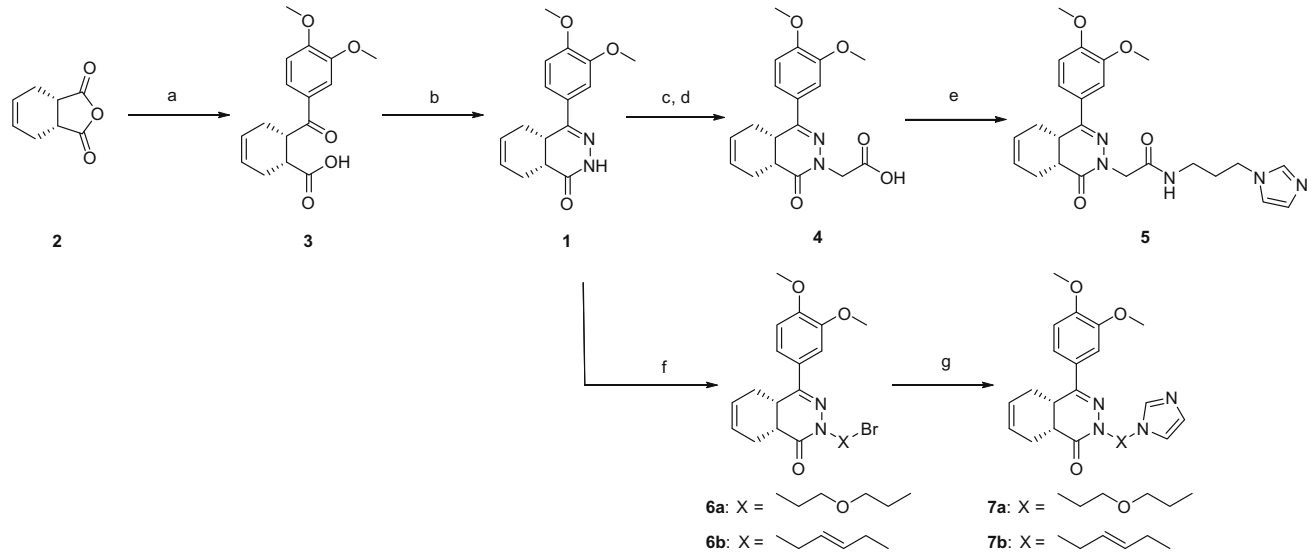


Figure 3. (a) X-ray structure of PDE4–zardaverine complex (1MKD[24]), (b) docked model of PDE4–**1** complex. (4a*S*,8a*R*)-4-(3,4-Dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (an enantiomer of **1**) was used for docking.

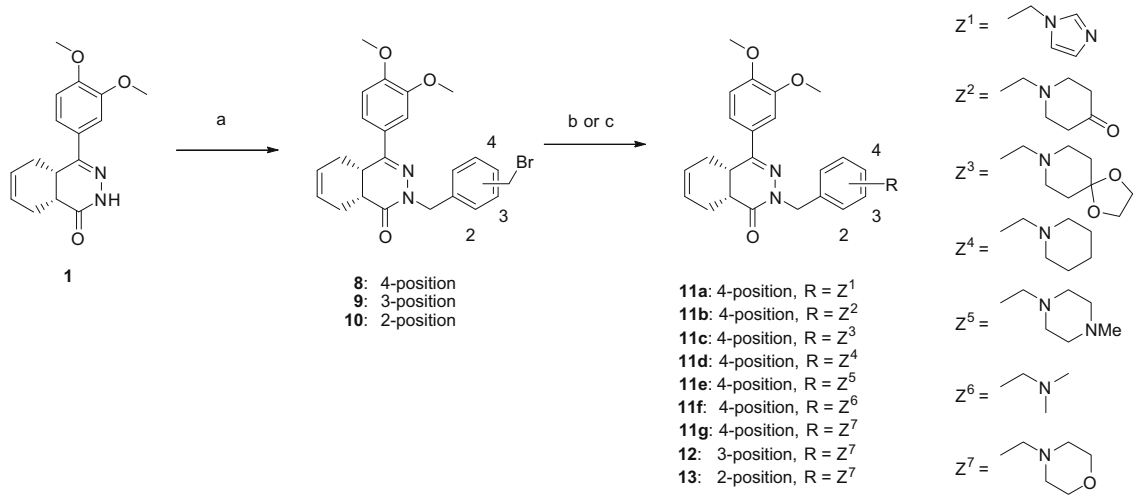
The main barrier for topical drug delivery is generally believed to be the stratum corneum of the skin. Because the stratum corneum is a lipophilic barrier, the lipophilicity of a drug is thought to be a key parameter controlling its penetration of the skin. Many approaches have therefore been taken to increase the lipophilicity of drugs with the aim of enhancing their activity on topical application.[27,28] Lipophilicity also tends to promote rapid metabolism,[29] which could help reduce the systemic side effects of drugs that happen to be absorbed into the systemic circulation by limiting their persistence in the circulation. These considerations suggested to us that chemical modifications directed towards increasing the lipophilicity of PDE4 inhibitors would be a good way to both enhance their topical activity and reduce their side effects. In our search for PDE4 inhibitors with favorable pharmacokinetic profiles, therefore, we decided to focus on the introduction of various kinds of lipophilic moiety at the 2-position of the phthalazinone ring.

2.2. Chemistry

The synthesis of racemic 4-(3,4-dimethoxyphenyl)-5,6,9,10-tetrahydrophthalazin-1-one derivatives is shown in Scheme 1. The keto acid **3**, obtained by conventional Friedel–Crafts acylation[23] of veratrole (1,2-dimethoxybenzene) by *cis*-1,2-cyclohexenedicarboxylic anhydride **2**, was condensed with hydrazine monohydrate to produce lead compound **1**. N-Alkylation of **1** with ethyl bromoacetate followed by hydrolysis under basic conditions gave carboxylic acid **4**, which was converted into amide **5** by condensation with aminopropylimidazole. Treatment of **1** with either of two alkyl dibromides in the presence of sodium hydride furnished the N-bromoalkyl-substituted phthalazinones **6a** and **6b**, which, on reaction with imidazole, yielded the desired compounds **7a** and **7b**. Tetrahydrophthalazinone **1** was coupled with the *o*-, *m*-, or



Scheme 1. Reagents: (a) veratrole, $AlCl_3$, CH_2Cl_2; (b) NH_2NH_2, EtOH; (c) NaH, $BrCH_2CO_2Et$, DMF; (d) aqueous NaOH, MeOH; (e) 1-(3-aminopropyl)imidazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, Et_3N, DMF; (f) bis(2-bromoethyl)ether (for **6a**), 1,4-dibromo-2-butene (for **6b**), NaH, DMF; (g) NaH, imidazole, DMF.



Scheme 2. Reagents: (a) (i) NaH, DMF (ii) α,α′-*p*-dibromoxylene (for **8**), α,α′-*m*-dibromoxylene (for **9**), or α,α′-*o*-dibromoxylene (for **10**); (b) NaH, imidazole, DMF; (c) various tertiary amines, K_2CO_3, THF.

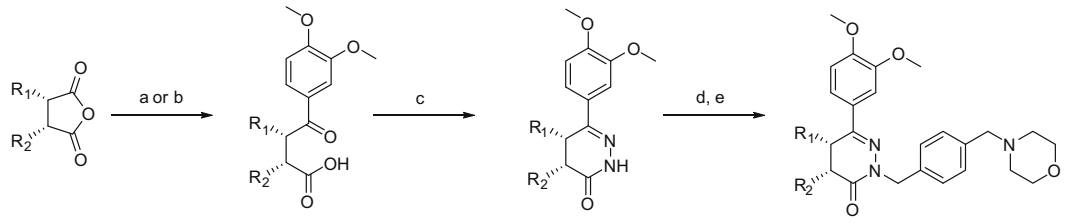
p-isomer of α,α′-dibromoxylene and the resulting benzyl bromides **8–10** were converted into the desired benzylamine-substituted phthalazinones **11a–g**, **12** and **13** by coupling with various tertiary amines (Scheme 2).

The synthetic route for the preparation of 6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one **17a** and the *cis*-4,5-cycloalkyl-6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-ones **17b–d** is summarized in Scheme 3. The keto acid **15a** was synthesized by Friedel–Crafts acylation of veratrole by succinic anhydride **14a**, and condensation of **15a** with hydrazine monohydrate yielded 6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one **16a**. The keto acids **15b–d** were similarly synthesized by Friedel–Crafts acylation of veratrole by *cis*-1,2-cycloalkyldicarboxylic acid anhydrides **14b–d**, and condensation of **15b–d** with hydrazine monohydrate similarly yielded the *cis*-4,5-cycloalkyl-6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-ones **16b–d**. Transformation of **16a–d** into the desired benzyl morpholines **17a–d** was carried out as shown in Schemes 1 and 2.

The optically active benzyl morpholine derivative **11g** was produced as shown in Scheme 4. The racemic keto acid (**±**)**-3** was optically resolved by using the chiral bases (*S*)-(−)-α-methylbenzylamine and (*R*)-(+)-α-methylbenzylamine as resolving agents as previously described.[30] The desired optically active compounds (**+**)**-11g** and (−)**-11g** were then prepared in enantiomerically pure form as outlined in Scheme 3.

2.3. Biological evaluation

The test compounds prepared were evaluated for their ability to inhibit the enzymatic activity of the PDE4D catalytic domain cloned from the human acute promyelocytic leukemia cell line HL-60. The concentration of test compound giving 50% inhibition of enzyme activity (IC_{50}) was estimated. In addition, the anti-inflammatory effect of the compounds was assessed by their ability to suppress the production of TNF-α by rat whole blood cells stimulated with lipopolysaccharide (LPS).[31]



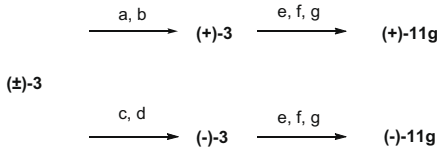
14a: R_1, R_2 = H
14b: R_1, R_2 = -CH_2-
14c: R_1, R_2 = -$(CH_2)_2$-
14d: R_1, R_2 = -$(CH_2)_4$-

15a: R_1, R_2 = H
15b: R_1, R_2 = -CH_2-
15c: R_1, R_2 = -$(CH_2)_2$-
15d: R_1, R_2 = -$(CH_2)_4$-

16a: R_1, R_2 = H
16b: R_1, R_2 = -CH_2-
16c: R_1, R_2 = -$(CH_2)_2$-
16d: R_1, R_2 = -$(CH_2)_4$-

17a: R_1, R_2 = H
17b: R_1, R_2 = -CH_2-
17c: R_1, R_2 = -$(CH_2)_2$-
17d: R_1, R_2 = -$(CH_2)_4$-

Scheme 3. Reagents: (a) veratrole, $AlCl_3$, CH_2Cl_2; (b) 4-bromoveratrole, magnesium turnings, THF; (c) NH_2NH_2, EtOH; (d) NaH, α,α′-*p*-dibromoxylene, DMF; (e) K_2CO_3, morpholine, THF.

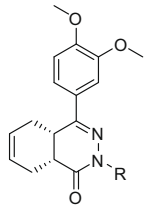


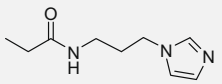
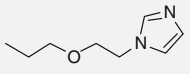
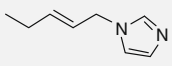
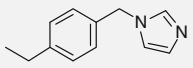
Scheme 4. Reagents: (a) (*S*)-(−)-α-methylbenzylamine, AcOEt; (b) aqueous HCl; (c) (*R*)-(+)-α-methylbenzylamine, AcOEt; (d) aqueous HCl; (e) NH_2NH_2, EtOH; (f) NaH, α,α′-*p*-dibromoxylene, DMF; (g) K_2CO_3, morpholine, THF.

The IC_{50} values for inhibition of PDE4 activity and TNF-α production for compounds **1** through **11a** are shown in Table 1. To evaluate the effect of filling the space available in the active-site pocket of PDE4 on the biological activity of PDE4 inhibitors, we identified the alkyl amide **5** as a starting point for further chemical modification. Alkyl amide **5** was designed to include an imidazole moiety at the end of the side chain of the phthalazinone ring system because the introduction of ionizable substituents is reported to enhance skin penetration.[32] Alkyl amide **5** showed moderate inhibition of PDE4 (IC_{50} = 14.3 nM), approximately sixfold more potent than that of **1**, but only relatively weak inhibition of TNF-α production by rat whole blood (IC_{50} = 112.5 nM). On the one hand, the high PDE4 inhibitory activity of **5** can be explained by the fact that it fills the active-site pocket of PDE4 in our 3D model. On the other hand, its low activity in the cell-based assay can be explained by its low lipophilicity (*c* log *P* = 0.8). With a view to identifying a compound with increased lipophilicity, we next varied the substituent at the 2-position of the phthalazinone ring system while keeping the imidazole moiety intact; that is, we synthesized compounds in which the alkyl amide linker of **5** was replaced with a diethyl ether linker (**7a**; *c* log *P* = 1.66), a 2-butene linker (**7b**; *c* log *P* = 2.35), or a xylene linker (**11a**; *c* log *P* = 3.51). The compound with the diethyl ether linker, **7a**, showed approximately threefold greater PDE4 inhibitory activity than **5**, while the compound with the 2-butene linker, **7b**, was the most potent, with 36-fold greater PDE4 inhibitory activity than **5** (IC_{50} = 0.4 nM). The compound with the xylene linker, **11a**, also had good activity (IC_{50} = 0.7 nM). As expected, a compound that better filled the available space in the active-site pocket of PDE4 (with a hydrophobic moiety at the 2-position of the phthalazinone ring system) was a better inhibitor. When the ability of the compounds to suppress the production of TNF-α was assessed, **7b** and **11a** were found to have over 150-fold greater activity than **5**, with IC_{50} values in the subnanomolar range. Because **7b** and **11a** both showed potent PDE4 inhibitory activity and potent TNF-α-suppressing activity, we selected the more lipophilic *N*-benzyl phthalazinone **11a** for further modification in the hope of identifying a compound with good in vivo efficacy.

We focused on the replacement of the imidazole ring of **11a** by various tertiary amines, mostly including a six-membered heterocyclic ring, because the imidazole ring is reported to inhibit cytochrome P450 and may be generally responsible for unfavorable drug–drug interactions.[33,34] Piperidone derivative **11b** (IC_{50} = 0.9 nM), 1,4-dioxa-8-azaspiro[4.5]decane derivative **11c** (IC_{50} = 1.3 nM) and the morpholine compound **11g** (IC_{50} = 0.9 nM) showed potent inhibition of PDE4 activity comparable to that of **11a**, while piperidine derivative **11d** and dimethylamino derivative **11f** had somewhat lower potency (Table 2). A similar profile was observed for the TNF-α-suppressing activity. 1,4-Dioxa-8-azaspiro[4.5]decane derivative **11c** and morpholine derivative **11g** showed excellent inhibition of both PDE4 activity and TNF-α production. These data suggest that the introduction of a suitable amine moiety at the end of the side chain of the phthalazinone ring system would improve the potency of both PDE4 inhibition and TNF-α suppression.

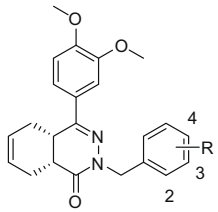
Table 1
Effect of substituents at the 2-position of the phthalazinone ring system on inhibition of PDE4 and suppression of TNF-α production

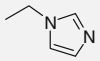
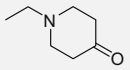
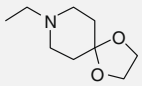
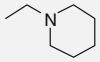
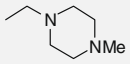
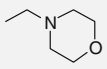
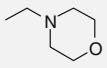
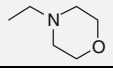


Compound	R	PDE4 inhibition IC_{50}[a] (nM)	TNF-α suppression IC_{50}[b] (nM)
1	H	85.0	207.3
5		14.3	112.5
7a		5.2	9.1
7b		0.4	0.7
11a		0.7	0.5

[a] PDE4 inhibition was assayed with recombinant human PDE4D catalytic domain.
[b] Suppression of TNF-α production by LPS-stimulated rat whole blood cells.

Table 2
Effect of various heterocyclic rings at the xylene linker on inhibition of PDE4 and suppression of TNF-α production



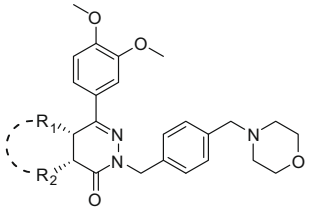
Compound	Position	R	PDE4 inhibition IC_{50}[a] (nM)	TNF-α suppression IC_{50}[b] (nM)
11a	4		0.7	0.5
11b	4		0.9	3.0
11c	4		1.3	0.9
11d	4		8.2	57.1
11e	4		2.0	14.5
11f	4		9.7	34.9
11g	4		0.9	0.9
12	3		1.4	3.0
13	2		1.3	1.0

[a,b] See corresponding footnotes to Table 1.

Because replacement of the imidazole ring by a morpholine ring resulted in excellent activity in both in vitro assays, we next investigated the effect on activity of changing the position of the morpholinomethyl moiety on the phenyl ring (**11g**, **12** and **13**). All three positions gave almost the same activity in both assays, with 3-substituent derivative **12** showing marginally lower activity in both assays.

The in vitro activities of dihydropyridazinone derivatives with an extra ring fused to the dihydropyridazinone ring were evaluated in 4-(morpholinomethyl)benzyl derivatives **11g** and **17a–d** (Table 3). All compounds with the fused ring system showed improved PDE4 inhibitory activity, and the activity increased with increasing size of the extra ring from three to six members. As expected from our 3D docking study of **1** and the PDE4 catalytic domain, the *cis*-cyclohexene and cyclohexane derivatives **11g** and **17d** strongly inhibited PDE4 (IC_{50} = 0.9 and 1.0 nM, respectively). This suggests that the *cis*-cyclohexene and cyclohexane rings of these compounds interact well with the active-site pocket of PDE4. The most potent TNF-α-production-suppressing activity was shown by cyclohexene derivative **11g** (IC_{50} = 0.9 nM), whose activity was 492-fold stronger than that of the dihydropyridazinone derivative

Table 3
Effect of an extra ring in dihydropyridazinone derivatives on inhibition of PDE4 and suppression of TNF-α production



Compound	R_1	R_2	PDE4 inhibition IC_{50}[a] (nM)	TNF-α suppression IC_{50}[b] (nM)
17a	H	H	45.9	442.4
17b			16.4	118.8
17c[c]			4.3	44.8
17d			1.0	4.6
11g			0.9	0.9

[a,b] See corresponding footnotes to Table 1.
[c] The biological activity of the hydrochloride salt was evaluated.

without an extra ring, **17a** (IC_{50} = 442.4 nM). We think that the cyclohexene ring of **11g** plays a dual role because it not only contributed to the PDE4 inhibitory activity of **11g** by improving its binding at the active site but also, by conferring increased lipophilicity, enhanced the ability of **11g** to penetrate the cell membrane of rat monocytes and lymphocytes in the TNF-α-suppression assay. We therefore selected **11g** for further study.

Next, the optically pure enantiomers of **11g** were individually evaluated (Table 4). In the PDE4 inhibition assay, the *cis*-(+)-enantiomer (**+**)**-11g** displayed an IC_{50} value of 0.3 nM, corresponding to 29 times the potency of the *cis*-(−)-enantiomer (−)**-11g**. A similar result was found in the TNF-α-production assay, with (**+**)**-11g** displaying 20 times the potency of (−)**-11g**.

The effect of topical application of the most potent compounds identified in the in vitro assays was evaluated in a mouse model of dermatitis induced by 2,4,6-trinitrochlorobenzene (TNCB) as previously described.[35,36] After topical administration of 20 mg of 1% test compound in a vaseline ointment, (**+**)**-11g** maleate, **11b** and **13** reduced the TNCB-induced ear swelling by almost two-thirds (Table 5), more than the control Protopic ointment (FK506 ointment; Astellas Pharma).

The in vitro metabolic half-life of (**+**)**-11g** maleate, expressed as the half-life of disappearance of the parent compound in the presence of a human liver microsomal fraction (1 mg protein/mL), was 6 min.

Topical administration of a compound may minimize side effects by reducing the exposure of the gut and CNS. However, even though topical doses are generally small, a large proportion of the dose may be absorbed into the systemic circulation and contribute to adverse events such as nausea. The low metabolic stability of

Table 4
Effect of **11g** stereochemistry on PDE4 inhibition and TNF-α suppression

Compound	PDE4 inhibition IC_{50}[a] (nM)	TNF-α suppression IC_{50}[b] (nM)
(+)-**11g**	0.3	4.2
(−)-**11g**	8.8	82.2

[a,b] See corresponding footnotes to Table 1.

Table 5
Effect of compounds in a mouse dermatitis model[a]

Compound	Reduction of dermatitis (%)
(+)-**11g**[b]	38.5
11b	36.8
13	36.8
Protopic	26.3

[a] See Section 4 for further details.
[b] The biological activity of the maleate salt was evaluated.

(**+**)-**11g** maleate in human liver S9 fractions leads us to expect that, even if it were largely absorbed after topical administration, it would disappear rapidly from the systemic circulation. The resulting low bioavailability of (**+**)-**11g** maleate should ensure a low risk of contributing to nausea.

3. Conclusion

With the help of 3D docking studies, we have designed and synthesized novel 2-substituted phthalazinone derivatives that potently inhibit PDE4 activity and TNF-α production by rat whole blood cells. Structure–activity relationship studies revealed that these inhibitory activities were strongly dependent on the nature of the substituent at the 2-position of the phthalazinone ring system, and that a benzyl moiety with an attached six-membered heterocyclic ring conferred particularly potent activity. We also investigated the effects of an extra ring in dihydropyridazinone derivatives and found that a *cis*-fused cyclohexene ring conferred potent activity, consistent with its filling the hydrophobic space of the active-site pocket of PDE4 as predicted by our 3D docking studies. The optimized compounds (**+**)-**11g** maleate, **11b** and **13** were topically active in a mouse dermatitis model, and their effects were stronger than those of Protopic ointment. Metabolic stability tests in vitro suggest that (**+**)-**11g** would be rapidly metabolized in vivo, a characteristic that would decrease the risk of side effects if it were absorbed into the systemic circulation.

4. Experimental

4.1. Chemistry

Reagents and solvents were used as obtained from the supplier without further purification. Melting points were determined on a Büchi B-545 melting-point apparatus, and are uncorrected. Column chromatography was carried out on a silica gel column (Fuji Silysia PSQ-100B or Fuji Silysia NH). Thin-layer chromatography (TLC) was performed on Merck TLC aluminum sheets Silica Gel 60 F_{254} with detection by UV quenching at 254 nm or spraying with phosphomolybdic acid. Synthetic yields of compounds were not optimized. ^{1}H NMR spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts (δ) are given in ppm from the internal standard, tetramethylsilane, and coupling constants are given in hertz (Hz). Mass spectra were obtained on a JEOL JMS-HX100 mass spectrometer. Infrared (IR) spectra were measured on a PerkinElmer® Spectrum100 FT-IR Spectrometer. Optical rotations were determined on a Horiba SEPA-200 high-sensitivity polarimeter with a 5-cm-path-length cell, and elemental analyses were performed on a J-Science Lab Micro Corder JM 10 elemental analyzer.

4.1.1. Preparation of 1, 5, 7a and 7b

4.1.1.1. *cis*-(±)-6-(3,4-Dimethoxybenzoyl)cyclohex-3-enecarboxylic acid (3). Veratrole (50.0 g, 361.5 mmol) was added dropwise to a suspension of aluminum chloride (53.0 g, 397.7 mmol) in CH_2Cl_2 (550 mL) at 0 °C. After stirring at 0 °C for 30 min, *cis*-4-cyclohexene-1,2-dicarboxylic anhydride (55.0 g, 361.5 mmol) was added and the reaction mixture was refluxed for 27 h. The mixture was then poured into ice water (500 mL) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from Et_2O to give **3** as white crystals (25.95 g, 25%). Mp 139.8–141.8 °C. Electrospray ionization mass spectrometry (ESI-MS) *m/z* 291.1 $[MH]^+$. IR: 1702.0, 1671.2, 1513.2, 1417.8, 1260.6, 1147.3, 1020.2 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 2.30–2.55 (3H, m), 2.79–3.10 (2H, m), 3.92 (3H, s), 3.95 (3H, s), 5.71 (2H, dd, *J* = 10.6, 29.0 Hz), 6.89 (1H, d, *J* = 8.4 Hz), 7.49–7.55 (2H, m). Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.19; H, 6.17. Found: C, 66.19; H, 6.25.

4.1.1.2. *cis*-(+)-6-(3,4-Dimethoxybenzoyl)cyclohex-3-enecarboxylic acid ((+)-3). A stirred solution of the γ-keto acid (**±**)-**3** (267.8 g, 0.922 mol) in ethyl acetate (AcOEt; 4.8 L) was treated with (*S*)-(−)-α-methylbenzylamine (117.4 mL, 0.922 mol), and the mixture was stirred for 22 h. The crystalline precipitate that formed was filtered off, washed with AcOEt, and recrystallized from AcOEt to give (**+**)-**3** as the (*S*)-(−)-α-methylbenzylamine salt (83.38 g, 22%). The diastereomeric salt was dissolved in $CHCl_3$ (1 L) and washed three times with 1 N HCl (300 mL). The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give (**+**)-**3** as a white powder (66.7 g, quant.). $[\alpha]_D^{25}$ +20.00 (*c* 0.50, $CHCl_3$).

4.1.1.3. *cis*-(−)-6-(3,4-Dimethoxybenzoyl)cyclohex-3-enecarboxylic acid ((−)-3). Compound (−)-**3** was prepared from the γ-keto acid (**±**)-**3** and purified by crystallization as the (*R*)-(+)-α-methylbenzylamine salt in a manner similar to that described for (−)-**3**. $[\alpha]_D^{25}$ −25.19 (*c* 0.50, $CHCl_3$).

4.1.1.4. *cis*-(±)-4-(3,4-Dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (1). To a solution of **3** (5.56 g, 19.2 mmol) in EtOH (28 mL) was added hydrazine monohydrate (1.86 mL, 38.4 mmol) at room temperature. The mixture was stirred at 80 °C for 15 h. On cooling, the product crystallized from the mixture and was filtered off and washed successively with isopropyl alcohol and Et_2O to give **1** as white crystals (3.57 g, 65%). Mp 178.0–179.9 °C. ESI-MS *m/z* 287.1 $[MH]^+$. IR: 1674.2, 1516.1, 1251.9, 1023.2 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 2.18–2.30 (3H, m), 2.85 (1H, t, *J* = 6.0 Hz), 2.95–3.05 (1H, m), 3.43 (1H, dt, *J* = 8.4 Hz, 5.4 Hz), 3.93 (3H, s), 3.94 (3H, s), 5.65–5.85 (2H, m), 6.87 (1H, d, *J* = 8.4 Hz), 7.23 (1H, dd, *J* = 8.4 Hz, 1.8 Hz), 7.46 (1H, d, *J* = 2.2 Hz), 8.62 (1H, br s). Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 67.21; H, 6.36; N, 9.88.

4.1.1.5. *cis*-(+)-4-(3,4-Dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((+)-1). Compound (**+**)-**1** was prepared from (**+**)-**3** in a manner similar to that described for **1**. Yield 73%, white crystals. $[\alpha]_D^{25}$ +849.60 (*c* 0.50, $CHCl_3$).

4.1.1.6. *cis*-(−)-4-(3,4-Dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((−)-1). Compound (−)-**1** was prepared from (−)-**3** in a manner similar to that described for **1**. $[\alpha]_D^{25}$ −890.40 (*c* 0.50, $CHCl_3$).

4.1.1.7. 2-[*cis*-4-(3,4-Dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl]acetic acid (4). Sodium hydride (60% dispersion in mineral oil; 8.5 mg, 2.11 mmol) was added to a solution of **1** (700 mg, 2.44 mmol) in dimethylformamide (DMF; 14 mL) at room temperature. After stirring for 30 min, bromoacetic acid ethyl ester (611 mg, 3.64 mmol) was added and stirring was continued for 15 h. The reaction mixture was then poured into water and extracted with AcOEt. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and

concentrated in vacuo. The product was purified by flash column chromatography (SiO_2, 12 g; AcOEt/*n*-hexane = 1:10→AcOEt/*n*-hexane = 1:3) to give 2-[*cis*-4-(3,4-dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl]acetic acid ethyl ester as colorless crystals (768 mg, 85%). Mp 113.7–114.8 °C. ESI-MS *m/z* 373.1 $[MH]^+$. IR: 1747.2, 1671.9, 1515.4, 1257.3, 1206.4, 1140.3, 1023.8 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.26 (3H, t, *J* = 7.0 Hz), 2.12–2.56 (3H, m), 2.89 (1H, t, *J* = 5.4 Hz), 3.00 (1H, br d, *J* = 18.2 Hz), 3.38 (1H, dt, *J* = 11.4 Hz, 5.6 Hz), 3.93 (6H, s), 4.14 (3H, q, *J* = 7.0 Hz), 4.63 (2H, dd, *J* = 73.6 Hz, 17.2 Hz), 5.71 (1H, d, *J* = 14.6 Hz), 5.79 (1H, d, *J* = 13.2 Hz), 6.87 (1H, d, *J* = 8.4 Hz), 7.25 (1H, dd, *J* = 8.4 Hz, 1.8 Hz), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{20}H_{24}N_2O_5{\cdot}0.3H_2O$: C, 63.58; H, 6.56; N, 7.41. Found: C. 63.26; H, 6.11; N, 7.26. To a solution of the ethyl ester (706 mg, 1.90 mmol) in MeOH (14 mL) was added aqueous 1 N NaOH (2.9 mL). The mixture was stirred at room temperature for 4 h, neutralized with aqueous 3 N HCl, concentrated in vacuo, and extracted with $CHCl_3$. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give **4** as white crystals (641 mg, 98%). Mp 175.2–178.5 °C. ESI-MS *m/z* 345.1 $[MH]^+$. IR: 1667.1, 1514.9, 1257.3, 1136.8, 1023.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.10–2.46 (3H, m), 2.89 (2H, t, *J* = 5.4 Hz), 3.00 (1H, br d, *J* = 17.6 Hz), 3.38 (1H, dt, *J* = 11.4 Hz, 5.0 Hz), 3.93 (3H, s), 3.95 (3H, s), 4.67 (2H, dd, *J* = 47.6 Hz, 18 Hz), 5.53–5.70 (2H, m), 6.86 (1H, d, *J* = 8.4 Hz), 7.24 (1H, d, *J* = 6.6 Hz), 7.44 (1H, s). Anal. Calcd for $C_{18}H_{20}N_2O_5{\cdot}0.25H_2O$: C, 61.97; H, 5.92; N, 8.03. Found: C, 62.06; H, 5.74; N, 7.99.

4.1.1.8. *N*-[3-(1*H*-Imidazol-1-yl)propyl]-2-[*cis*-4-(3,4-dimethoxyphenyl)-1-oxo-4a,5,8,8a-tetrahydrophthalazin-2(1*H*)-yl]acetamide (5). To a solution of **4** (200 mg, 0.58 mmol) in DMF (4 mL) was added 1-(3-aminopropyl)imidazole (87 mg, 0.7 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (133 mg, 0.7 mmol), 1-hydroxybenzotriazole (107 mg, 0.7 mmol) and Et_3N (0.24 mL, 1.74 mmol). The mixture was stirred at room temperature for 15 h, diluted with water and extracted with AcOEt. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (NH–SiO_2, 12 g; AcOEt/*n*-hexane = 1:1→MeOH/AcOEt = 1:5) to give **1** as a colorless powder (123.8 mg, 47%). ESI-MS *m/z* 452.3 $[MH]^+$. IR: 1144.7, 1662.5, 1515.3, 1257.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.99 (2H, t, *J* = 7.0 Hz), 2.14–2.36 (3H, m), 2.84–3.00 (1H, m), 3.00 (1H, br d, *J* = 18.0 Hz), 3.270 (2H, ddd, *J* = 12.8 Hz, 6.2 Hz, 2.4 Hz), 3.42 (1H, dt, *J* = 16.2 Hz, 5.8 Hz), 3.93 (6H, s), 3.96 (2H, t, *J* = 7.4 Hz), 4.53 (2H, dd, *J* = 38.8 Hz, 15.4 Hz), 6.75 (2H, br d, *J* = 24.6 Hz, 10.6 Hz), 6.21 (1H, br t, *J* = 7.4 Hz), 6.87 (1H, d, *J* = 8.6 Hz), 6.92 (1H, s), 7.05 (1H, s), 7.26 (1H, dd, *J* = 8.4 Hz, 2.2 Hz), 7.46 (2H, s). Anal. Calcd for $C_{24}H_{29}N_5O_4{\cdot}0.8H_2O$: C, 61.87; H, 6.62; N, 15.03. Found: C, 61.87; H, 6.49; N, 15.00.

4.1.1.9. *cis*-2-[2-(2-Bromoethoxy)ethyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (6a). Sodium hydride (60% dispersion in mineral oil; 8.5 mg, 2.11 mmol) was added to a solution of **1** (504 mg, 1.76 mmol) in DMF (8 mL) at 0 °C. After stirring for 10 min, bis(2-bromoethyl) ether (1.23 mL, 8.80 mmol) was added and stirring was continued for 15 min. The reaction mixture was poured into water, neutralized with aqueous 1 N HCl and extracted with AcOEt. The organic layer washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (SiO_2, 40 g; $CHCl_3$→$CHCl_3$/MeOH = 100:1) to give **6a** as a colorless oil (823 mg, quant.). ESI-MS *m/z* 437.2 $[MH]^+$. IR 2005.4, 1667.5, 1516.1, 1257.2 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.10–2.30 (3H, m), 2.81 (1H, t, *J* = 5.4 Hz), 3.00 (1H, dd, *J* = 19.6 Hz, 3.0 Hz), 3.30–3.50 (3H, m), 3.75–3.90 (5H, m), 3.93 (3H, s), 3.95 (3H, s), 4.20–4.36 (1H, m), 5.60–5.85 (2H, m), 6.87 (1H, d, *J* = 8.4 Hz), 7.20 (1H, dd, *J* = 8.4 Hz, 2.2 Hz), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{20}H_{25}N_2O_4{\cdot}1H_2O$: C, 52.76; H, 5.98; N, 6.15. Found: C, 52.47; H, 5.45; N, 5.99.

4.1.1.10. *cis*-2-[(*E*)-4-Bromobut-2-enyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (6b). Compound **6b** was prepared from **1** and 1,4-dibromo-2-butene in a manner similar to that described for **6a**. Yield 55%, colorless crystals. Mp 119.7–121.0 °C. ESI-MS *m/z* 419.1 $[MH]^+$. IR: 1668.0, 1515.6, 1257.2, 1023.6 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.00–2.30 (3H, m), 2.81 (1H, t, *J* = 5.0 Hz), 2.90–3.05 (1H, m), 3.36 (1H, dt, *J* = 11.6 Hz, 6.2 Hz), 3.93 (3H, s), 3.95 (3H, s), 3.80–4.00 (2H, m), 5.65–5.90 (2H, m), 5.90–6.00 (2H, m), 6.87 (1H, d, *J* = 8.4 Hz), 7.20 (1H, dd, *J* = 8.4 Hz, 2.8 Hz), 7.48 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{20}H_{23}BrN_2O_3{\cdot}0.25H_2O$: C, 56.68; H, 5.59; N, 6.61. Found: C, 56.44; H, 5.45; N, 6.54.

4.1.1.11. *cis*-2-{2-[2-(1*H*-Imidazol-1-yl)ethoxy]ethyl}-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (7a). To a solution of imidazole (52 mg, 0.75 mmol) in tetrahydrofuran (THF; 2 mL) was added NaH (60% dispersion in oil; 33 mg, 0.925 mmol) at room temperature, and the mixture was stirred for 30 min. A solution of **6a** (108 mg, 0.25 mmol) in THF (1 mL) was added dropwise and stirring was continued for 18 h. The resulting mixture was diluted with ice water and extracted with AcOEt. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (SiO_2, 12 g; AcOEt→AcOEt/MeOH = 5:1) to give **7a** as a colorless oil (40 mg, 38%). ESI-MS *m/z* 425.0 $[MH]^+$. IR: 1663.9, 1515.6, 1257.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.05–2.30 (3H, m), 2.78 (1H, t, *J* = 5.4 Hz), 2.98 (1H, br d, *J* = 17.2 Hz), 3.34 (1H, dt, *J* = 11.8 Hz, 6.4 Hz), 3.60–3.80 (5H, m), 3.93 (3H, s), 3.95 (3H, s), 4.04 (2H, t, *J* = 4.8 Hz), 4.14–4.29 (1H, m), 5.60–5.85 (2H, m), 6.98 (1H, d, *J* = 8.6 Hz), 6.93 (2H, s), 7.27 (1H, dd, *J* = 8.4 Hz, 1.8 Hz), 7.43–7.49 (2H, m). Anal. Calcd for $C_{23}H_{28}N_4O_4{\cdot}0.6H_2O$: C, 63.46; H, 6.76; N, 12.87. Found: C, 63.30; H, 6.61; N, 13.35.

4.1.1.12. *cis*-2-[(*E*)-4-(1*H*-Imidazol-1-yl)but-2-enyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (7b). Compound **7b** was prepared from **6b** in a manner similar to that described for **7a** except that the crude solid was washed with Et_2O and filtered. Yield 59%, white crystals. Mp 112–115 °C. ESI-MS *m/z* 407.3 $[MH]^+$. IR: 3368.2, 1664.2, 1515.3, 1257.8, 1141.1, 1023.1 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.05–2.30 (3H, m), 2.81 (1H, m), 2.90–3.05 (1H, m), 3.35 (1H, dt, *J* = 11.8 Hz, 5.8 Hz), 3.93 (6H, s), 4.45–4.55 (4H, m), 5.65–5.90 (4H, m), 6.87 (1H, d, *J* = 8.4 Hz), 6.90 (1H, s), 7.04 (1H, s), 7.24 (1H, dd, *J* = 8.0 Hz, 2.2 Hz), 7.44 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{23}H_{26}N_4O_3{\cdot}0.2H_2O$: C, 67.36; H, 6.49; N, 13.66. Found: C, 67.49; H, 6.70; N, 13.57.

4.1.2. Preparation of 11a–g, 12 and 13

4.1.2.1. *cis*-(±)-2-[4-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (8). Compound **8** was prepared from **1** and α,α′-*p*-dibromoxylene in a manner similar to that described for **6a**. Yield 50%, white powder. ESI-MS *m/z* 469.2 $[MH]^+$. IR: 1667.0, 1515.7, 1257.8, 1137.3, 1023.1 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.35 (3H, m), 2.82 (1H, t, *J* = 5.8 Hz), 3.03 (1H, br d, *J* = 21.2 Hz), 3.34 (1H, dt, *J* = 11.6 Hz, 5.8 Hz), 3.92 (3H, s), 3.94 (3H, s), 4.47 (2H, s), 5.04 (2H, dd, *J* = 39.8 Hz, 14.6 Hz), 5.60–5.85 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.20–7.40 (5H, m), 7.43 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{24}H_{25}BrN_2O_3{\cdot}0.25H_2O$: C, 60.83; H, 5.42; N, 5.91. Found: C, 60.84; H, 5.27; N, 5.71.

4.1.2.2. *cis*-(+)-2-[4-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((+)-8). Compound (+)-**8** was prepared from (+)-**1** in a manner similar to that described for **8**. $[\alpha]_D^{25}$ +614.80 (*c* 0.50, $CHCl_3$).

4.1.2.3. *cis*-(−)-2-[4-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((−)-8). Compound (−)-**8** was prepared from (−)-**1** in a manner similar to that described for **8**. $[\alpha]_D^{25}$-882.79 (*c* 1.0, $CHCl_3$).

4.1.2.4. *cis*-2-[3-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (9). Compound **9** was prepared from **1** and α,α′-*m*-dibromoxylene in a manner similar to that described for **6a**. Yield 53%, white powder. ESI-MS *m/z* 469.1 $[MH]^+$. IR: 2364.0, 2004.8, 1666.07, 1515.9, 1257.9, 1138.3, 1024.0 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.35 (3H, m), 2.83 (1H, t, *J* = 5.8 Hz), 3.02 (1H, d, *J* = 19.4 Hz), 3.35 (1H, dt, *J* = 11.4 Hz, 5.8 Hz), 3.92 (3H, s), 3.93 (3H, s), 4.47 (2H, s), 5.03 (2H, dd, *J* = 50.2 Hz, 11.4 Hz), 5.60–5.85 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.20–7.35 (4H, m), 7.40–7.45 (2H, m). Anal. Calcd for $C_{24}H_{25}BrN_2O_3{\cdot}0.4H_2O$: C, 60.49; H, 5.46; N, 5.88. Found: C, 60.47; H, 5.18; N, 5.80.

4.1.2.5. *cis*-2-[2-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (10). Compound **9** was prepared from **1** and α,α′-*o*-dibromoxylene in a manner similar to that described for **6a**. Yield 62%, pale yellow powder. ESI-MS *m/z* 469.1 $[MH]^+$. IR: 1665.2, 1516.6, 1265.5, 1020.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.80–2.30 (3H, m), 2.84 (1H, t, *J* = 5.8 Hz), 3.05 (1H, d, *J* = 18.6 Hz), 3.34 (1H, dt, *J* = 11.4 Hz, 6.0 Hz), 3.92 (3H, s), 3.93 (3H, s), 4.85 (2H, dd, *J* = 12.6 Hz, 10.0 Hz), 5.2 (2H, dd, *J* = 17.4 Hz, 14.4 Hz), 5.73 (2H, m), 6.86 (1H, d, *J* = 8.4 Hz), 7.20–7.50 (6H, m). Anal. Calcd for $C_{24}H_{25}BrN_2O_3{\cdot}0.25H_2O$: C, 60.83; H, 5.42; N, 5.91. Found: C, 60.69; H, 5.28; N, 5.72.

4.1.2.6. *cis*-2-{4-[(1*H*-Imidazol-1-yl)methyl]benzyl}-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11a). Compound **11a** was prepared from **8** in a manner similar to that described for **7a**. Yield 49%, white powder. ESI-MS *m/z* 457.3 $[MH]^+$. IR: 3298.1, 1651.0, 1514.6, 1257.7 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.80–2.30 (3H, m), 2.81 (1H, t, *J* = 5.8 Hz), 3.02 (1H, br d, *J* = 21.6 Hz), 3.34 (1H, dt, *J* = 11.8 Hz, 6.2 Hz), 3.89 (3H, s), 3.92 (3H, s), 5.00 (2H, dd, *J* = 43.0 Hz, 14.4 Hz), 5.08 (2H, s), 5.60–5.90 (2H, m), 6.86 (1H, d, *J* = 8.0 Hz), 6.88 (1H, s), 7.07–7.11 (3H, m), 7.23 (1H, dd, *J* = 8.4 Hz, 2.2 Hz), 7.36–7.44 (3H, m), 7.52 (1H, s) Anal. Calcd for $C_{27}H_{28}N_4O_3{\cdot}1.25H_2O$: C, 67.69; H, 6.42; N, 11.70. Found: C, 67.52; H, 6.34; N, 11.46.

4.1.2.7. *cis*-4-(3,4-Dimethoxyphenyl)-2-{4-[(4-oxopiperidin-1-yl)-methyl]benzyl}-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11b). To a solution of **8** (250 mg, 0.533 mmol) in THF (5 mL) was added 4-piperidone monohydrate (409 mg, 2.665 mmol) and potassium carbonate (737 mg, 5.33 mmol). The mixture was stirred at room temperature for 12 h. The precipitate that formed was removed by filtration, the filtrate was extracted with AcOEt, and the organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (SiO_2, 40 g; $CHCl_3$→$CHCl_3$/MeOH = 100:1) to give **11b** as a colorless powder (175 mg, 67%). ESI-MS *m/z* 488.3 $[MH]^+$. IR: 1669.9, 1515.2, 1342.9, 1257.4, 1138.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.35 (3H, m), 2.47 (4H, t, *J* = 5.8 Hz), 2.72 (4H, t, *J* = 5.8 Hz), 2.83 (1H, t, *J* = 5.8 Hz), 3.02 (1H, d, *J* = 16.8 Hz), 3.34 (1H, dt, *J* = 11.2 Hz, 5.8 Hz), 3.58 (2H, s), 3.92 (3H, s), 3.93 (3H, s), 5.05 (2H, dd, *J* = 43.2 Hz, 14.2 Hz), 5.60–5.85 (2H, m), 6.86 (1H, d, *J* = 8.6 Hz), 7.22–7.39 (5H, m), 7.46 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{29}H_{33}N_3O_4{\cdot}0.1H_2O$: C, 71.17; H, 6.84; N, 8.59. Found: C, 70.90; H, 6.68; N, 8.58.

4.1.2.8. *cis*-2-[4-(1,4-Dioxa-8-azaspiro[4.5]decan-8-ylmethyl)-benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11c). Compound **11c** was prepared from **8** and 1,4-dioxa-8-azaspiro[4.5]decane in a manner similar to that described for **11b**. Yield 56%, white powder. ESI-MS *m/z* 532.3 $[MH]^+$. IR: 3346.1, 1666.7, 1514.9, 1257.0 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.72 (4H, t, *J* = 5.8 Hz), 1.90–2.30 (3H, m), 2.50 (4H, t, *J* = 6.2 Hz), 2.82 (1H, t, *J* = 6.2 Hz), 3.02 (1H, br d, *J* = 18.0 Hz), 3.36 (1H, dt, *J* = 11.4 Hz, 5.8 Hz), 3.48 (2H, s), 3.92–3.94 (10H, m), 5.02 (2H, dd, *J* = 40.8 Hz, 14.4 Hz), 5.60–5.85 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.20–7.36 (5H, m), 7.45 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{31}H_{37}N_3O_5{\cdot}0.5H_2O$: C, 68.87; H, 7.08; N, 7.77. Found: C, 68.72; H, 6.80; N, 7.73.

4.1.2.9. *cis*-4-(3,4-Dimethoxyphenyl)-2-[4-(piperidin-1-ylmethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11d). Compound **11d** was prepared from **8** and piperidine hydrochloride in a manner similar to that described for **11b**. Yield 74%, colorless powder. ESI-MS *m/z* 474.4 $[MH]^+$. IR: 1671.4, 1514.8, 1257.3 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.40–1.62 (6H, m), 1.85–2.45 (8H, m), 2.82 (1H, t, *J* = 5.8 Hz), 3.03 (1H, br d, *J* = 17.6 Hz), 3.33 (1H, dt, *J* = 11.8 Hz, 5.8 Hz), 3.45 (2H, s), 3.91 (3H, s), 3.92 (3H, s), 5.02 (2H, dd, *J* = 42.2 Hz, 14.2 Hz), 5.60–5.85 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.20–7.36 (5H, m), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{29}H_{35}N_3O_3{\cdot}0.5H_2O$: C, 72.17; H, 7.52; N, 8.71. Found: C, 71.98; H, 7.56; N, 8.54.

4.1.2.10. *cis*-4-(3,4-Dimethoxyphenyl)-2-{4-[(4-methylpiperazin-1-yl)methyl]benzyl}-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11e). Compound **11e** was prepared from **8** and 1-methylpiperazine in a manner similar to that described for **11b**. Yield 43%, pale yellow oil. ESI-MS *m/z* 489.3 $[MH]^+$. IR: 2936.7, 1669.5, 1515.5, 1257.7 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.30 (3H, m), 2.28 (3H, s), 2.40–2.55 (8H, m), 2.82 (1H, br t, *J* = 6.0 Hz), 3.03 (1H, br d, *J* = 16.0 Hz), 3.25–3.40 (1H, m), 3.46 (2H, s), 3.92 (6H, s), 5.00 (2H, dd, *J* = 42.0 Hz, 12 Hz), 5.60–5.90 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.24 (2H, d, *J* = 8.0 Hz), 7.35 (2H, d, *J* = 8.0 Hz), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{29}H_{36}N_4O_3{\cdot}0.5H_2O$: C, 69.99; H, 7.49; N, 11.26. Found: C, 69.97; H, 7.29; N, 11.10.

4.1.2.11. *cis*-4-(3,4-Dimethoxyphenyl)-2-{4-[(dimethylamino)-methyl]benzyl}-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11f). Compound **11f** was prepared from **8** and dimethylamine hydrochloride in a manner similar to that described for **11b**. Yield 30%, pale yellow oil. ESI-MS *m/z* 434.1 $[MH]^+$. IR: 1669.4, 1516.0, 1257.4 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.30 (3H, m), 2.23 (6H, s), 2.82 (1H, br s), 3.03 (1H, br d, *J* = 17.0 Hz), 3.25–3.40 (1H, m), 3.93 (2H, s), 3.92 (6H, s), 4.97 (2H, dd, *J* = 49.0 Hz, 14.2 Hz), 5.60–5.90 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.23 (2H, d, *J* = 8.4 Hz), 7.35 (2H, d, *J* = 8.0 Hz), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{26}H_{31}N_3O_3{\cdot}0.5H_2O$: C, 70.56; H, 7.29; N, 9.49. Found: C, 70.89; H, 7.29; N, 9.49.

4.1.2.12. *cis*-(±)-4-(3,4-Dimethoxyphenyl)-2-[4-(morpholinomethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (11g). Compound **11g** was prepared from **8** and morpholine in a manner similar to that described for **11b**. Yield 54%, white powder. ESI-MS *m/z* 456.3 $[MH]^+$. IR: 3277.7, 1688.7, 1515.0, 1257.6 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.95–2.30 (3H, m), 2.42 (4H, t, *J* = 4.8 Hz), 2.82 (1H, t, *J* = 6.2 Hz), 3.04 (1H, br d, *J* = 18.8 Hz), 3.35 (1H, dt, *J* = 11.4 Hz, 6.0 Hz), 3.46 (2H, s), 3.69 (4H, t, *J* = 4.8 Hz), 3.92 (3H, s), 3.93 (3H, s), 5.04 (2H, dd, *J* = 43.6 Hz, 14.2 Hz), 5.60–5.90 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.20–7.37 (5H, m), 7.45 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{28}H_{33}N_3O_4{\cdot}0.2H_2O$: C, 70.18; H, 7.03; N, 8.77. Found: C, 70.12; H, 7.10; N, 8.75.

4.1.2.13. *cis*-(+)-4-(3,4-Dimethoxyphenyl)-2-[4-(morpholinomethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((+)-11g). Compound (+)-**11g** was prepared from (+)-**8** in a manner similar to that described for **11b**. $[\alpha]_D^{25}$ +417.39 (*c* 1.0, $CHCl_3$).

4.1.2.14. *cis*-(−)-4-(3,4-Dimethoxyphenyl)-2-[4-(morpholinomethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one ((−)-11g). Compound (−)-**11g** was prepared from (−)-**8** in a manner similar to that described for **11b**. $[\alpha]_D^{25}$ −419.19 (*c* 1.0, $CHCl_3$).

4.1.2.15. *cis*-4-(3,4-Dimethoxyphenyl)-2-[3-(morpholinomethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (12). Compound **12** was prepared from **9** and morpholine in a manner similar to that described for **11b**. Yield 59%, white powder. ESI-MS *m/z* 476.3 $[MH]^+$. IR: 1669.4, 1514.9, 1257.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.80–2.30 (3H, m), 2.39 (4H, t, *J* = 4.8 Hz), 2.83 (1H, t, *J* = 5.8 Hz), 3.04 (1H, d, *J* = 18.2 Hz), 3.37 (1H, dt, *J* = 11.8 Hz, 5.8 Hz), 3.46 (2H, s), 3.64 (4H, t, *J* = 4.8 Hz), 3.92 (6H, s), 5.03 (2H, dd, *J* = 71.6 Hz, 14.2 Hz), 5.72 (2H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.21–7.44 (5H, m), 7.44 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{28}H_{33}N_3O_4{\cdot}0.2H_2O$: C, 70.18; H, 7.02; N, 8.77. Found: C, 70.15; H, 7.07; N, 8.72.

4.1.2.16. *cis*-4-(3,4-Dimethoxyphenyl)-2-[2-(morpholinomethyl)benzyl]-4a,5,8,8a-tetrahydrophthalazin-1(2*H*)-one (13). Compound **13** was prepared from **10** and morpholine in a manner similar to that described for **11b**. Yield 42%, white powder. ESI-MS *m/z* 476.3 $[MH]^+$. IR: 1670.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.90–2.53 (3H, m), 2.46 (4H, t, *J* = 4.2 Hz), 2.82 (1H, t, *J* = 2.6 Hz), 3.03 (1H, br d, *J* = 18.8 Hz), 3.35 (1H, dt, *J* = 11.8 Hz, 6.0 Hz), 3.65 (2H, s), 3.67 (4H, t, *J* = 4.8 Hz), 3.90 (3H, s), 3.92 (3H, s), 5.25 (2H, dd, *J* = 31.6 Hz, 15.0 Hz), 5.60–5.85 (2H, m), 6.85 (1H, d, *J* = 8.6 Hz), 7.20–7.27 (4H, m), 7.38–7.41 (2H, m). Anal. Calcd for $C_{28}H_{33}N_3O_4{\cdot}0.7H_2O$: C, 68.89; H, 7.10; N, 8.61. Found: C, 68.95; H, 6.84; N, 8.62.

4.1.3. Preparation of 17a–d

4.1.3.1. 4-(3,4-Dimethoxyphenyl)-4-oxobutanoic acid (15a). Compound **15a** was prepared from veratrole and succinic anhydride in a manner similar to that described for **3**. Yield 12%, white crystals. Mp 126.3–130.5 °C. ESI-MS *m/z* 261.2 $[M+Na]^+$. IR: 1709.5, 1671.1, 1591.2, 1516.0, 1427.6, 1276.7, 1162.0 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.81 (2H, t, *J* = 6.6 Hz), 3.29 (2H, t, *J* = 6.6 Hz), 3.94 (3H, s), 3.96 (3H, s), 6.90 (1H, d, *J* = 8.4 Hz), 7.60 (1H, d, *J* = 2.2 Hz), 7.62 (1H, dd, *J* = 8.6 Hz, 2.0 Hz).

4.1.3.2. *cis*-2-(3,4-Dimethoxybenzoyl)cyclopropanecarboxylic acid (15b). Compound **15b** was prepared from veratrole and 3-oxabicyclo[3.1.0]hexane-2,4-dione in a manner similar to that described for **3**. Yield 28%, white crystals. Mp 133.1–134.9 °C. ESI-MS *m/z* 251.1 $[MH]^+$. IR: 1702.9, 1670.7, 1513.9, 1419.3, 1262.0, 1018.4 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.55 (1H, dt, *J* = 8.4 Hz, 4.6 Hz), 1.90 (1H, dd, *J* = 12.0 Hz, 6.6 Hz), 2.33 (1H, dd, *J* = 15.8 Hz, 8.8 Hz), 2.92 (1H, dd, *J* = 15.4 Hz, 8.4 Hz), 3.94 (3H, s), 3.97 (3H, s), 6.92 (1H, d, *J* = 8.6 Hz), 7.56 (1H, s), 7.72 (1H, d, *J* = 8.4 Hz). Anal. Calcd for $C_{13}H_{14}O_5{\cdot}0.1H_2O$: C, 61.95; H, 5.68. Found: C, 61.93; H, 5.76.

4.1.3.3. *cis*-2-(3,4-Dimethoxybenzoyl)cyclobutanecarboxylic acid (15c). Compound **15c** was prepared from veratrole and perhydrocyclobuta[c]furan-1,3-dione in a manner similar to that described for **3**. Yield 46%, pale yellow crystals. Mp 102.4–105.2 °C. ESI-MS *m/z* 265.1 $[MH]^+$. IR: 2978.5, 2350.6, 2005.1, 1704.2, 1595.0, 1515.2, 1263.9, 1021.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.20–2.50 (4H, m), 3.40–3.55 (1H, m), 3.92 (3H, s), 3.94 (3H, s), 4.20–4.35 (1H, m), 6.85 (1H, d, *J* = 8.4 Hz), 7.41 (1H, dd, *J* = 8.2 Hz, 1.8 Hz), 7.50 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{14}H_{16}O_5{\cdot}0.1H_2O$: C, 63.20; H, 6.14. Found: C, 63.29; H, 5.94.

4.1.3.4. *cis*-2-(3,4-Dimethoxybenzoyl)cyclohexanecarboxylic acid (15d). A solution of 4-bromoveratrole (24.1 g, 111 mmol) in THF (77 mL) was added dropwise to a suspension of magnesium turnings (2.7 g, 111 mmol) in THF (200 mL) at room temperature and the mixture was stirred for 1.5 h. at 85 °C. After cooling at 0 °C, the solution was added dropwise to a solution of *cis*-1,2-cyclohexanedicarboxylic anhydride (17.1 g, 111 mmol) in THF (277 mL) at 0 °C. Stirring was continued for 1 h at 0 °C and the mixture was then allowed to warm to room temperature and was kept at room temperature for 15 h. The reaction was quenched by the addition of a saturated NH_4Cl solution and the reaction mixture was concentrated in vacuo and extracted with AcOEt. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was crystallized from isopropyl alcohol to give **15d** as white crystals (6.0 g, 19%). Mp 130.5–132.3 °C. ESI-MS *m/z* 293.2 $[MH]^+$. IR: 1699.1, 1669.7, 1513.3, 1417.8, 1260.1, 1147.1, 1020.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.30–1.60 (3H, m), 1.70–2.35 (6H, m), 2.65–2.75 (1H, m), 3.92 (1H, s), 3.94 (1H, s), 6.87 (1H, d, *J* = 8.2 Hz), 7.47 (1H, dd, *J* = 8.2 Hz, 2.2 Hz), 7.53 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.39; H, 6.76.

4.1.3.5. 6-(3,4-Dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one (16a). A suspension of **15a** (1.65 g, 6.93 mmol) in EtOH (10 mL) was added to hydrazine monohydrate (0.67 mL, 13.9 mmol) at room temperature and the mixture was refluxed for 4 h. After cooling to room temperature, the crystallized product was filtered off and washed successively with isopropyl alcohol, aqueous 1 N HCl, and H_2O, again with isopropyl alcohol, and finally with Et_2O to give **16a** as white crystals (1.46 g, 90%). Mp 169.7–172.5 °C. ESI-MS *m/z* 235.0 $[MH]^+$. IR: 3251.9, 1671.8, 1517.6, 1419.9, 1338.0, 1271.1, 1230.3, 1201.7, 1170.6, 1120.45, 1022.3, 765.5, 576.7 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.61 (2H, dd, *J* = 8.4 Hz, 6.6 Hz), 2.98 (2H, dd, *J* = 8.6 Hz, 6.8 Hz), 3.93 (3H, s), 3.94 (3H, s), 6.87 (1H, d, *J* = 8.6 Hz), 7.19 (1H, dd, *J* = 8.0 Hz, 1.8 Hz), 7.42 (1H, d, *J* = 1.8 Hz), 8.73 (1H, br s). Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.47; H, 5.83; N, 12.06.

4.1.3.6. *cis*-5-(3,4-Dimethoxyphenyl)-3,4-diazabicyclo[4.1.0]-hept-4-en-2-one (16b). Compound **16b** was prepared from **15b** in a manner similar to that described for **16a**. Yield 71%, white crystals. Mp 147.0–149.1 °C. ESI-MS *m/z* 247.2 $[MH]^+$. IR: 1664.5, 1516.5, 1424.7, 1372.6, 1268.6, 1216.8, 1172.7, 1137.6, 1023.4, 579.3, 458.0 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.50 (1H, dt, *J* = 8.8 Hz, 5.2 Hz), 1.91 (1H, q, *J* = 6.6 Hz), 2.33 (1H, q, *J* = 8.4 Hz), 2.88 (1H, q, *J* = 8.8 Hz), 3.93 (3H, s), 3.96 (3H, s), 6.86 (1H, d, *J* = 8.4 Hz), 7.54 (1H, d, *J* = 2.2 Hz), 7.73 (1H, dd, *J* = 8.4 Hz, 2.2 Hz). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.11; H, 5.74; N, 11.25.

4.1.3.7. *cis*-5-(3,4-Dimethoxyphenyl)-3,4-diazabicyclo[4.2.0]-oct-4-en-2-one (16c). Compound **16c** was prepared from **15c** in a manner similar to that described for **16a**. Yield 62%, pale yellow crystals. Mp 184.6–187.1 °C. ESI-MS *m/z* 261.2 $[MH]^+$. IR: 3233.7, 1665.4, 1515.4, 1462.4, 1420.6, 1338.8, 1265.9, 1171.5, 1136.6, 1022.9, 567.4 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.35–2.70 (4H, m), 3.30–3.50 (1H, m), 3.80–3.95 (1H, m), 3.91 (3H, s), 3.93 (3H, s), 6.83 (1H, d, *J* = 8.4 Hz), 7.00 (1H, dd, *J* = 8.4 Hz, 2.0 Hz), 7.38 (1H, d, *J* = 2.2 Hz). Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.68; H, 6.20; N, 10.99.

4.1.3.8. *cis*-4-(3,4-Dimethoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one (16d). Compound **16d** was prepared from

15d in a manner similar to that described for **16a**. Yield 78%, white crystals. Mp 166.7–169.0 °C. ESI-MS *m/z* 289.2 $[MH]^+$. IR: 2934.1, 1670.2, 1515.9, 1336.8, 1251.9, 1132.8, 1024.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.35–1.90 (7H, m), 2.53–2.65 (1H, m), 2.75–2.80 (1H, m), 3.06–3.20 (1H, m), 3.92 (3H, s), 3.94 (3H, s), 6.87 (1H, d, J = 8.4 Hz), 7.20 (1H, dd, J = 8.4 Hz, 2.2 Hz), 7.45 (1H, d, J = 1.8 Hz), 8.50 (1H, br s). Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.25; H, 7.08; N, 9.75.

4.1.3.9. 6-(3,4-Dimethoxyphenyl)-2-[4-(morpholinomethyl)benzyl]-4,5-dihydropyridazin-3(2*H*)-one (17a). 2-[4-(Bromomethyl)benzyl]-6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one was prepared from **16a** and α,α′-*p*-dibromoxylene in a manner similar to that described for **6a**. Yield quant., white crystals. Mp 130.2–141.6 °C. ESI-MS *m/z* 417.0 $[MH]^+$. IR: 3404.2, 1669.5, 1517.9, 1344.9, 1271.0, 1024.4 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.62 (2H, dd, J = 8.8 Hz, 6.6 Hz), 2.94 (2H, dd, J = 8.4 Hz, 6.6 Hz), 3.92 (6H, s), 4.48 (2H, s), 5.02 (2H, s), 6.86 (1H, d, J = 8.4 Hz), 7.18 (1H, dd, J = 8.4 Hz, 2.2 Hz), 7.31–7.45 (6H, m). Anal. Calcd for $C_{20}H_{21}BrN_2O_3$: C, 57.56; H, 5.07; N, 6.71. Found: C, 57.34; H, 4.90; N, 6.65. Compound **17a** was prepared from 2-[4-(bromomethyl)benzyl]-6-(3,4-dimethoxyphenyl)-4,5-dihydropyridazin-3(2*H*)-one in a manner similar to that described for **11b**. Yield quant., white powder. FAB-MS *m/z* 424 $[MH]^+$. IR: 3343.9, 1663.8, 1513.2, 1269.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.42 (4H, t, J = 4.8 Hz), 2.61 (2H, dd, J = 8.8 Hz, 6.6 Hz), 2.92 (2H, dd, J = 8.8 Hz, 6.6 Hz), 3.47 (2H, s), 3.69 (4H, t, J = 4.8 Hz), 3.92 (6H, s), 5.01 (2H, s), 6.85 (1H, d, J = 8.4 Hz), 7.19 (1H, dd, J = 8.4 Hz, 2.2 Hz), 7.23–7.45 (6H, m). Anal. Calcd for $C_{24}H_{29}N_3O_4{\cdot}0.75H_2O$: C, 65.96; H, 7.03; N, 9.62. Found: C, 65.86; H, 6.74; N, 9.51.

4.1.3.10. *cis*-5-(3,4-Dimethoxyphenyl)-3-[4-(morpholinomethyl)benzyl]-3,4-diazabicyclo[4.1.0]hept-4-en-2-one (17b). *cis*-3-[4-(Bromomethyl)benzyl]-5-(3,4-dimethoxyphenyl)-3,4-diazabicyclo[4.1.0]hept-4-en-2-one was prepared from **16b** in a manner similar to that described for **6a**. Yield 64%, white powder. ESI-MS *m/z* 429.1 $[MH]^+$. IR: 1650.2, 1608.0, 1514.9, 1421.2, 1255.2, 1023.4 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.82 (1H, dd, J = 10.0 Hz, 5.2 Hz), 1.80 (1H, dt, J = 9.0 Hz, 4.4 Hz), 2.21–2.26 (1H, m), 2.50–2.62 (1H, m), 3.92 (3H, s), 3.93 (3H, s), 4.48 (2H, s), 4.98 (2H, dd, J = 33.0 Hz, 14.4 Hz), 6.89 (1H, d, J = 8.2 Hz), 7.25–7.42 (6H, m). Anal. Calcd for $C_{21}H_{21}BrN_2O_3{\cdot}0.1H_2O$: C, 58.51; H, 4.96; N, 6.50. Found: C, 58.36; H, 4.93; N, 6.24. Compound **17b** was prepared from *cis*-3-[4-(bromomethyl)benzyl]-5-(3,4-dimethoxyphenyl)-3,4-diazabicyclo[4.1.0]hept-4-en-2-one in a manner similar to that described for **11b**. Yield 95%, white powder. ESI-MS *m/z* 436.3 $[MH]^+$. IR: 1658.5, 1515.6, 1373.1, 1269.3, 1116.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.81 (1H, dd, J = 10.0 Hz, 5.2 Hz), 1.80 (1H, dt, J = 30.0 Hz, 16.4 Hz), 2.27 (1H, ddd), 2.42 (4H, t, J = 4.6 Hz), 2.53 (1H, ddd), 3.46 (2H, s), 3.69 (4H, t, J = 4.4 Hz), 3.93 (6H, s), 5.00 (2H, dd, J = 29.2 Hz, 14.2 Hz), 6.89 (1H, d, J = 8.4 Hz), 7.25–7.35 (5H, m), 7.38 (1H, d, J = 1.4 Hz). Anal. Calcd for $C_{25}H_{29}N_3O_4{\cdot}0.7H_2O$: C, 67.01; H, 6.84; N, 9.38. Found: C, 66.94; H, 6.68; N, 9.55.

4.1.3.11. *cis*-5-(3,4-Dimethoxyphenyl)-3-[4-(morpholinomethyl)benzyl]-3,4-diazabicyclo[4.2.0]oct-4-en-2-one hydrochloride (17c). *cis*-3-[4-(Bromomethyl)benzyl]-5-(3,4-dimethoxyphenyl)-3,4-diazabicyclo[4.2.0]oct-4-en-2-one was prepared from **16c** in a manner similar to that described for **6a**. Yield 71%, white crystals. Mp 113.3–115.8 °C. ESI-MS *m/z* 443.2 $[MH]^+$. IR: 2938.5, 1655.1, 1514.5, 1387.0, 1343.3, 1265.32, 1174.15, 1141.2, 1023.7, 602.1 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.25–2.70 (4H, m), 3.35–3.50 (1H, m), 3.80–3.95 (1H, m), 3.90 (6H, s), 4.49 (2H, s), 5.02 (2H, s), 6.81 (1H, d, J = 8.4 Hz), 7.00 (1H, dd, J = 8.4 Hz, 2.2 Hz), 7.30–7.50 (5H, m). Anal. Calcd for $C_{22}H_{23}BrN_2O_3{\cdot}0.25H_2O$: C, 59.00; H, 5.29; N, 6.26. Found: C, 59.10; H, 5.19; N, 6.24. Compound **17c** was prepared from *cis*-3-[4-(bromomethyl)benzyl]-5-(3,4-dimethoxyphenyl)-3,4-diazabicyclo[4.2.0]oct-4-en-2-one in a manner similar to that described for **11b** and converted to its HCl salt. Yield 79%, white powder. FAB-MS *m/z* 450 $[MH]^+$. IR: 3397.9, 2312.7, 1647.1, 1514.3, 1348.2, 1265.2, 1033.1, 772.9 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.30–2.95 (6H, m), 3.25–3.50 (3H, m), 3.90 (3H, s), 3.91 (3H, s), 3.80–4.00 (3H, m), 4.12 (2H, d, J = 4.4 Hz), 4.37 (2H, t, J = 11.8 Hz), 5.05 (2H, s), 6.83 (1H, d, J = 8.4 Hz), 7.03 (1H, dd, J = 8.4 Hz, 2.0 Hz), 7.33 (1H, d, J = 1.8 Hz), 7.51 (2H, d, J = 8.2 Hz), 7.62 (2H, d, J = 8.4 Hz), 13.24 (1H, br s). Anal. Calcd for $C_{26}H_{31}N_3O_4{\cdot}HCl{\cdot}2H_2O$: C, 59.82; H, 6.95; N, 8.05. Found: C, 59.73; H, 6.80; N, 8.02.

4.1.3.12. *cis*-4-(3,4-Dimethoxyphenyl)-2-[4-(morpholinomethyl)benzyl]-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one (17d). *cis*-2-[4-(Bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one was prepared from **16d** in a manner similar to that described for **6a**. Yield 43%, colorless oil. ESI-MS *m/z* 471.1 $[MH]^+$. IR: 1661.4, 1513.2, 1337.6, 1253.7, 1127.9, 1022.6 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.20–1.50 (3H, m), 1.60–1.90 (3H, m), 2.50–2.65 (1H, m), 2.70–2.75 (1H, m), 3.05–3.20 (1H, m), 3.91 (6H, s), 4.47 (2H, s), 5.03 (2H, dd, J = 34.8 Hz, 14.8 Hz), 6.85 (1H, d, J = 8.4 Hz), 7.20 (1H, dd, J = 8.6 Hz, 2.0 Hz), 7.30–7.45 (6H, m). Anal. Calcd for $C_{24}H_{27}BrN_2O_3{\cdot}0.5H_2O$: C, 60.00; H, 5.87; N, 5.83. Found: C, 59.76; H, 5.52; N, 5.68. Compound **17d** was prepared from *cis*-2-[4-(bromomethyl)benzyl]-4-(3,4-dimethoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one in a manner similar to that described for **11b**. Yield 59%, white powder. FAB-MS *m/z* 478 $[MH]^+$. IR: 3277.0, 1663.6, 1514.8, 1255.5 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.20–1.50 (4H, m), 1.50–1.70 (2H, m), 1.70–1.90 (1H, m), 2.42 (4H, t, J = 4.8 Hz), 2.50–2.65 (1H, m), 2.70–2.75 (1H, m), 3.05–3.20 (1H, m), 3.46 (2H, s), 3.69 (4H, t, J = 4.8 Hz), 3.92 (6H, s), 5.02 (2H, dd, J = 36.2 Hz, 14.8 Hz), 6.85 (1H, d, J = 8.4 Hz), 7.15–7.40 (5H, m), 7.44 (1H, d, J = 1.8 Hz). Anal. Calcd for $C_{26}H_{31}N_3O_2{\cdot}0.2H_2O$: C, 69.89; H, 7.41; N, 8.73. Found: C, 69.75; H, 7.20; N, 8.57.

4.2. Biological assays

4.2.1. Animals

For the TNF-α-suppression assay, male Sprague-Dawley rats were used at 10 weeks of age. For the dermatitis model, male BALB/c mice were used at eight weeks of age. All animals were supplied by Japan SLC and were used after quarantine and acclimation for a week after delivery to the laboratory. The animals were housed in rooms at 20–26 °C with a relative humidity of 35–75% under a 12-h light–dark cycle (lights on, 8:00–20:00) and a ventilation frequency of at least 15 times/h. The animals were fed standard laboratory chow (F-2; Funabashi Farm) and were allowed free access to tap water. All experiments were conducted in compliance with the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as revised on 1 June 2006).

4.2.2. Cloning and production of the human PDE4D catalytic domain

The catalytic domain of human PDE4D was cloned from HL-60 cells to include bp 703–1737 of locus NM_006203 (NCBI Reference Sequence).[24] Amplification was performed by polymerase chain reaction (PCR) with the primers 5′-ATGCACAGCTCTAGTCTGAC-3′ (forward) and 5′-TTTACTAGTTTAGCTCTGAGGGATTGTGCTC-3′ (reverse). The PCR product was ligated into the EcoRV/SpeI-digested expression vector pEU3-NII (Toyobo). The vector containing the insert was amplified in *Escherichia coli* JM109 cells and transcribed with T7 RNA polymerase. The resulting mRNA was translated in vitro with the PROTEIOS™ wheat germ cell-free protein synthesis

core kit Ver. 2 (Toyobo) according to the manufacturer's instructions to give the PDE4D catalytic domain.

4.2.3. Assay and determination of IC_{50} of test compounds

The hydrolysis of cAMP catalyzed by the PDE4D catalytic domain was measured in a total volume of 30 μL containing enzyme and cAMP (10 pmol) in Tris–HCl (pH 7.6), 100 mM NaCl, 150 mM $MgCl_2$, and 0.5% polyethylene glycol 6000 in the presence or absence of test compound. After incubation at 30 °C for 90 min, the reaction was quenched by incubation at 75 °C for 1 min and cAMP was determined with the HitHunter™ cAMP II Assay Kit (DiscoveRx). The IC_{50} was estimated from dose–response curves as the concentration of test compound required to inhibit cAMP hydrolysis by 50% under these conditions.

4.2.4. Inhibition of TNF-α production by test compounds in LPS-treated rat whole blood

The ability of test compounds to suppress TNF-α production by rat whole blood treated with LPS was evaluated as previously described.[37] Under ether anesthesia, blood was withdrawn from a 10-week-old male Sprague-Dawley rat through the abdominal vein into a tube containing 1.6 mg/mL EDTA. A 10 mM stock solution of test compound in dimethyl sulfoxide was diluted as necessary before use. Rat blood (80 μL) was dispensed into each well of a 96-well plate, test compound (10 μL) was added with thorough mixing, and the plate was incubated at 37 °C in 5% CO_2 for 30 min. LPS (10 μL of a 100 μg/mL solution) was then added with thorough mixing and incubation was continued for 5 h. Phosphate-buffered saline (100 μL) was then added to each well, the plate was centrifuged at 4000 rpm for 2 min, and the resulting supernatants were collected and stored frozen at −80 °C to await determination of TNF-α by ELISA. The IC_{50} value of the test compound was calculated as the concentration required for 50% inhibition of TNF-α production under these conditions.

4.2.5. Effect of test compounds in a dermatitis model

The ability of test compounds to reduce dermatitis was investigated in a mouse model of dermatitis induced by TNCB.[31,32] Eight-week-old male BALB/C mice were sensitized by applying TNCB/acetone solution (1% w/v, 20 μL) dropwise to each side of the right pinna. From seven days after sensitization, TNCB/acetone solution was applied to both sides of the right pinna every other day (three times per week) for a further three weeks. TNCB/acetone solution alone was applied for the first two of these three weeks so as to fully induce dermatitis. For the treated group ($n = 4$), 20 mg of 1% test compound in a vaseline ointment was applied to each side of the right pinna five times over the last week in addition to the TNCB/acetone solution. The diseased control group ($n = 4$) was treated in the same way except that vaseline containing no test compound was applied during the last week. In both groups, the left pinna was not treated in any way and served as a normal, non-inflamed pinna for comparison. Six hours after the last application, the thickness of the right and left pinnae were measured with a Sony μ-mate digital micrometer, and the percentage reduction of the ear swelling was calculated as

$$\left\{1 - \frac{[\text{mean thickness of right pinna} - \text{mean thickness of left pinna}]_{\text{treated group}}}{[\text{mean thickness of right pinna} - \text{mean thickness of left pinna}]_{\text{diseased control group}}}\right\} \times 100$$

4.2.6. Metabolic stability of 11g

The maleate salt of **11g** was incubated in a reaction mixture (500 μL) containing human liver S9 fraction (1 mg protein/mL) and an NADPH-generating system (2.5 mM β-$NADP^+$, 20 mM glucose-6-phosphate, 4 U/mL glucose-6-phosphate dehydrogenase, and 10 mM $MgCl_2$) in 100 mM potassium phosphate, pH 7.4. After preincubation at 37 °C for 5 min, the reaction was initiated by the addition of 5 μL of **11g** maleate in methanol to give a final concentration of 2 μM. After incubation at 37 °C for 0, 2 or 10 min, the reaction was stopped by the addition of acetonitrile (500 μL). The reaction mixture was then centrifuged at 13,000*g* for 5 min and a sample of the supernatant (25 μL) was analyzed by HPLC. The HPLC system was a Waters 2695 separations module fitted with a Waters 2487 dual-wavelength absorbance detector. The column was an Inertsil ODS-3 (5 μm, 4.6 × 150 mm; GL Science) operated at a flow rate of 1.0 mL/min. The mobile phase was 0.1% ammonium acetate/acetonitrile (50:50) and detection was at 314 nm. The metabolic stability of **11g** was evaluated by its elimination half-life ($t_{1/2}$). The peak area of **11g** on the HPLC chromatogram was converted to percentage remaining by taking the peak area at zero-time of incubation as 100%. The elimination rate constant (k) was estimated by linear regression of the logarithm of the percentage remaining on incubation time, and $t_{1/2}$ was calculated as −0.693/k.

4.3. Molecular modeling

Molecular modeling was performed with the MOE software package Version 2003.02 (Chemical Computing Group, Inc.). Active sites were identified with the Site Finder module, and surfaces were generated with the Surfaces and Maps module. Inhibitors were manually docked into the active-site pocket of PDE4 by using the coordinates of the PDE4–zardaverine complex (PDB entry 1MKD) as a reference. Figures were prepared with PyMOL Version 1.1 (DeLano Scientific LLC).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.08.014.

References and notes

1. Torphy, T. J. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 351.
2. Dent, G.; Giembycz, M. A.; Evans, P. M.; Rabe, K. F.; Barnes, P. J. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1167.
3. Gantner, F.; Kupferschmidt, R.; Schudt, C.; Wendel, A.; Hatzelmann, A. *Br. J. Pharmacol.* **1997**, *121*, 221.
4. Spina, D. *Curr. Opin. Invest. Drugs* **2000**, *1*, 204.
5. Spina, D. *Drugs* **2003**, *63*, 2575.
6. Sturton, G.; Fitzgerald, M. *Chest* **2002**, *121*, 192S.
7. Barnette, M. S. *Prog. Drug Res.* **1999**, *53*, 193.
8. Chujor, S. N. C.; Hammerschmid, F.; Lam, C. *J. Invest. Dermatol.* **1998**, *110*, 287.
9. Harzenetter, M. D.; Novotny, A. R.; Gais, P.; Molina, C. A.; Altmayr, F.; Holzmann, B. *J. Immunol.* **2007**, *179*, 607.
10. Cho, E. S.; Yu, J. H.; Kim, M. S.; Yim, M. *Yonsei Med. J.* **2005**, *46*, 149.
11. Jin, S.-L. C.; Conti, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7628.
12. Keller, T. H.; Bray-French, K.; Demnitz, F. W. J.; Müller, T.; Pombo-Villar, E.; Walker, C. *Chem. Pharm. Bull.* **2001**, *49*, 1009.
13. Pérez-Torres, S.; Miró, X.; Palacios, J. M.; Cortés, R.; Puigdoménech, P.; Mengod, G. *J. Chem. Neuroanat.* **2000**, *20*, 349.
14. Jacobitz, S.; McLaughlin, M. M.; Livi, G. P.; Burman, M.; Torphy, T. J. *Mol. Pharmacol.* **1996**, *50*, 891.
15. Schneider, H. H.; Schmiechen, R.; Brezinski, M.; Seidler, J. *Eur. J. Pharmacol.* **1986**, *127*, 105.
16. Torphy, T. J.; Stadel, J. M.; Burman, M.; Cieslinski, L. B.; McLaughlin, M. M.; White, J. R.; Livi, G. P. *J. Biol. Chem.* **1992**, *267*, 1798.
17. Arumugam, K.; Jabaris, S. S. L.; Manojit, P. *J. Med. Chem.* **2008**, *51*, 5471.
18. Spina, D. *Br. J. Pharmacol.* **2008**, *155*, 308.

19. Ehinger, A. M.; Gorr, G.; Hoppmann, J.; Telser, E.; Ehinger, B.; Kietzmann, M. *Eur. J. Pharmacol.* **2000**, *392*, 93.
20. Bäumer, W.; Gorr, G.; Hoppmann, J.; Ehinger, A. M.; Ehinger, B.; Kietzmann, M. *Eur. J. Pharmacol.* **2002**, *446*, 195.
21. Duplantier, A. J.; Bachert, E. L.; Cheng, J. B.; Cohan, V. L.; Jenkinson, T. H.; Kraus, K. G.; McKechney, M. W.; Pillar, J. D.; Watson, J. W. *J. Med. Chem.* **2007**, *50*, 344.
22. Stinchcomb, A. L.; Swaan, P. W.; Ekabo, O.; Harris, K. K.; Browe, J.; Hammell, D. C.; Cooperman, T. A.; Pearsall, M. *J. Pharm. Sci.* **2002**, *91*, 2571.
23. (a) Van der Mey, M.; Van der Laan, I. J.; Timmerman, H.; Hatzelmann, A.; Boss, H.; Hafner, D.; Beume, R.; Kley, H.-P. Phthalazinones WO 98/31674, July 23, 1998.; (b) Van der Mey, M.; Hatzelmann, A.; Van der Laan, I. J.; Sterk, G. J.; Thibaut, U.; Timmerman, H. *J. Med. Chem.* **2001**, *44*, 2511.
24. Lee, M. E.; Markowitz, J.; Lee, J.-O.; Lee, H. *FEBS Lett.* **2002**, *530*, 53.
25. Huai, Q.; Wang, H.; Sun, Y.; Kim, H.-Y.; Liu, Y.; Ke, H. *Structure* **2003**, *11*, 865.
26. Huai, Q.; Colicelli, J.; Ke, H. *Biochemistry* **2003**, *42*, 13220.
27. Wang, J.; Sung, K. C.; Huang, J.-F.; Yeh, C.-H.; Fang, J.-Y. *J. Pharm. Pharmacol.* **2007**, *59*, 917.
28. Bonina, F. P.; Rimoli, M. G.; Avallone, L.; Barbato, F.; Amato, M.; Puglia, C.; Ricci, M.; De Caprariis, P. *J. Pharm. Sci.* **2002**, *91*, 171.
29. (a) Lewis, D. F. V.; Jacobs, M. N.; Dickins, M. *Drug Discovery Today* **2004**, *9*, 530; (b) van De Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem.* **2001**, *44*, 1313.
30. Van der Mey, M.; Boss, H.; Couwenberg, D.; Hatzelmann, A.; Sterk, G. J.; Goubitz, K.; Schenk, H.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2526.
31. Chambers, R. J.; Marfat, A.; Cheng, J. B.; Cohan, V. L.; Damon, D. B.; Duplantier, A. J.; Hibbs, T. A.; Jenkinson, T. H.; Johnson, K. L.; Kraus, K. G.; Pettipher, E. R.; Salter, E. D.; Shirley, J. T.; Umland, J. P. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 739.
32. (a) Rautio, J.; Nevalainen, T.; Taipale, H.; Vepsäläinen, J.; Gynther, J.; Laine, K.; Järvinen, T. *Eur. J. Pharm. Sci.* **2000**, *11*, 157; (b) Rautio, J.; Nevalainen, T.; Taipale, H.; Vepsäläinen, J.; Gynther, J.; Pedersen, T.; Järvinen, T. *Pharm. Res.* **1999**, *16*, 1172.
33. Velaparthi, U.; Liu, P.; Balasubramanian, B.; Carboni, J.; Attar, R.; Gottardis, M.; Li, A.; Greer, A.; Zoeckler, M.; Wittman, M. D.; Vyas, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3072.
34. Maurice, M.; Pichard, L.; Daujat, M.; Fabre, I.; Joyeux, H.; Domergue, J.; Maurel, P. *FASEB J.* **1992**, *6*, 752.
35. Kitagaki, H.; Fujisawa, S.; Watanabe, K.; Hayakawa, K.; Shiohara, T. *J. Invest. Dermatol.* **1995**, *105*, 749.
36. Kitagaki, H.; Ono, N.; Hayakawa, K.; Kitazawa, T.; Watanabe, K.; Shiohara, T. *J. Immunol.* **1997**, *159*, 2484.
37. Beck, G.; Bottomley, G.; Bradshaw, D.; Brewster, M.; Broadhurst, M.; Devos, R.; Hill, C.; Johnson, W.; Kim, H. J.; Kirtland, S.; Kneer, J.; Lad, N.; Mackenzie, R.; Martin, R.; Nixon, J.; Price, G.; Rodwell, A.; Rose, F.; Tang, J. P.; Walter, D. S.; Wilson, K.; Worth, E. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 390.