1,4-Cyclohexanecarboxylates: Potent and Selective Inhibitors of Phosophodiesterase 4 for the Treatment of Asthma

Siegfried B. Christensen,*,† Aimee Guider,† Cornelia J. Forster,† John G. Gleason,† Paul E. Bender,† Joseph M. Karpinski,† Walter E. DeWolf, Jr.,† Mary S. Barnette,‡ David C. Underwood,‡ Don E. Griswold,‡ Lenora B. Cieslinski,‡ Miriam Burman,‡ Steven Bochnowicz,‡ Ruth R. Osborn,‡ Carol D. Manning,‡ Marilyn Grous,‡ Leonard M. Hillegas,‡ Joan O'Leary Bartus,§ M. Dominic Ryan,|| Drake S. Eggleston,|| R. Curtis Haltiwanger,|| and Theodore J. Torphy‡,⊥

Research and Development, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

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Evaluation of a variety of PDE4 inhibitors in a series of cellular and in vivo assays suggested a strategy to improve the therapeutic index of PDE4 inhibitors by increasing their selectivity for the ability to inhibit PDE4 catalytic activity versus the ability to compete for high affinity [^{3}H]rolipram-binding sites in the central nervous system. Use of this strategy led ultimately to the identification of *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid (**1**, SB 207499, Ariflo™), a potent second-generation inhibitor of PDE4 with a decreased potential for side effects versus the archetypic first generation inhibitor, (*R*)-rolipram.

Introduction

The central role of cyclic nucleotides in regulating the function of airway smooth muscle, inflammatory cells and immune cells is well established.[1−8] Compelling evidence exists that cyclic 3′,5′-adenosine monophosphate (cAMP) is the second messenger that both mediates airway smooth muscle relaxation and exerts a broad inhibitory effect on the activity of immune and inflammatory cells.[7−10] Mechanistically, the intracellular concentration of cAMP can be elevated either by stimulation of adenylyl cyclase to increase the rate at which cAMP is synthesized, as with β-adrenoceptor agonists, or by inhibiting cyclic nucleotide phosphodiesterases (PDEs) to decrease the rate at which cAMP is metabolized.

Cyclic nucleotide PDEs comprise a family of distinct, cell-associated isozymes that inactivate cAMP or cyclic 3′,5′-guanosine monophosphate (cGMP) by catalyzing hydrolysis of the 3′-phosphoester bond to form the corresponding inactive 5′-nucleotide products. To date, at least seven families of PDE isozymes have been identified, each of which is encoded by a distinct gene or family of genes.[11,12] Though similar in their primary hydrolytic role, these isozymes differ with respect to their substrate specificity (or lack thereof), structure, subunit organization, kinetic characteristics, allosteric regulation by endogenous activators and inhibitors, tissue and cellular distribution, and sensitivity to synthetic inhibitors.[8,13−15] Recognition of these differences has greatly stimulated interest in PDEs as drug targets, since these differences invite the possibility of targeting compounds specifically for the isozyme that predominates in the tissue or cell of interest. In particular, the dominant role of the cAMP-specific PDE (PDE4) in nearly all immune and inflammatory cells, and to a somewhat lesser extent in airway smooth muscle, has made this isozyme an attractive target for novel antiasthmatic and antiinflammatory therapy.[8,10,14−17]

As would be predicted from the predominance of PDE4 in immune and inflammatory cells and the putative biological role of cAMP in these cells, PDE4 inhibitors exert broad antiinflammatory effects in vitro.[18−30] This suppression of inflammatory cell activity has been extended to several in vivo models of inflammation.[31−35] Thus, the antiinflammatory and immunomodulatory activity displayed by PDE4 inhibitors in a variety of in vitro and in vivo settings, coupled with the relaxant activity of PDE4 inhibitors on human isolated bronchi,[36−38] support the concept that PDE4 inhibition represents a unique approach toward the therapy of asthma. However, first-generation PDE4 inhibitors also possess certain dose-limiting side effects resulting from an extension of their pharmacologic mechanism of action (i.e., inhibition of PDE4 in inappropriate tissues). The side effects of primary concern are nausea and vomiting,[39−41] increased gastric acid secretion,[41,42] and potential psychotropic activity.[41,43−46]

The primary objective of our research effort has been to identify novel, selective (for PDE4 versus other PDE isozymes), orally active second-generation PDE4 inhibitors as antiinflammatory and antiasthmatic agents with decreased potential for the side effects reported for the archetypic PDE4 inhibitor rolipram (Figure 1). To achieve this goal, we adopted a strategy focused on improving the selectivity of PDE4 inhibitors for their ability to inhibit human monocyte-derived PDE4 catalytic activity (herein designated LPDE4 activity)[47] versus their ability to compete for high affinity (nanomolar) [^{3}H]rolipram-binding sites in the central nervous system (CNS; this activity is herein designated HPDE4 activity),[48] two divergent biochemical properties of rolipram. Though the precise molecular nature of LPDE4 and HPDE4 activity remains uncertain, kinetic and radioligand binding studies on full-length and truncated forms of recombinant human PDE4 suggest that these

† Department of Medicinal Chemistry.
‡ Department of Pulmonary Pharmacology.
§ Department of Molecular Virology and Host Defense.
|| Department of Physical and Structural Chemistry.
⊥ Department of Immunopharmacology.

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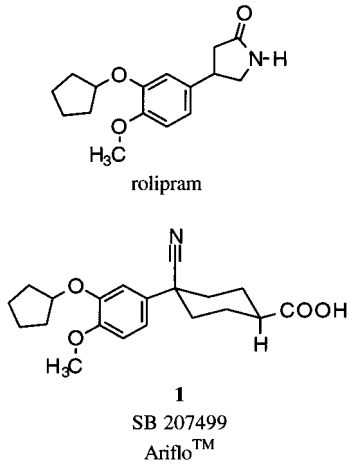


Figure 1. Structures of rolipram and **1**.

activities arise from two distinct forms of the enzyme.[47,49,50] While both forms appear to display catalytic activity, rolipram binds to HPDE4 with a high affinity (K_d ~2 nM) and to LPDE4 with a low affinity (K_d ~100 nM). These forms differ with respect to their relative sensitivity to inhibitors[47,51,52] and to their biological roles.[51,52]

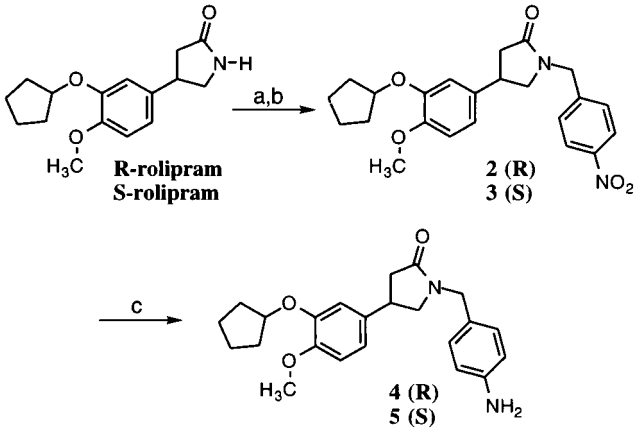
Herein, we briefly describe the derivation of our hypothesis that **potent LPDE4 inhibitors with reduced HPDE4 activity versus first-generation inhibitors would display good antiinflammatory activity with a decreased potential for side effects.** We also describe the use of this strategy to design *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid [IUPAC name: *c*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-*r*-1-cyclohexanecarboxylic acid] (**1**, SB 207499, Ariflo™, Figure 1), a potent and selective (versus other PDE isozymes) second-generation inhibitor of PDE4 with a significant improvement in side-effect profile relative to the first-generation inhibitor, (*R*)-rolipram.

Results and Discussion

Chemistry. Rolipram was synthesized and resolved as described previously.[53] Independent treatment of (*R*)- and (*S*)-rolipram with sodium hydride in anhydrous DMF in the presence of 15-crown-5, followed by reaction with 4-nitrobenzyl bromide, provided compounds **2** and **3**, respectively (Scheme 1). Subsequent reduction of **2** to **4** and **3** to **5** was accomplished with 10% palladium on carbon in the presence of excess ammonium formate in MeOH.[54]

The synthesis of **1** is outlined in Scheme 2. Reductive bromination of aldehyde **6**[55] with 1,1,3,3-tetramethyldisiloxane and lithium bromide in the presence of chlorotrimethylsilane[56] was followed by reaction of the product with sodium cyanide in DMF[57] to provide benzyl nitrile **7**. Double Michael reaction[58] of **7** in the presence of excess methyl acrylate in acetonitrile using Triton-B as base provided the pimelate **8**. Dieckmann cyclization[59] with sodium hydride in hot DME provided β-keto ester **9**, which was saponified and decarboxylated to cyclohexanone **10** with sodium chloride in hot aqueous DMSO.[60] Cyclohexanone **10** was homologated by Peterson-type reaction with excess 2-lithio-2-(trimethylsilyl)-1,3-dithiane[61] to produce ketene dithioacetal **11**. Mercuric chloride-mediated methanolysis[61] of **11** provided an approximately 11:1 mixture of the cis and trans esters (**12** and **13**, respectively), which was separated by flash chromatography on silica gel. Saponification of the individual esters with potassium hydroxide in a THF/methanol/water solvent mixture, followed by acidification with aqueous HCl, then provided the corresponding carboxylic acids **1** and **14**.

Scheme 1[a]



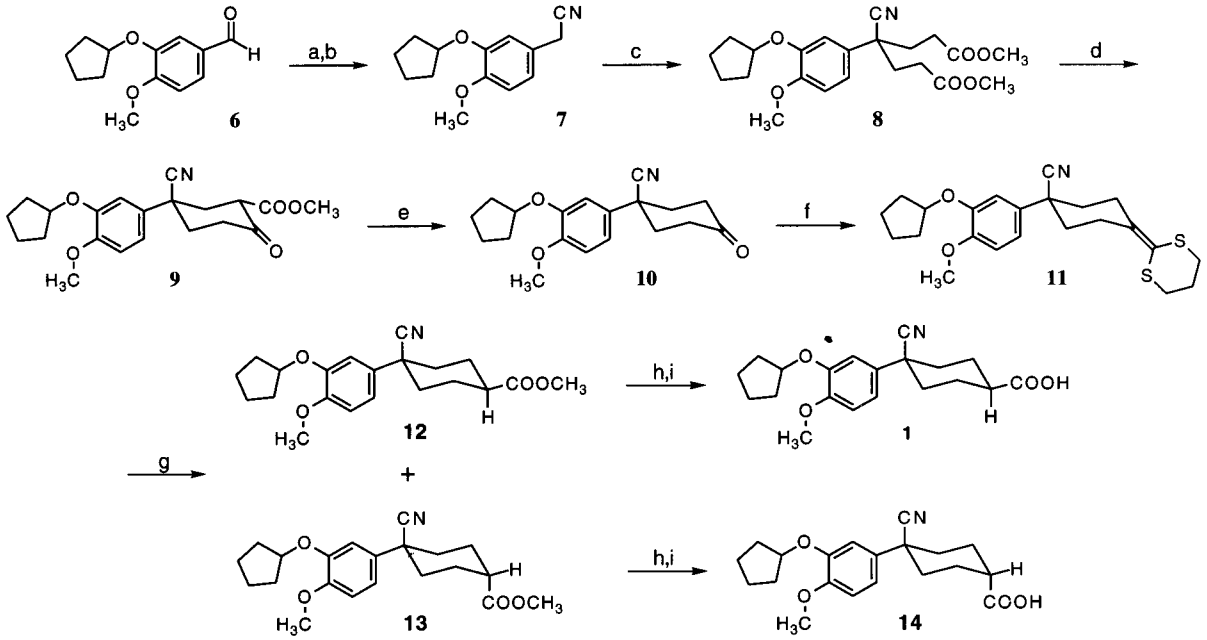
[a] Reagents: (a) NaH, 15-crown-5, DMF; (b) 4-nitrobenzyl bromide, THF; (c) 10% Pd/C, HCO_2NH_4, MeOH.

The synthesis of tetrazole **17** was accomplished by the method of Duncia[62] outlined in Scheme 3. EDC-mediated coupling of **1** with 3-aminopropionitrile in methylene chloride in the presence of HOBt provided cyanoethyl amide **15**. Reaction of **15** with $TMSN_3$ in the presence of DEAD and PPh_3 in THF provided protected tetrazole **16**, which was deprotected with sodium hydroxide in aqueous THF and then acidified with aqueous HCl to provide **17**.

Ketone **10** served as a convenient vehicle for the examination of substituent effects at the benzylic position of this class of cyclohexanone PDE4 inhibitors. Thus, reductive decyanation[63] of cyano ketone **10** with sodium in liquid ammonia was accompanied by concomitant reduction of the ketone to provide alcohol **18** (Scheme 4). Oxidation of **18** with PCC[64] to ketone **19**, followed by homologation as for ketone **10** in Scheme 2, produced ketene dithioacetal **20**. Hydrogen peroxide-mediated hydrolysis of **20**, followed by basic decomposition of the excess peroxide and then acidification with aqueous HCl, provided the descyanocyclohexane carboxylic acid **21**.[65]

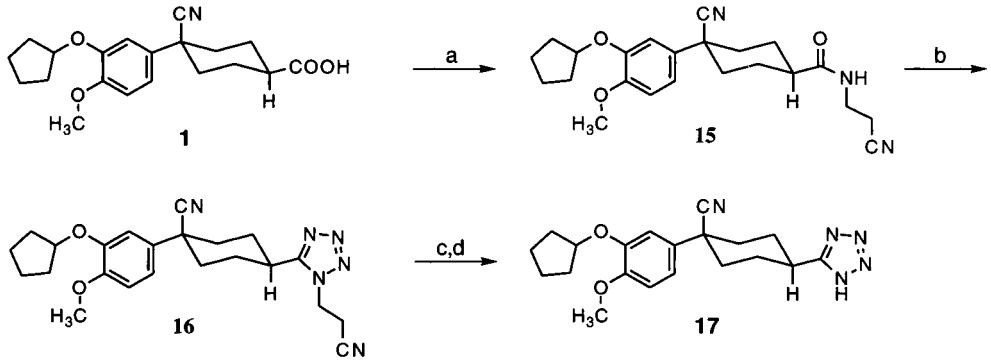
Alternatively, treatment of **10** with methyl orthoformate[66] and catalytic *p*-toluenesulfonic acid in methanol at reflux provided dimethyl ketal **22**, the key intermediate used to prepare five additional benzylically modified cyclohexanones (Scheme 5). Basic hydrogen peroxide-mediated hydrolysis[67] of the nitrile of **22** in aqueous methanol produced ketal amide **23**, which was deketalized to benzylic amide **24** in acidic acetone. Reduction of the nitrile of **22** with DIBAl-H[68] in toluene provided ketal aldehyde **25**. Simple hydrolysis of **25** with aqueous HCl in ethyl acetate produced benzylic aldehyde **26**. Homologation of **25** with the anion of dimethyl (diazomethyl)phosphonate[69] generated at −78 °C in THF with potassium *tert*-butoxide produced ketal acetylene **27**, which was hydrolyzed as with **23** to benzylic acetylene **28**. Reduction of ketal aldehyde **25** with

Scheme 2[a]



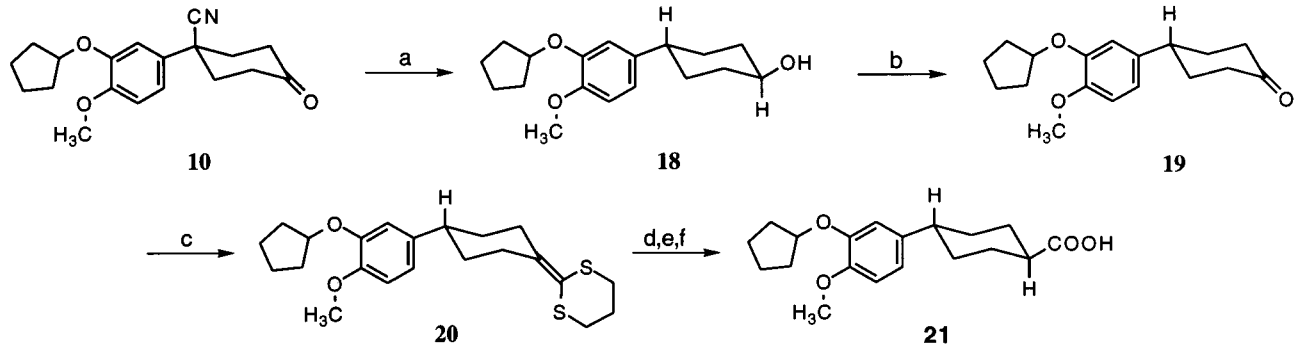
[a] Reagents: (a) LiBr, $(Me_2HSi)_2O$, TMSCl, MeCN; (b) NaCN, DMF; (c) excess methyl acrylate, MeCN, Triton-B; (d) NaH, DME, 85 °C; (e) NaCl, H_2O, DMSO, 150 °C; (f) lithium 2-(trimethylsilyl)-1,3-dithiane, THF, −78 °C; (g) $HgCl_2$, $HClO_4$, MeOH, 65 °C; (h) KOH, aqueous MeOH, THF; (i) aqueous HCl.

Scheme 3[a]



[a] Reagents: (a) $H_2N(CH_2)_2CN$, HOBt, EDC, CH_2Cl_2; (b) $TMSN_3$, PPh_3, DEAD, THF; (c) NaOH, aqueous THF; (d) aqueous HCl.

Scheme 4[a]



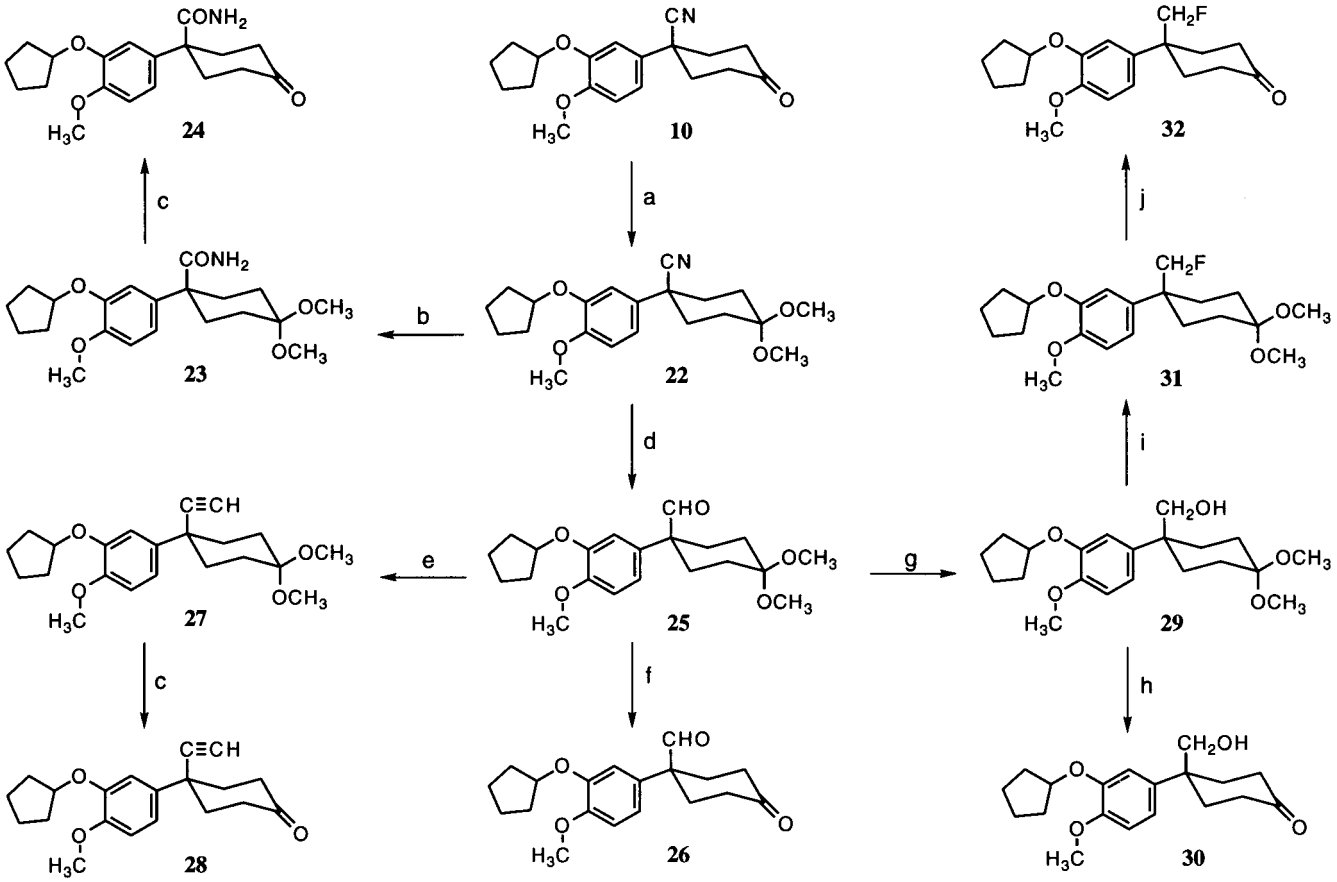
[a] Reagents (a) Na, NH_3; (b) PCC, CH_2Cl_2; (c) lithium 2-(trimethylsilyl)-1,3-dithiane, THF, −78 °C; (d) TFA, H_2O_2, 85 °C; (e) NaOH; (f) aqueous HCl.

sodium borohydride[70] in DME provided benzylic hydroxymethyl ketal **29**, which was hydrolyzed to the benzylic (hydroxymethyl)cyclohexanone **30**. Reaction of **29** with DAST[71] in methylene chloride at −78 °C produced the benzylic fluoromethyl ketal **31**, which was hydrolyzed with aqueous HCl in ethyl acetate to the benzylic (fluoromethyl)cyclohexanone **32**.

Compounds **33** and **34** (Table 1) were synthesized by published procedures.[72,73]

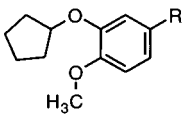
Structural assignment for cis ester **12** and trans ester **13** was based initially on the expectation that the thermodynamically more stable product (cis ester **12**) would be the major product, and on the resonance in the NMR spectrum in $CDCl_3$ for the α-methine proton

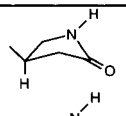
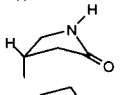
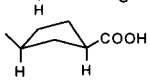
Scheme 5[a]



[a] Reagents: (a) $HC(OMe)_3$, *p*-TsOH (cat.), MeOH, reflux; (b) H_2O_2, K_2CO_3, MeOH/H_2O; (c) *p*-TsOH (cat.), $COMe_2$; (d) DIBAl-H, toluene; (e) $(MeO)_2P(O)CHN_2$, KO^{t}Bu, THF, −78 °C; (f) 3 N HCl, EtOAc; (g) $NaBH_4$, DME; (h) 1 N HCl, ether, reflux; (i) DAST, CH_2Cl_2, −78 °C; (j) 1 N HCl, EtOAc.

Table 1. Activity Profile of First-Generation PDE4 Inhibitors



Compd	R	LPDE4[a] IC50 (nM)	HPDE4[b] IC50 (nM)	H/L[c]	Antigen provocation[d] ID50 (mg/kg, i.v.)	Antigen provocation[e] ID50 (mg/kg, p.o.)	Hypothermia[f] ED50 (mg/kg, p.o.)	Therapeutic index[g]
R-rolipram		300	5	0.017	0.2	2	0.2	0.1
S-rolipram		1100	45	0.04	0.2	2	>10	>5
33		500	45	0.09	nt[h]	nt	nt	--
34		1200	5	0.004	0.5	3	0.5	0.17

[a] Inhibition of monocyte-derived human recombinant LPDE4 produced by yeast; IC50 values are the average of three independent determinations, with variation in individual values of <5%. [b] Competition for [^{3}H]-rolipram binding in rat brain cytosol; IC50 values are the average of two independent determinations, with variation in individual values of <5%. [c] Selectivity, *i.e.*, the value for HPDE4 divided by the value for LPDE4. [d] Antigen-induced bronchoconstriction in guinea pigs (i.v., 10 min pre-treatment); ID50 values are based on at least three point line analysis with n = 4-6 animals per point. [e] Antigen-induced bronchoconstriction in guinea pigs (p.o., 1 hr pre-treatment); ID50 values are based on at least three point line analysis with n = 4-6 animals per point. [f] ED50 for reversal of reserpine-induced hypothermia in the mouse (p.o.); ED50 values represent the mean response obtained from 6-8 animals. [g] Therapeutic index represents the value for hypothermia divided by the value for antigen provocation (p.o.). [h] Not tested.

at 2.36 ppm, a sharp, well-resolved triplet of triplets displaying the apparent coupling constants ($J_{ax,ax}$ = 12 Hz; $J_{ax,eq}$ = 4 Hz) diagnostic of the axial proton in a cyclohexane chair conformation.[74] In contrast, the

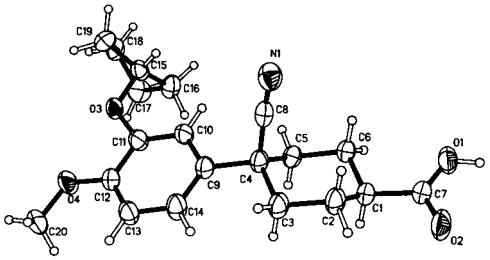


Figure 2. A view of **1** from its crystal structure showing the numbering scheme employed. For clairity, only the higher occupancy positions for the disordered cyclopentyl ring are shown. Anisotropic displacement ellipsoids for non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed spheres of arbitrary size.

corresponding signal for trans ester **13** was a broadened, distorted apparent six-line manifold with $J_{app} = 4$ Hz, indicative of an equatorial proton in a cyclohexane chair conformation.

Confirmation of the relative configuration of substituents in cis ester **12** was obtained with the crystal structure of its derived acid **1** (Figure 2). In the solid state, the cyclohexane ring system of **1** possesses an undistorted chair conformation. The nitrile and carboxylic acid moieties adopt a cisoid orientation relative to one another, with the nitrile oriented axially and the carboxylic acid oriented equatorially. The phenyl ring of the 3-(cyclopentyloxy)-4-methoxyphenyl moiety occupies an equatorial orientation about the cyclohexane chair, with a dihedral angle of 26.5° between the phenyl ring and the mean plane of the cyclohexane ring. The methyl group of the phenyl methoxy moiety is virtually in-plane with the phenyl ring. The cyclopentyl substituent occupies two different orientations, the major one (68%) illustrated in Figure 2 and the minor one in which the ring system is rotated approximately +20° about the phenyl carbon−oxygen bond.

The structural rigidity in solution of the cyclohexanone ring of descyano ketone **19** was apparent from its NMR spectrum obtained in $CDCl_3$. Specifically, the resonance for the benzylic methine proton at 2.95 ppm was a sharp, well-resolved triplet of triplets, displaying the apparent coupling constants ($J_{ax,ax} = 12$ Hz; $J_{ax,eq} = 3$ Hz) characteristic of an axial proton in a cyclohexane chair conformation. This unambiguously placed the 3-(cyclopentyloxy)-4-methoxyphenyl moiety in an equatorial orientation relative to the "pseudoequatorial" carbonyl of the cyclohexanone ring.

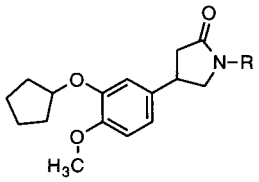
Consistently, the configuration of the descyanocyclohexanecarboxylic acid **21** again was evident from its NMR spectrum in $CDCl_3$. Here, a pair of triplet of triplets centered at 2.45 and 2.40 ppm, with apparent coupling constants of $J_{ax,ax} = 12$ Hz and $J_{ax,eq} = 4$ Hz, clearly signaled axial orientations for the cyclohexane C-4 and C-1 protons, respectively, and, therefore, a transoid orientation for the equatorially oriented 3-(cyclopentyloxy)-4-methoxyphenyl and carboxylic acid moieties. These conclusions were confirmed by more extensive 1D TOCSY and 1D NOE difference experiments conducted in benzene-d_6 at 300 K to improve signal dispersion (data not shown).

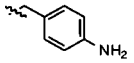
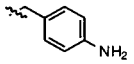
Structure−Activity Relationships. The multiplicity of PDE isozymes precluded a substrate-based design strategy for an isozyme-selective PDE inhibitor. Thus, the initial steric and electronic structural requirements for selective PDE4 inhibition were derived from the inhibitory and specificity profiles of a series of active site directed (competitive) PDE4 inhibitors, appropriately modified congeners of rolipram. Using portions of rolipram as a template, additional functionalities expected to modify physicochemical properties while maintaining or improving potency were explored. These studies identified the 3-(cyclopentyloxy)-4-methoxyphenyl moiety **and** an appropriately positioned hydrogen bond acceptor moiety as the minimum pharmacophoric requirements for potent PDE4 inhibition, consistent with results subsequently reported by other researchers.[75] Thus, in our studies, cyclopentanone **33**, a racemate, was comparable to (*R*)-rolipram in inhibition of LPDE4 activity (Table 1), revealing that the NH function of the rolipram pyrrolidinone ring was unnecessary for inhibition. Both (*R*)-rolipram and **33** were more potent than (*S*)-rolipram by a factor of 2−4. The carboxyl function of cyclopentanecarboxylate **34** served as a slightly poorer hydrogen bond acceptor, with **34** (again, a racemate) 4-fold less potent than (*R*)-rolipram as an inhibitor of LPDE4 activity. With respect to HPDE4 activity, **33** and (*S*)-rolipram were some 9-fold less potent than (*R*)-rolipram, while **34** was comparable to (*R*)-rolipram in HPDE4 activity.

In an effort to investigate the pharmacologic roles of LPDE4 and HPDE4 activity, three of these four compounds were evaluated for their ability to inhibit antigen-induced bronchoconstriction in guinea pigs, an antiallergic model,[34] and to reverse reserpine-induced hypothermia in the mouse, a standard model of psychotropic activity.[44,76] Upon either intravenous or oral administration, (*R*)-rolipram, (*S*)-rolipram, and **34** were essentially equipotent inhibitors of bronchoconstriction in the guinea pig model (Table 1). In terms of their ability to reverse reserpine-induced hypothermia in the mouse, **34** was again equipotent to (*R*)-rolipram, while (*S*)-rolipram was essentially inactive in this model, consistent with reports on (*R*)- and (*S*)-rolipram by earlier workers.[77] While the approach used here to compare antiallergic activity in the guinea pig with reversal of hypothermia in the mouse using inhibitors administered by the oral route is not without pitfalls, particularly with regard to the impact that differences in pharmacokinetics and drug disposition can have in cross-species comparisons, results with these three compounds suggested to us a tentative correlation between inhibition of LPDE4 activity and peripheral (antiinflammatory) effects on one hand and between HPDE4 activity and central (CNS) effects on the other.

Parallel studies conducted with the rolipram enantiomers and their N-substituted derivatives strengthened this tentative correlation. Thus, **4**, the 4-aminobenzyl derivative of (*R*)-rolipram, displayed LPDE4 activity comparable to that of the parent with an 8-fold loss in HPDE4 activity. Its enantiomer, **5**, possessed an even more interesting profile in vitro, with a 6-fold improvement in LPDE4 activity and a 65-fold loss of HPDE4 activity relative to (*R*)-rolipram. Given that neither **4** nor **5** possessed significant oral activity in the guinea pig model, these compounds were evaluated pharmacologically in a different way. Thus, the ability of these compounds to inhibit antigen-induced bronchoconstric-

Table 2. Activity Profile of the Rolipram Enantiomers and *N*-(4-Aminobenzyl) Derivatives



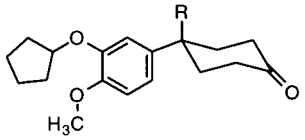
Compd	Absolute Configuration	R	LPDE4[a] IC50 (nM)	HPDE4[a] IC50 (nM)	H/L[a]	Antigen provocation[a] ID50 (mg/kg, i.v.)	Emesis[b] ED50 (mg/kg, i.v.)	Therapeutic index[c]
rolipram	R	H	300	5	0.017	0.2	0.037	0.19
4	R		195	40	0.2	10	0.75	0.08
rolipram	S	H	1100	45	0.04	0.2	>0.3	>1.5
5	S		52	320	6.1	0.5	>3.0	>6

[a] See corresponding footnotes from Table 1. [b] Emesis in dogs (i.v.); ED50 values were generated from a cumulative frequency curve of n=5 dogs. [c] Therapeutic index represents the value for emesis divided by the value for antigen provocation.

tion in guinea pigs was compared with their ability to induce emesis in dogs[78] when administered by the intravenous route, another cross-species comparison subject to the caveat noted above. In these studies, **5** was comparable in potency to (*R*)- and (*S*)-rolipram in the guinea pig model, with **4** displaying unexpectedly poor in vivo activity relative to its LPDE4 potency in vitro. Compounds **4**, **5**, and (*S*)-rolipram were significantly less potent than (*R*)-rolipram in their ability to elicit emesis in the dog, consistent with their reduced HPDE4 activity versus (*R*)-rolipram in vitro. Overall, the trend of the data summarized in Tables 1 and 2 suggested that despite the caveat of cross-species comparison, decreasing the HPDE4 activity of PDE4 inhibitors, and/or improving the selectivity of PDE4 inhibitors for LPDE4 activity at the expense of HPDE4 activity, could result in an improved therapeutic index.

Thus, our synthetic efforts focused on construction of a structurally simplified pharmacophore possessing the requisite improvement in LPDE4 activity versus HPDE4 activity. The five-membered pyrrolidinone ring system of rolipram, the cyclopentanone of compound **33**, and the cyclopentane ring bearing the carboxyl moiety of compound **34** all possess significant conformational flexibility. Ring puckering allows the 3-(cyclopentyloxy)-4-methoxyphenyl group to adopt either a pseudoaxial or pseudoequatorial orientation with respect to the carbonyl or carboxyl moieties of these compounds about the five-membered ring. While molecular mechanics-based conformational analysis (amber*/Macromodel/Batchmin) coupled with molecular orbital calculations (Gaussian94, 6-31G*//6-31G*) suggested that the pseudoequatorial conformation of each compound would be energetically preferred, unambiguous assignment of an active conformation of these five-membered rings was not possible. For this, we sought a ring scaffold that would (1) decrease conformational mobility, allowing more rigid placement of key pharmacophore units in order to derive a more well-defined pharmacophore model; (2) avoid the issue of chirality to simplify both structure activity relationship (SAR) interpretation and synthetic efficiency; (3) provide flexibility in both the synthesis and SAR development of alternative spacial relationships among key pharmacophore units; and (4) possess LPDE4 activity comparable to or better than that of (*R*)-rolipram and **33** with a better selectivity for LPDE4 activity versus HPDE4 activity than these compounds.

Table 3. Activity Profile of Cyclohexanone Derivatives

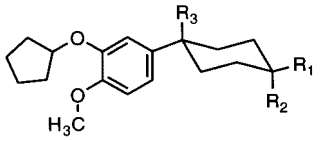


compd	R	LPDE4[a] IC_{50} (nM)	HPDE4[a] IC_{50} (nM)	H/L[a]
10	CN	44	120	2.7
28	C≡CH	80	300	3.8
19	H	120	70	0.6
24	$CONH_2$	320	700	2.2
26	CHO	340	500	1.5
32	CH_2F	1180	6000	5.1
30	CH_2OH	2500	7000	2.8

[a] See corresponding footnotes from Table 1.

A 1,4-substituted cyclohexane ring fulfilled all of these criteria, as evidenced by the achiral cyclohexanone **19** (Table 3). Relative to (*R*)-rolipram, the structurally rigid **19** (vide supra) possesses 2.5-fold better LPDE4 activity with a 25-fold reduction in HPDE4 activity, a ca. 60-fold improvement. Likewise, relative to **33** (cf. Table 2), **19** possesses 4-fold better LPDE4 activity with a 1.5-fold reduction in HPDE4 activity, resulting in a nominal 6-fold improvement in selectivity for LPDE4 versus HPDE4 activity. Comprehensive SAR studies of substituents possessing a wide range of steric and electronic properties at the benzylic position of cyclohexanone **19** revealed that a third pharmacophore element, a nitrile, improved both LPDE4 activity and selectivity. As illustrated in Table 3 with selected examples, cyano ketone **10** is roughly 2.5-fold more potent and 4.5-fold more selective than **19** for LPDE4 activity. Replacement of the cyano moiety of **10** by an acetylene (**28**), an amide (**24**), an aldehyde (**26**), a

Table 4. Activity Profile of **1** and Related Compounds



compd	R_1	R_2	R_3	LPDE4[a] IC_{50} (nM)	HPDE4[a] IC_{50} (nM)	H/L[a]
1	COOH	H	CN	95	120	1.3
14	H	COOH	CN	3410	500	0.15
12	CO_2CH_3	H	CN	530	1000	1.9
13	H	CO_2CH_3	CN	1070	700	0.7
17	5-tetrazole	H	CN	740	110	0.15
21	COOH	H	H	1200	120	0.1

[a] See corresponding footnotes from Table 1.

fluoromethyl group (**32**), or a hydroxymethyl group (**30**) produced progressively poorer LPDE4 activity, accompanied in these cases by a parallel loss in HPDE4 activity. It is assumed by analogy to compound **19** that, in solution, the 3-(cyclopentyloxy)-4-methoxyphenyl moiety of **10** adopts the equatorial orientation with the nitrile in the axial orientation, as it is in the solid state (data not shown), and that this is the conformation producing LPDE4 inhibition.

Maintaining the benzylic nitrile, further examinations of substituent effects focused on maintaining the LPDE4 activity and selectivity versus HPDE4 of **10** while improving pharmaceutical properties within this class, which prompted the synthesis of **1**. Compound **1** displays a 2-fold reduction in inhibition of LPDE4 relative to **10** with identical HPDE4 activity (Table 4). The importance of proper spatial positioning of the carboxyl moiety relative to the 3-(cyclopentyloxy)-4-methoxyphenyl and cyano moieties of the cyclohexane ring is illustrated by comparison of **1** with its trans isomer, **14**. LPDE4 potency is profoundly influenced, with **14** some 36-fold less potent than **1**, while the differential effect on HPDE4 activity is a comparatively modest 4-fold loss. In the corresponding pair of diastereomeric esters, differences between the isomers for both activities are much less pronounced because of the poor intrinsic potency of cis ester **12**. Given that the inhibition of LPDE4 activity by **12** is essentially equivalent to that of the tetrazole **17**, which, like **1**, is ionized and negatively charged at physiological pH, the poor activity of **12** and **13** most likely results from a steric constraint on inhibitor functionality in this region of the enzyme active site. In contrast, the 8−9-fold decrease in HPDE4 activity for **12** versus **1** and **17** suggests that it is the lack of charge associated with **12,** rather than steric factors, which is important for the decrease in this activity.

Finally, to confirm our previous observations concerning the importance of the benzylic nitrile function in the cyclohexanone series, we undertook synthesis and evaluation of descyano compound **21** from **1**. As expected based on the previous SAR (cf. Table 3), the LPDE4 activity of **21** was some 12-fold less than that of **1**, although in this case removal of the nitrile had no effect on HPDE4 activity, with **21** equipotent to **1**.

Biological Profile of 1. Compound **1** is inactive against PDEs 1, 2, 3, and 5 at concentrations up to 10 μM. Compound **1** potently and competitively inhibits LPDE4 with a $K_i = 92 \pm 6$ nM. Compound **1** is nearly equipotent with respect to LPDE4 activity ($IC_{50} = 95$ nM) and HPDE4 activity ($IC_{50} = 120$ nM). In contrast, the HPDE4 activity ($IC_{50} = 5$ nM) of (*R*)-rolipram is 60-fold greater than its LPDE4 activity ($IC_{50} = 300$ nM). This represents a roughly 75-fold improvement over (*R*)-rolipram in terms of the selectivity of **1** for LPDE4 versus HPDE4 (Table 5).

To illustrate the functional consequences of this improvement in the selectivity of **1** versus (*R*)-rolipram for LPDE4 versus HPDE4 activity (Table 5), the ability of **1** and (*R*)-rolipram to decrease tumor necrosis factor-α production by human isolated monocytes ($hTNF_\alpha$)[51] was compared with their ability to increase acid secretion by isolated rabbit parietal glands.[52] (*R*)-Rolipram and **1** inhibit lipopolysaccharide (LPS)-induced $hTNF_\alpha$ release in a concentration-dependent manner, with IC_{50} values of 70 and 110 nM, respectively. (*R*)-Rolipram is a potent acid secretagogue in rabbit parietal glands ($EC_{50} = 4$ nM). In marked contrast, **1** is a much weaker acid secretagogue, with an $EC_{50} = 800$ nM. Thus, while (*R*)-rolipram is 17-fold more potent as an acid secretagogue than as an inhibitor of $hTNF_\alpha$ generation, **1** is 7-fold more potent as an inhibitor of $hTNF_\alpha$ generation than as an acid secretagogue, a 120-fold improvement. These results are consistent with our previous conclusions that inhibition of LPDE4 activity is associated with suppression of $hTNF_\alpha$ production,[51] an antiinflammatory effect, whereas inhibition of HPDE4 activity correlates with acid secretion,[52] a side effect.

While these in vitro assay systems suggested an improved therapeutic potential for **1**, it was essential to determine if this improvement was maintained in vivo. Our first approach was to determine the antiallergic potency of **1** versus (*R*)-rolipram against antigen-induced bronchoconstriction in the anesthetized guinea pig[34] and to compare this value with its emetic potency

Table 5. Comparative Potencies of **1** and (*R*)-Rolipram

compd	LPDE4[a] IC_{50} (nM)	HPDE4[a] IC_{50} (nM)	$hTNF_\alpha$[b] IC_{50} (nM)	acid secretion[c] EC_{50} (nM)	antigen provocation[a] ID_{50} (mg/kg, iv)	emesis[d] ED_{50} (mg/kg, iv)	$hTNF_\alpha$[e] ID_{50} (mg/kg, po)	hypothermia[f] ED_{50} (mg/kg, po)
(*R*)-rolipram	300	5	70	4	0.2	0.04	5.1	0.2
1	95	120	110	800	1.8	4.6	4.9	2.3
potency ratio[g]	3	0.04	0.6	0.005	0.1	0.01	1	0.09

[a] See corresponding footnotes from Table 1. [b] IC_{50} for inhibition of LPS-induced $hTNF_\alpha$ generation from human monocytes; IC_{50} values are the average of four to seven independent determinations. [c] EC_{50} for stimulation of acid secretion from rabbit parietal glands; EC_{50} values are the average of three to five independent determinations. [d] Emesis in dogs (iv); see corresponding footnote from Table 2. [e] ID_{50} for inhibition of LPS-induced $hTNF_\alpha$ generation in the mouse (po); ID_{50} values represent the mean response obtained from three to eight animals. [f] ED_{50} for reversal of reserpine-induced hypothermia in the mouse (po); see corresponding footnote from Table 1. [g] Potency ratio represents the potency of **1** relative to (*R*)-rolipram, i.e., the value for (*R*)-rolipram divided by the value for **1**.

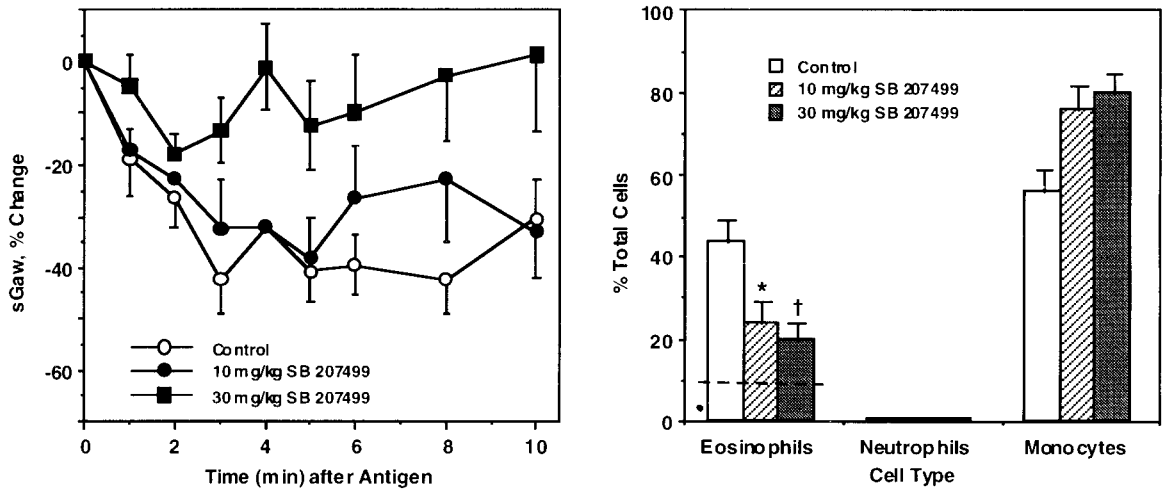


Figure 3. Inhibitory effect of **1** on antigen-induced bronchoconstriction and eosinophil infiltration in conscious guinea pigs. Sensitized guinea pigs pretreated with chlorpheniramine (0.5 mg/kg) were placed in a plethysmograph and given an oral dose of **1** (10 or 30 mg/kg, dissolved in 90% PEG/10% H_2O) 1 h prior to the administration of antigen (ovalbumin) by the aerosol route. Pulmonary function (SGaw) was monitored over the next 10 min (left). The animals were removed and bronchoalveolar lavage was performed 24 h after antigen challenge (right). The cells were isolated from the lavage fluid and differential cell counts analyzed in stained cytospin preparations. Data are expressed as percent of total cell number. The dashed line represents the percentage of eosinophils in unchallenged animals. The results obtained are the mean ±SE of six to seven animals/group. *†Significantly less than vehicle control: *($P < 0.05$), †($P < 0.01$).

in dogs.[78] After intravenous administration, (*R*)-rolipram and **1** inhibit antigen-induced bronchoconstriction with ID_{50} values of 0.2 and 1.8 mg/kg, respectively (Table 5). A noteworthy observation from these studies is that **1** has no effect on mean arterial pressure, pulse pressure, or heart rate. (*R*)-Rolipram produces emesis in the dog at 5-fold lower doses ($ED_{50} = 0.04$ mg/kg, iv) than those that inhibit bronchoconstriction in the guinea pig. In contrast, intravenous **1** produces emesis in the dog with an $ED_{50} = 4.6$ mg/kg, 3-fold greater than its ID_{50} for inhibiting antigen-induced bronchoconstriction in the guinea pig. This yields an improved therapeutic potential of slightly greater than 10-fold compared with (*R*)-rolipram. These results demonstrate that the postulated improvement in therapeutic potential based upon in vitro measurements is reflected in an in vivo setting, though again the role of potential differences in pharmacokinetics and drug disposition is unknown in this cross-species comparison.

To confirm this improvement in therapeutic potential without relying on a cross-species comparison, and to demonstrate that this improvement could be maintained upon administration by the oral route, our second approach was to compare the ability of **1** and (*R*)-rolipram to inhibit LPS-induced $hTNF_\alpha$ generation from human monocytes adoptively transferred into mice[79] with their ability to reverse reserpine-induced hypothermia in the same species. Upon oral administration, (*R*)-rolipram and **1** inhibit LPS-induced $hTNF_\alpha$ production in a dose-dependent manner and with approximately equal potencies ($ED_{50} \approx 5$ mg/kg). However, when administered orally, (*R*)-rolipram ($ED_{50} = 0.2$ mg/kg) and **1** ($ED_{50} = 2.3$ mg/kg) differ markedly in their ability to reverse reserpine-induced hypothermia. (*R*)-Rolipram is at least 20-fold more potent at reversing reserpine-induced hypothermia than inhibiting $hTNF_\alpha$ production in vivo in the mouse, while **1** is only 2-fold more potent in reversing reserpine-induced hypothermia than it is at inhibiting $hTNF_\alpha$ production. This 10-fold improvement over (*R*)-rolipram confirms our previous in vivo results using the cross-species comparison.

Oral pharmacodynamics of **1** were assessed in both the guinea pig and the mouse. Orally administered **1** (30 mg/kg) inhibits antigen-induced bronchoconstriction in conscious guinea pigs with an estimated half-life of 4−5 h. In the mouse, orally administered **1** (30 mg/kg) inhibits the production of $mTNF_\alpha$ with an estimated half-life of >10 h.

The activity of **1** also was examined in a guinea pig model of the early-phase/late-phase response to antigen (Figure 3).[34] In this model, conscious sensitized guinea pigs are placed in a plethysmograph and given an aerosol challenge with ovalbumin. This results in mast cell degranulation and the rapid development of an early-phase response marked by bronchoconstriction. This early-phase response resolves over a period of 30−60 min. Beginning approximately 6 h after the antigen challenge and continuing over a 24-h period, there is a fulminant infiltration of inflammatory cells into the airway. The most prominent of these cells, which can be monitored by bronchoalveolar lavage, is the eosinophil. This infiltration of eosinophils into the airway is analogous to the late-phase response in human asthmatic patients. Previously, we have demonstrated that (*R*)-rolipram administered orally 1 h prior to challenge inhibited antigen-induced bronchoconstriction and eosinophil influx in a dose-dependent manner.[34] However, at the doses used (1, 3 and 10 mg/kg), (*R*)-rolipram also produces signs of CNS excitement (e.g., hyperpnea, agitation, tremor) consistent with its previously reported action in the guinea pig.[80] Similar to (*R*)-rolipram, oral administration of **1** (30 mg/kg) 1 h prior to antigen challenge inhibits eosinophil influx by 65% (Figure 3). In contrast to (*R*)-rolipram, **1** does not produce any signs of CNS excitement at this dose. Thus, **1** prevents the development of a late-phase reaction in the guinea pig but, unlike rolipram, does so at a dose that produces no overt CNS effects.

In summary (Table 5), **1** is a potent and competitive inhibitor of PDE4, which is selective for PDE4 over the other PDE isozymes and is 75-fold more selective than (*R*)-rolipram with respect to LPDE4 activity versus HPDE4 activity. In three models established to evaluate the side-effect potential (acid secretagogue, emetic and psychotropic effects) of PDE4 inhibitors, the overall trend of the data suggests substantial improvement for **1** versus the first generation inhibitor (*R*)-rolipram (10−120-fold, Table 5). Compound **1** is orally active in both guinea pig and mouse models with excellent pharmacodynamic properties.

Conclusions

Correlation of the central (CNS) effects of rolipram with HPDE4 rather than LPDE4 activity, along with the apparent separation between the central and peripheral (antiinflammatory) pharmacological effects of PDE4 inhibitors, led us to a strategy focused on increasing the potency of compounds for LPDE4 activity while decreasing their HPDE4 activity. Our original observation that these divergent biological activities could be separated by appropriate modification of PDE4 inhibitors have been extended in subsequent SAR studies with a number of different PDE4 inhibitors.[47,50,81−86] One crucial feature of this divergence in terms of drug discovery is that HPDE4 activity is not present, or is present at very low densities, in many tissues that contain a considerable amount of LPDE4 activity. In particular, HPDE4 is undetectable in most immune and inflammatory cells, while the CNS appears to contain a high proportion of this activity.[48,51,86,87] This prompted investigations of the relevance of HPDE4 to the functional actions of PDE4 inhibitors by ourselves and others using standard pharmacological approaches. Thus, as judged by the rank-order potency of a variety of PDE4 inhibitors for HPDE4 versus LPDE4 activity, we and others have demonstrated that HPDE4 activity strongly correlates with the following functional effects: (1) behavioral activity in rodents;[76] (2) stimulation of acid secretion from rabbit parietal glands;[52] (3) emesis in dogs[78] and ferrets;[86] (4) elevation of cAMP content in guinea-pig eosinophils;[88] (5) relaxation of guinea-pig airway;[89] and (6) inhibition of superoxide production from human neutrophils.[51] In contrast, by the same criteria, inhibition of LPDE4 is linked with inhibiting the following processes: (1) guinea pig mast cell degranulation;[34] (2) antigen-driven proliferation of human T-cells;[30] (3) TNF-α generation in human monocytes;[25,51] and (4) superoxide production from guinea pig eosinophils.[90] Eosinophils are unique in that both LPDE4 and HPDE4 activities are present and functionally important.[88,90]

While the precise molecular nature of HPDE4 vis-à-vis LPDE4, as well as the consequent mechanistic rationale for the differential rank-order potency of PDE4 inhibitors in producing these effects, remains unknown, the demonstration that individual PDE4 inhibitors produce different pharmacological effects with distinct rank-order potencies has defined our strategy for a new generation of compounds. Thus, *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid (**1**, SB 207499, Ariflo™), a potent and selective (versus other PDE isozymes) inhibitor of PDE4, is among the first of a new generation of PDE4 inhibitors specifically designed to possess an improved therapeutic potential relative to first generation inhibitors (e.g.,(*R*)-rolipram). Whether this comparative improvement conveys clinical success remains to be determined. Compound **1** continues to progress in clinical trials as a novel therapy for the treatment of asthma.

Experimental Section

(1) Crystallographic Studies. A colorless plate of **1** with approximate dimensions 0.6 × 0.2 × 0.1 mm was mounted on a glass fiber with paratone for examination. Lattice parameters were determined at 223 K on an Enraf Nonius CAD-4 diffractometer equipped with graphite monochromated molybdenum radiation (λ Mo Kα = 0.710 73 Å) from the setting angles of 25 high-order reflections. The systematic absences, $0k0$ for k odd and $h0l$ for l odd, indicated space group $P2_1/c$. Intensity data were collected on the diffractometer using variable speed $\omega-2\theta$ scans where the final scan speed was determined for each reflection from a prescan of its intensity. Scan speeds varied from 1.27 to 5.5 deg min^{-1}. Background estimates were made by extending the scan 25% on each side of the peak. Three intensity standards, monitored at regular intervals, showed a negligible variation. Data were corrected for backgrounds, Lorentz, and polarization effects. A total of 4384 data were collected to $2\theta \leq 55°$ ($-18 \leq h \leq 18$; $0 \leq k \leq 18$; $0 \leq l \leq 12$), which gave 4128 unique data after averaging of symmetry equivalents ($R_{int} = 0.02$). The structure was solved by direct methods.[91] Atomic positions were refined with anisotropic displacement parameters for all nondisordered positions and for the highest occupancy conformer (68%) of the cyclopentyloxy substituent. The lower occupancy positions (32%) for the cyclopentyloxy group were refined with isotropic temperature factors.[92] The function minimized employing full-matrix least-squares procedures was $\sum w(F_o^2 - F_c^2)^2$. Weights, w, were eventually assigned to the data as $w = 1/[\sigma^2(F_o^2) + (0.0934P)^2 + 0.87P]$ where $P = [\mathrm{MAX}(F_o^2,0) + 2F_c^2]/3$. Positions for the hydrogen atoms were determined from difference Fourier maps. The positional and isotropic displacement parameters for the hydrogen attached to the heteroatom were refined. Other hydrogen atoms were included in idealized positions riding on the atom to which they are attached with isotropic displacement factors assigned as 1.2 times U_{eq} of the attached atom. The refinement (on F^2) converged (max $\Delta/\sigma = 0.002$) to values of the conventional crystallographic residuals $R = 0.056$ for observed data and $R = 0.114$ (w$R_2 = 0.178$) for all data. A final difference Fourier map was featureless with maximal residual density peaks between ± 0.24 e Å^{-3}. Values of the neutral atom scattering factors, including $\Delta f'$ and $\Delta f''$ for all non-hydrogen atoms, were from the *International Tables for X-ray Crystallography*.[93] Crystal data: $C_{20}H_{25}NO_4$, M_r (343.43), monoclinic, $P2_1/c$, a (14.244(2) Å), b (14.239(1) Å), c (9.292(3) Å), β (107.06(1)°), V (1801.7(4) Å^3), Z (4), ρ_{calc} (1.266 g cm^{-3}), μ (0.088 mm^{-1}), F(000) (736).

(2) Chemistry. Melting points were determined on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded at ambient temperature on a Bruker AM-250 or AM-400 spectrometer in the indicated solvent, with chemical shifts reported as apparent centers of multiplets in ppm (δ) downfield from internal TMS and apparent first-order coupling constants reported in hertz. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter in a 1 dm cell. Elemental analyses were determined using a Perkin-Elmer 240C; the amorphous and/or hygroscopic nature of certain compounds resulted in incorporation of fractions of water molecules into their elemental analyses. Flash chromatography was conducted using silica gel 60 (70−230 mesh ASTM) from E. Merck.

(*R*)-(+)-1-(4-Aminobenzyl)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]pyrrolidin-2-one (4). (*R*)-(−)-rolipram (1.8 g, 6.5 mmol) was added to a suspension of NaH (196 mg of an 80% dispersion) in dry DMF (65 mL) containing 15-crown-5-ether (1.28 mL). The suspension was stirred under an argon

atmosphere overnight at room temperature and then was heated at 50−60 °C for 90 min to provide a solution of the anion. 4-Nitrobenzyl bromide (2.79 g, 12.9 mmol) was dissolved in dry THF (70 mL), and the solution of anion was added. The reaction mixture was stirred overnight, and the THF was removed in vacuo. The resulting solution was poured into ice water, was acidified with 3 N HCl, and was extracted with EtOAc. The organic extract was washed six times with water, was dried (Na_2SO_4), and was evaporated. The residue was purified by flash chromatography, eluting with 1−3% MeOH/$CHCl_3$, to provide **2** as a yellow resin (570 mg, 21%): $[\alpha]^{25}_D$ (0.61, methanol) = +48.8°; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21 (d, J = 9 Hz, 2H), 7.44 (d, J = 9 Hz, 2H), 6.80 (d, J = 8 Hz, 1H), 6.70 (dd, J = 8, 2 Hz, 1H), 6.67 (s,1H), 4.71 (m, 1H), 4.67 (d, J = 15 Hz, 1H), 4.52 (d, J = 15 Hz, 1H), 3.81 (s, 3H), 3.62 (t, J = 9 Hz, 1H), 3.52 (p, J = 7 Hz, 1H), 3.26 (dd, J = 9, 9 Hz, 1H), 2.90 (dd, J = 9, 18 Hz, 1H), 2.64 (dd, J = 9, 18 Hz, 1H), 1.85−1.60 (br m, 8H).

A solution of **2** (503 mg, 1.23 mmol) in anhydrous THF (6 mL) was treated with a suspension of ammonium formate (1.2 g, 19 mmol) and 10% Pd/C (120 mg) in MeOH. The suspension was stirred for 2 h under argon. The reaction was filtered through Celite and was washed with MeOH. The solvent was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and water. After extracting, the organic layer was washed twice with water, was dried (K_2CO_3), and was concentrated in vacuo. The resin was purified by flash chromatography, eluting with a gradient of 50−100% EtOAc/CH_2Cl_2, to provide **4** as a colorless oil (396 mg, 82%): $[\alpha]^{25}_D$ (0.56, methanol) = +72.5°; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.07 (d, J = 8 Hz, 2H), 6.79 (d, J = 8 Hz, 1H), 6.72−6.60 (m, 4H), 4.71 (m, 1H), 4.44 (d, J = 14 Hz, 1H), 4.31 (d, J = 14 Hz, 1H), 3.82 (s, 3H), 3.66 (s, 2H), 3.57 (t, J = 9.6 Hz, 1H), 3.42 (p, J = 8 Hz, 1H), 3.20 (dd, J = 9.6, 9.6 Hz, 1H), 2.83 (dd, J = 8 Hz, J = 16 Hz, 1H), 2.55 (dd, J = 8 Hz, J = 16 Hz, 1H), 1.85−1.62 (br m, 8H). Anal. ($C_{23}H_{28}N_2O_3 \cdot {}^1/_5H_2O$) C, H, N.

(*S*)-(−)-1-(4-Aminobenzyl)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]pyrrolidin-2-one (5). Compound **3** was produced from (*S*)-(+)-rolipram by the reaction sequence described for the preparation of **2**. The residue was purified by successive flash chromatography, first eluting with 1−2% MeOH/$CHCl_3$, then eluting with 3:1 EtOAc/hexanes, to provide **3** as a yellow resin (505 mg, 19%): $[\alpha]^{25}_D$ (0.61, methanol) = −48.5°; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21 (d, J = 9 Hz, 2H), 7.44 (d, J = 9 Hz, 2H), 6.80 (d, J = 8 Hz, 1H), 6.70 (dd, J = 8, 2 Hz, 1H), 6.67 (s,1H), 4.71 (m, 1H), 4.67 (d, J = 15 Hz, 1H), 4.52 (d, J = 15 Hz, 1H), 3.81 (s, 3H), 3.62 (t, J = 9 Hz, 1H), 3.52 (p, J = 7 Hz, 1H), 3.26 (dd, J = 9, 9 Hz, 1H), 2.90 (dd, J = 9, 18 Hz, 1H), 2.64 (dd, J = 9, 18 Hz, 1H), 1.85−1.60 (br m, 8H).

Compound **5** was produced from **3** by the reaction sequence described for the preparation of **4**. The resin was purified by flash chromatography, eluting with a gradient of 50−75% EtOAc/CH_2Cl_2, to provide **5** as a colorless resin (342 mg, 81%): $[\alpha]^{25}_D$ (0.63, methanol) = −72.5°; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.07 (d, J = 8 Hz, 2H), 6.79 (d, J = 8 Hz, 1H), 6.72−6.60 (m, 4H), 4.71 (m, 1H), 4.44 (d, J = 14 Hz, 1H), 4.31 (d, J = 14 Hz, 1H), 3.82 (s, 3H), 3.66 (s, 2H), 3.57 (t, J = 9.6 Hz, 1H), 3.42 (p, J = 8 Hz, 1H), 3.20 (dd, J = 9.6, 9.6 Hz, 1H), 2.83 (dd, J = 8 Hz, J = 16 Hz,1H), 2.55 (dd, J = 8 Hz, J = 16 Hz, 1H), 1.85−1.62 (br m, 8H). Anal. ($C_{23}H_{28}N_2O_3 \cdot {}^1/_5H_2O$) C, H, N.

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]acetonitrile (7). To a solution of **6**[55] (20 g, 90.8 mmol) in CH_3CN (100 mL) was added LiBr (15 g, 173 mmol) followed by the dropwise addition of TMSCl (17.4 mL, 137 mmol). After 15 min, the reaction mixture was cooled to 0 °C, 1,1,3,3-tetramethyldisiloxane (26.7 mL, 151 mmol) was added dropwise, and the resulting mixture was allowed to warm to room temperature. After 3 h of stirring, the mixture was separated into two layers. The lower layer was removed, was diluted with CH_2Cl_2, and was filtered through Celite. The filtrate was concentrated under reduced pressure, was dissolved in CH_2Cl_2, and was refiltered. The solvent was removed in vacuo to provide a light tan oil. To a solution of this crude α-bromo-3-(cyclopentyloxy)-4-methoxytoluene in DMF (160 mL) under an argon atmosphere was added NaCN (10.1 g, 206 mmol), and the resulting mixture was stirred at room temperature for 18 h, then was poured into cold water (600 mL), and was extracted three times with ether. The organic extract was washed three times with water and once with brine and was dried (K_2CO_3). The solvent was removed in vacuo, and the residue was purified by flash chromatography, eluting with 10% EtOAc/hexanes, to provide **7** as an off-white solid (17.7 g, 84%): mp 32−34 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (m, 3H), 4.80 (m, 1H), 3.85 (s, 3H), 3.68 (s, 2H), 2.0−1.8 (m, 6H), 1.65 (m, 2H). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

Dimethyl 4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]pimelate (8). To a solution of **7** (7 g, 30.3 mmol) in CH_3CN (200 mL) under an argon atmosphere was added a 40% solution of Triton B in CH_3OH (1.4 mL, 3.03 mmol), and the mixture was heated to reflux. Methyl acrylate (27 mL, 303 mmol) was added carefully, and the reaction mixture was maintained at reflux for 5 h and then was cooled. The mixture was diluted with ether, was washed once with 1 N HCl and once with brine, and was dried ($MgSO_4$), and the solvent was removed in vacuo. The solid residue was triturated with 5% EtOH/hexane to provide **8** as a white solid (9 g, 74%): mp 81−82 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.97 (m, 3H), 4.78 (m, 1H), 3.85 (s, 3H), 3.62 (s, 6H), 2.6−2.1 (m, 8H), 2.0−1.8 (m, 6H), 1.6 (m, 2H). Anal. ($C_{22}H_{29}NO_6$) C, H, N.

2-Carbomethoxy-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexan-1-one (9). To a solution of **8** (5.9 g, 14.6 mmol) in dry DME (120 mL) under an argon atmosphere was added NaH (80% suspension in mineral oil, 1.05 g, 43.8 mmol). The mixture was heated to reflux for 4.5 h, then was cooled to room temperature, and was stirred for 16 h. Water was added, and the reaction mixture was partitioned between ether and acidic water. The organic extract was dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by flash chromatography, eluting with 3:1 hexanes/EtOAc, to provide **9** as a white foam (4.9 g, 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.8 (s, 1H), 6.99 (m, 2H), 6.87 (d, J = 8 Hz, 1H), 4.81 (m, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 2.98 (d, J = 16 Hz, 1H), 2.80 (m, 1H), 2.65 (d, J = 16 Hz, 1H), 2.48 (br d, J = 16 Hz, 1H), 2.26 (m, 1H), 2.16 (m, 1H), 2.0−1.7 (m, 6H), 1.6 (m, 2H). Anal. ($C_{19}H_{23}NO_3 \cdot {}^1/_4H_2O$) C, H, N.

4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexan-1-one (10). A mixture of **9** (0.80 g, 2.15 mmol), DMSO (16 mL), water (1 mL), and NaCl (0.8 g) under an argon atmosphere was heated at 140−145 °C for 5 h. The reaction mixture was cooled and was concentrated in vacuo. The residue was purified by flash chromatography, eluting with 3:1 hexanes/EtOAc, to provide a yellow solid. Trituration with hexanes/EtOAc yielded **10** as a white solid (0.52 g, 77%): mp 111−112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (m, 2H), 6.87 (d, J = 8 Hz, 1H), 4.80 (m, 1H), 3.85 (s, 3H), 2.90 (dt, J = 6, 15 Hz, 2H), 2.56 (dt, J = 2, 15 Hz, 2H), 2.47 (dt, J = 3, 14 Hz, 2H), 2.24 (dt, J = 4, 14 Hz, 2H), 2.0−1.8 (m, 6H), 1.63 (m, 2H). Anal. ($C_{19}H_{23}NO_3$) C, H, N.

2-[4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexylidene]-1,3-dithiane (11). To a solution of 2-(trimethylsilyl)-1,3-dithiane (9.25 mL, 48.7 mmol) in dry THF (80 mL) at 0 °C under an argon atmosphere was added rapidly *n*-butyllithium (2.5 M in hexanes, 19.2 mL, 48 mmol). After 10 min, the mixture was cooled to −78 °C and a solution of **10** (7.53 g, 23 mmol) in THF (40 mL) was added. After 10 min, aqueous NaCl was added, and the mixture was allowed to warm to room temperature and was diluted with water. This mixture was combined with the product of three substantially similar reactions conducted on **10** (3.04, 6.01, and 6.1 g, 48.3 mmol total), the combined mixture was extracted three times with CH_2Cl_2, the extract was dried ($MgSO_4$), and the solvent was evaporated. Purification by flash chromatography, eluting with 10% EtOAc/hexanes, provided **11** as a white solid (26 g, 87%): mp 115−116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (m, 2H), 6.84 (d, J = 7 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.30 (d, J = 15 Hz, 2H), 2.91 (t, J = 6 Hz, 4H), 2.38 (dt, J = 3, 14 Hz,

2H), 2.22 (br d, J = 14 Hz, 2H), 2.16 (m, 2H), 2.0−1.7 (m, 8H), 1.65 (m, 2H). Anal. ($C_{23}H_{29}N_2S_2$) C, H, N.

Methyl *cis*-and *trans*-4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylate (12 and 13). Perchloric acid (70%, 13.8 mL, 160 mmol) and mercuric chloride (34.1 g, 126 mmol) were added to a solution of **11** (13 g, 31.3 mmol) in CH_3OH (0.5 L) under an argon atmosphere, and the mixture was heated at reflux for 2 h and then was allowed to stir at room temperature for 42 h. The mixture was diluted with CH_2Cl_2 and was filtered through Celite, and the filtrate was combined with that of a similar reaction conducted concurrently on the same scale. The mixture was neutralized with aqueous $NaHCO_3$ and was extracted three times with CH_2Cl_2. The organic extract was washed three times with aqueous sodium sulfite, was dried ($MgSO_4$), and was evaporated. Purification by flash chromatography, eluting with 15% EtOAc/hexanes, provided the cis ester (**12**) as a white solid (12.4 g, 56%): mp 119−120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, J = 2 Hz, 1H), 6.96 (dd, J = 2, 8 Hz, 1H), 6.85 (d, J = 8 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.72 (s, 3H), 2.36 (tt, J = 4, 12 Hz, 1H), 2.25 (br d, J = 13 Hz, 2H), 2.18 (dd, J = 4, 13 Hz, 2H), 2.1−1.7 (m, 10H), 1.6 (m, 2H). Anal. ($C_{21}H_{27}NO_4$) C, H, N. The trans ester (**13**) was also isolated from this mixture as a solid (1.04 g, 5%): mp 50−51 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, J = 2 Hz, 1H), 6.95 (dd, J = 2, 8 Hz, 1H), 6.84 (d, J = 8 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 3.71 (s, 3H), 2.80 (m, J_{app} = 4 Hz, 1H), 2.24 (m, 2H), 2.1−1.8 (m, 12H), 1.6 (m, 2H). Anal. ($C_{21}H_{27}NO_4 \cdot {}^3/_4H_2O$) C, H, N.

***cis*-4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-cyclohexane-1-carboxylic Acid (1).** To a solution of **12** (0.12 g, 0.34 mmol) in CH_3OH (0.9 mL, containing just enough THF to solubilize the compound) under an argon atmosphere was added a solution of KOH (0.06 g, 0.9 mmol) in water (0.7 mL). The resulting mixture was stirred at room temperature for 1.5 h, then was poured into water, and was extracted with EtOAc. The aqueous phase was acidified with 10% hydrochloric acid and was extracted twice with EtOAc. The organic phase from the acid extraction was dried (Na_2SO_4) and was concentrated under reduced pressure to provide **1** as a solid. The solid was purified by flash chromatography, eluting with 4% $CH_3OH/CHCl_3$, to provide a white solid (0.05 g, 44%): mp 157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, J = 2 Hz, 1H), 6.97 (dd, J = 2, 8 Hz, 1H), 6.86 (d, J = 8 Hz, 1H), 4.81 (m, 1H), 3.85 (s, 3H), 2.42 (tt, J = 4, 12 Hz, 1H), 2.25 (t, J = 16 Hz, 4H), 2.04 (dd, J = 2, 13 Hz, 2H), 1.98−1.75 (m, 6H), 1.63 (m, 2H); ^{1}H NMR (400 MHz, CD_3OD) δ 7.03 (m, 2H), 6.94 (d, J = 9 Hz, 1H), 4.83 (m, 1H), 3.80 (s, 3H), 2.41 (m, 1H), 2.15 (d, J = 9 Hz, 4H), 2.0−1.7 (m, 10H), 1.6 (m, 2H). Anal. ($C_{20}H_{25}NO_4$) C, H, N.

***trans*-4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-cyclohexane-1-carboxylic Acid (14).** To a solution of **13** (0.68 g, 1.9 mmol) in CH_3OH (8 mL, containing just enough THF to solubilize the compound) under an argon atmosphere was added water (4 mL) and KOH (0.32 g, 5.7 mmol). The resulting mixture was stirred at room temperature for 24 h, was acidified with 10% HCl, and was extracted three times with 10% CH_3OH/CH_2Cl_2. The organic extract was dried ($MgSO_4$) and was concentrated under reduced pressure. Purification by flash chromatography, eluting with 4% CH_3OH/CH_2Cl_2, provided a white semisolid (0.52 g), which was triturated with ether to yield **14** as a white solid (0.43 g, 66%): mp 157−158 °C; ^{1}H NMR (250 MHz, CD_3OD) δ 6.97 (m, 3H), 4.80 (m, 1H), 3.80 (s, 3H), 2.76 (m, 1H), 2.19 (m, 2H), 2.0−1.7 (m, 12H), 1.6 (m, 2H). Anal. ($C_{20}H_{25}NO_4$) C, H, N.

***cis*-4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-cyclohexane-1-*N*-(2-cyanoethyl)carboxamide (15).** To a solution of **1** (0.55 g, 1.6 mmol), HOBt (0.24 g, 1.76 mmol), and 3-aminopropionitrile (0.11 g, 1.6 mmol) in CH_2Cl_2 (10 mL) at 0 °C under an argon atmosphere was added EDAC·HCl (0.34 g, 1.76 mmol), and the mixture was allowed to warm to room temperature. After 6 h, the mixture was diluted with CH_2Cl_2, was washed twice with 10% aqueous K_2CO_3 and twice with 10% HCl, and was dried ($MgSO_4$). The solvent was evaporated, and the residue was crystallized from hexanes/EtOAc to provide **15** as a solid (0.54 g, 85%): mp 146−147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.0 (d, J = 2 Hz, 1H), 6.96 (dd, J = 8, 2 Hz, 1H), 6.85 (d, J = 8 Hz, 1H), 6.0 (m, 1H), 4.8 (m, 1H), 3.85 (s, 3H), 3.54 (q, J = 6 Hz, 2H), 2.67 (t, J = 6 Hz, 2H), 2.27 (m, 3H), 2.13 (m, 2H), 2.03 (m, 2H), 1.8−2.0 (m, 9H), 1.6 (m, 2H). Anal. ($C_{23}H_{29}N_3O_3$) C, H, N.

***cis*-1-(2-Cyanoethyl)-5-[4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexyl]tetrazole (16).** To a solution of **15** (0.15 g, 0.37 mmol), PPh_3 (0.19 g, 0.73 mmol) and $TMSN_3$ (0.097 mL, 0.73 mmol), in dry THF (2 mL) at room temperature under an argon atmosphere was added dropwise DEAD (0.12 mL, 0.73 mmol), and the mixture was stirred in the dark for 24 h. Ceric ammonium nitrate (0.81 g, 1.48 mmol) in water (10 mL) was added slowly at 0 °C to decompose the excess $TMSN_3$, the mixture was extracted three times with CH_2Cl_2, the extract was dried ($MgSO_4$), and the solvent was evaporated. Purification by flash chromatography, eluting with 2:1 EtOAc/hexanes, followed by recrystallization from hexanes/EtOAc, provided **16** as a white solid (0.03 g, 19%): mp 149−150 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.03 (m, 2H), 6.87 (d, J = 8 Hz, 1H), 4.83 (m, 1H), 4.62 (t, J = 6 Hz, 2H), 3.86 (s, 3H), 3.15 (t, J = 6 Hz, 2H), 3.03 (m, 1H), 2.4 (m, 3H), 2.15 (m, 2H), 2.1−1.71 (m, 9H), 1.6 (m, 2H). Anal. ($C_{23}H_{28}N_6O_2$) C, H, N.

***cis*-4-Cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-(5-tetrazolyl)cyclohexane (17).** A mixture of **16** (0.098 g, 0.23 mmol) and NaOH (0.018 g, 0.46 mmol) in 10:1 THF/water (5 mL) at room temperature under an argon atmosphere was stirred overnight. The mixture was acidified with 3 N HCl and was extracted three times with EtOAc, the extract was dried ($MgSO_4$), and the solvent was evaporated. Purification by flash chromatography, eluting with 80:20:2 $CHCl_3$/CH_3OH/water, followed by trituration with hexanes/EtOAc, provided **17** as a white solid (0.038 g, 45%): mp 190−191 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.0 (m, 2H), 6.88 (d, J = 8 Hz, 1H), 4.83 (m, 1H), 3.86 (s, 3H), 3.3 (m, 1H), 2.37 (m, 3H), 2.2 (m, 2H), 2.0−1.8 (m, 9H), 1.52 (m, 2H). Anal. ($C_{20}H_{25}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

4-[3-(Cyclopentyloxy)-4-methoxyphenyl]cyclohexan-1-one (19). Ammonia (30 mL) was condensed at −78 °C into a flask containing 10 hexane-washed Na spheres (3−8 mm). To this stirred mixture was added a solution of **10** (0.50 g, 1.6 mmol) in THF (10 mL). After 0.2 h, solid NH_4Cl was added, followed by ether and water. The mixture was warmed to room temperature over 2 h, was extracted twice with ether, was dried ($MgSO_4$), and was evaporated. Purification by flash chromatography, eluting with 4:1 hexanes/EtOAc, provided **18** as a white solid (0.26 g, 56%): mp 72 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (d, J = 9 Hz, 1H), 6.71 (m, 2H), 4.75 (m, 1H), 3.82 (s, 3H), 3.67 (m, 1H), 2.43 (m, 1H), 2.10 (m, 2H), 2.94−1.83 (m, 7H), 1.58−1.39 (m, 8H).

To a suspension of PCC (0.29 g, 1.34 mmol) in CH_2Cl_2 (2 mL) was added a solution of **18** (0.26 g, 0.9 mmol) in CH_2Cl_2 (4 mL). After 4 h, the mixture was diluted with ether, was stirred for 0.5 h, and was filtered through Celite, and the filtrate was concentrated in vacuo. Purification by flash chromatography, eluting with 4:1 hexanes/EtOAc, provided **19** as a white solid (0.24 g, 94%): mp 66−67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, J = 9 Hz, 1H), 6.75 (m, 2H), 4.78 (m, 1H), 3.83 (s, 3H), 2.95 (tt, J = 3, 12 Hz, 1H), 2.51 (m, 4H), 2.21 (br m, 2H), 2.0−1.8 (m, 8H), 1.60 (m, 2H). Anal. ($C_{18}H_{24}O_3 \cdot {}^1/_7H_2O$) C, H, N.

2-[4-[3-(Cyclopentyloxy)-4-methoxyphenyl]cyclohexylidene]-1,3-dithiane (20). To a solution of 2-(trimethylsilyl)-1,3-dithiane (0.48 mL, 2.49 mmol) in dry tetrahydrofuran (5 mL) at 0 °C under an argon atmosphere was added rapidly *n*-butyllithium (2.45 M in hexanes, 1.0 mL, 2.49 mmol). After 0.25 h, the mixture was cooled to −78 °C and a solution of **19** (0.24 g, 0.83 mmol) in THF (3 mL) was added. After 0.5 h, aqueous NaCl was added, and the mixture was warmed to room temperature and was diluted with water. The mixture was extracted three times with CH_2Cl_2, the extract was dried ($MgSO_4$), and the solvent was evaporated. Purification by flash chromatography, eluting with 10% EtOAc/hexanes, followed by trituration from ether/hexanes, provided **20** as a

white solid (0.1 g, 31%): mp 90−92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (d, J = 9 Hz, 1H), 6.71 (m, 2H), 4.76 (m, 1H), 3.81 (s, 3H), 3.28 (d, J = 13 Hz, 2H), 2.88 (m, 4H), 2.64 (m, 1H), 2.15 (m, 2H), 2.01−1.80 (m, 10H), 1.58 (m, 2H), 1.45 (m, 2H). Anal. ($C_{22}H_{30}O_2S_2$) C, H.

***trans*-4-[3-(Cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic Acid (21).** To a suspension of **20** (0.09 g, 0.23 mmol) in 4:1 CH_3CN/water (5 mL) was added TFA (0.06 mL, 0.78 mmol). After 1 h of heating at 60−65 °C, the mixture was cooled to 30 °C, 30% aqueous H_2O_2 (0.32 mL, 3.13 mmol) was added, and the temperature was increased to 80 °C. After 0.75 h, the mixture was cooled to 40 °C and 10% aqueous NaOH (3 mL) was added. The mixture was acidified with 3 N HCl, was extracted three times with CH_2Cl_2, and was dried ($MgSO_4$), and the solvent was evaporated. Purification by flash chromatography, eluting with 95:5 $CHCl_3$/CH_3OH, provided **21** as a white solid (0.03 g, 45%): mp 156−158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (d, J = 9 Hz, 1H), 6.72 (m, 2H), 4.77 (m, 1H), 3.82 (s, 3H), 2.45 (tt, J = 4, 12 Hz, 1H), 2.40 (tt, J = 4, 12 Hz, 1H), 2.15 (br d, J = 11 Hz, 2H), 2.01−1.78 (m, 7H), 1.70−1.45 (m, 7H). Anal. ($C_{19}H_{26}O_4$) C, H.

4-(Aminocarbonyl)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexan-1-one (24). A mixture of compound **10** (0.5 g, 1.6 mmol), trimethyl orthoformate (0.21 mL, 1.9 mmol), and a catalytic amount of *p*-TsOH in CH_3OH (20 mL) was heated gently under an argon atmosphere for 2 h. The mixture was cooled, was partitioned between aqueous sodium carbonate and EtOAc, and was extracted twice with EtOAc. The organic extract was dried (K_2CO_3), and the solvent was removed in vacuo to provide compound **22** as an oil (0.57 g, 99%). A solution of **22** (0.34 g, 0.95 mmol) and powdered potassium carbonate (0.7 g, 5.1 mmol) in CH_3OH (20 mL) and water (4 mL) at 0 °C was treated with H_2O_2 (30% solution, 2.55 mL). The mixture was allowed to warm to room temperature, and after 7 days, brine was added and the mixture was extracted with CH_2Cl_2. The organic extract was washed twice with brine and was dried (K_2CO_3), and the solvent was removed in vacuo. Purification by flash chromatography provided the ketal amide **23** (0.055 g, 15%), along with recovered **22** (0.25 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.95 (m, 2H), 6.83 (d, J = 8 Hz, 1H), 5.30 (br s, 2H), 4.75 (m, 1H), 3.83 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 2.40 (m, 2H), 2.13 (m, 3H), 1.87−1.53 (m, 11H).

A mixture of **23** (0.055 g, 0.15 mmol) and *p*-TsOH (catalytic amount) in 20% aqueous acetone (5 mL) was stirred under an argon atmosphere at reflux for 8 h. The mixture was cooled, was diluted with water, and was extracted with CH_2Cl_2. The organic extract was dried ($MgSO_4$), and the solvent was removed in vacuo to provide **24** as a hygroscopic, amorphous material (0.035 g, 72%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.0 (m, 2H), 6.90 (d, J = 8 Hz, 1H), 5.35 (m, 2H), 4.75 (m, 1H), 3.85 (s, 3H), 2.7−2.5 (m, 4H), 2.45−2.30 (m, 4H), 1.95−1.80 (m, 6H), 1.63 (m, 2H). Anal. ($C_{19}H_{25}NO_4 \cdot {}^3/_8H_2O$) C, H, N.

4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-4-formylcyclohexan-1-one (26). A solution of **22** (0.57 g, 1.6 mmol) in toluene (20 mL) at room temperature under an argon atmosphere was treated with a solution of DIBAl-H (1.5 M in toluene, 2.7 mL, 4 mmol). After 2 h, a solution of saturated aqueous sodium bisulfite was added, and the mixture was extracted twice with EtOAc. The organic extract was washed with 5% aqueous Na_2CO_3 and was dried (K_2CO_3), and the solvent was removed in vacuo to provide **25** as an oil (0.55 g, 96%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.33 (s, 1H), 6.85 (d, J = 1 Hz, 2H), 6.79 (s, 1H), 4.75 (m, 1H), 3.83 (s, 3H), 3.19 (s, 3H), 3.16 (s, 3H), 2.0−1.8 (m, 10H), 1.6 (m, 6H).

Compound **25** (0.1 g, 0.28 mmol) in EtOAc (2 mL) was treated with 3 N HCl (5 mL), and the mixture was stirred vigorously and gently heated for 10 min. The mixture was extracted twice with EtOAc, the organic extract was washed with 5% aqueous Na_2CO_3 and was dried (K_2CO_3), and the solvent was removed in vacuo. This material, combined with that obtained from an identical reaction, was purified by flash chromatography, eluting with 2% EtOAc/$CHCl_3$, to provide **26** as a white solid (0.1 g, 57%): mp 55−57 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.50 (s, 1H), 6.90 s, 2H), 6.82 (s, 1H), 4.75 (m, 1H), 3.85 (s, 3H), 2.60−2.25 (m, 8H), 2.0−1.75 (m, 6H), 1.62 (m, 2H). Anal. ($C_{19}H_{24}O_4$) C, H.

4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-4-ethynylcyclohex-1-one (28). To a solution of KO^{t}Bu (0.155 g, 1.38 mmol) in dry THF (5 mL) under an argon atmosphere at −78 °C was added a solution of dimethyl (diazomethyl)phosphonate[69] (ca. 88% pure, 0.24 g, 1.38 mmol). After 0.25 h, a solution of **25** (0.42 g, 1.15 mmol) in dry THF (5 mL) was added dropwise, and the mixture was allowed to stir at −78 °C under an argon atmosphere for 5 h. Aqueous HOAc was added, and the mixture was concentrated, was partitioned between CH_2Cl_2 and water, and was extracted twice. The organic extract was dried ($MgSO_4$) and was evaporated. Purification by flash chromatography, eluting with 3:1 hexanes/EtOAc, provided **27** as an oil (0.13 g, 32%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.16 (d, J = 2 Hz, 1H), 7.06 (dd, J = 2, 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 4.80 (m, 1H), 3.83 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 2.40 (s, 1H), 2.08−1.75 (m, 14H), 1.60 (m, 2H).

A mixture of **27** (0.13 g, 0.36 mmol) and *p*-TsOH (catalytic amount) in acetone (5 mL) was stirred under an argon atmosphere at room temperature for 1.5 h. The mixture was concentrated, was diluted with EtOAc, and was washed with water. The organic extract was dried ($MgSO_4$) and the solvent was removed in vacuo to provide **28** as an oil (0.11 g, 97%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.16 (d, J = 2 Hz, 1H), 7.07 (dd, J = 2, 8 Hz, 1H), 6.84 (d, J = 8 Hz, 1H), 4.80 (m, 1H), 3.84 (s, 3H), 2.99 (dt, J = 6, 14 Hz, 2H), 2.56 (s, 1H), 2.44 (d, J = 14 Hz, 2H), 2.30−2.10 (m, 6H), 2.00−1.75 (m, 6H), 1.60 (m, 2H). Anal. ($C_{20}H_{24}O_3 \cdot {}^1/_2H_2O$) C, H.

4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-4-(hydroxymethyl)cyclohexan-1-one (30). To a solution of **25** (0.24 g, 0.66 mmol) in DME (5 mL) under an argon atmosphere was added $NaBH_4$ (0.05 g, 1.3 mmol), and the mixture was stirred at room temperature for 0.75 h. Water was added, the mixture was partitioned between ether and water and was extracted twice with ether, and the organic extract was dried (K_2CO_3) and was evaporated to provide **29** as an oil (0.19 g, 79%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.85, (m, 3H), 4.75 (m, 1H), 3.84 (s, 3H), 3.47 (m, 2H), 3.19 (s, 3H), 3.13 (s, 3H), 2.12 (m, 2H), 1.95−1.75 (m, 8H), 1.75−1.60 (m, 4H), 1.43 (m, 2H). Compound **29** (0.15 g, 0.41 mmol) in ether (2 mL) was treated with 1 N HCl (2 mL), and the mixture was stirred vigorously and gently heated for 10 min. The mixture was extracted with ether, the organic extract was washed with 5% aqueous Na_2CO_3 and was dried (K_2CO_3), and the solvent was removed in vacuo. Purification by flash chromatography, eluting with 25% EtOAc/$CHCl_3$, provided **30** as a wax (0.06 g, 56%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.03−6.87 (m, 3H), 4.78 (m, 1H), 3.88 (s, 3H), 3.53 (m, 2H), 2.60−2.47 (m, 2H), 2.40−2.30 (m, 3H), 2.0−1.73 (m, 9H), 1.60 (m 2H). Anal. ($C_{19}H_{26}O_4$) C, H.

4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-4-(fluoromethyl)cyclohexan-1-one (32). A solution of **29** (0.37 g, 1.02 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of (diethylamido)sulfur trifluoride (0.14 mL, 1.02 mmol) at −78 °C under an argon atmosphere. The mixture was allowed to warm to room temperature, and after 0.75 h, 5% aqueous Na_2CO_3 was added. The mixture was extracted with $CHCl_3$, the organic extract was dried ($MgSO_4$), and the solvent was removed in vacuo to provide **31** as a yellow oil (0.3 g, 80%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.85−6.65, (m, 3H), 4.75 (m, 1H), 3.83 (s, 3H), 3.23 (s, 3H), 3.16 (s, 3H), 2.80 (d, J = 24 Hz, 2H), 2.0−1.5 (m, 16H).

Compound **31** (0.35 g, 0.95 mmol) in EtOAc (2 mL) was treated with 1 N HCl (2 mL), and the mixture was stirred vigorously and gently heated for 10 min. The mixture was extracted with EtOAc, the organic extract was washed with 5% aqueous Na_2CO_3 and was dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by flash chromatography, eluting with 25% EtOAc/hexanes, followed by trituration with ether/hexanes, provided **32** as a white solid (0.075 g, 24%): mp 72−74 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.83−6.70, (m, 3H), 4.75 (m, 1H), 3.80 (s, 3H), 2.80 (d, J = 24 Hz, 2H), 2.65 (dt, J = 8, 18 Hz, 2H), 2.35−2.13 (m, 4H), 2.0−1.75 (m, 8H), 1.6 (m, 2H). Anal. ($C_{19}H_{25}FO_3$) C, H.

(3) Biology. LPDE4 activity and HPDE4 activity were assayed as described previously using monocyte-derived human recombinant LPDE4 produced in yeast and HPDE4 derived from rat brain preparations.[47] K_i values were determined as described previously.[47] PDEs 1, 2, and 5 were isolated from canine tracheal smooth muscle and assayed as described previously.[94] PDE3 was isolated from guinea-pig cardiac ventricle and was assayed as described previously.[95] The remaining in vitro and in vivo assays were conducted according to the procedures described for (*R*)-rolipram and/or other PDE4 inhibitors in the references cited in the text of this manuscript.

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Supporting Information Available: Tables of elemental analyses and crystallographic data (crystal data and structural refinements, bond distances, bond angles, positional parameters and anisotropic displacement parameters) (6 pages). Ordering information is given on any current masthead page.

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