Selective Type IV Phosphodiesterase Inhibitors as Antiasthmatic Agents. The Syntheses and Biological Activities of 3-(Cyclopentyloxy)-4-methoxybenzamides and Analogues

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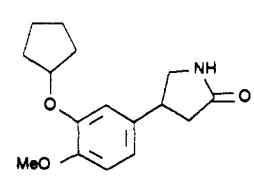
The syntheses and biological activities of a number of benzamide derivatives, designed from rolipram, which are selective inhibitors of cyclic AMP-specific phosphodiesterase (PDE IV), are described. The effects of changes to the alkoxy groups, amide linkage, and benzamide *N*-phenyl ring on the inhibition of the cytosolic PDE IV from pig aorta have been investigated. As a result, some highly potent and selective PDE IV inhibitors have been identified. The most potent compounds have been further evaluated for their inhibitory potencies against PDE IV obtained from and superoxide O_2^- generation from guinea pig eosinophils in vitro. Selected compounds have also been examined for their activities in inhibiting histamine-induced bronchospasm in anaesthetized guinea pigs. 3-(Cyclopentyloxy)-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (**15j**) showed exceptional potency in all tests and may have therapeutic potential in the treatment of asthma.

Introduction

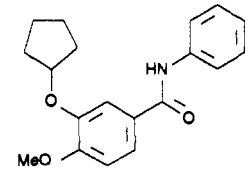
Asthma is characterized by variable airflow obstruction, pulmonary eosinophilia, and airway hyperresponsiveness. These symptoms are believed to be a consequence of a chronic inflammation of the airway mucosa, and in addition to eosinophils, many other inflammatory cells such as mast cells, lymphocytes, and macrophages are also present in increased numbers in asthmatic airway tissue.[1,2] Proinflammatory mediators and proteins synthesized and released by these cells fuel the ongoing inflammatory reaction and contribute to airway smooth muscle contraction, mucus secretion, plasma protein extravasation, and eventually oedema formation.[1,2]

β_2-Adrenoceptor agonists are widely used in asthma therapy and, when inhaled, produce instant bronchodilation. They have, however, little effect on the underlying inflammation, and for that reason, their regular, long-term use has been debated.[3,4] Glucocorticoids, on the other hand, have well-documented antiinflammatory properties, and they can, very effectively, reduce airway hyperresponsiveness. With long-term treatment of mild asthma, the degree of airway reactivity reverts back to the normal range.[5] Perceived disadvantages of glucocorticoids include their lack of immediate bronchodilator actions and significant suppression of the hypothalamic–pituitary–adrenal (HPA) axis and of bone growth in children, which are caused by systemic exposure.[6] By inference, a new drug with bronchodilator and glucocorticoid-like anti-inflammatory properties should be most useful in asthma therapy.

Currently, much attention is focused on the antiasthma potential of drugs with cyclic nucleotide phosphodiesterase (PDE) inhibitory actions. PDE is the predominant enzyme involved in cAMP and cGMP catabolism, and as a consequence, reduced PDE activity results in enhanced intracellular levels of these nucleotides.[7-9] cAMP is a common second messenger, generally associated with dampening effects on airway smooth muscle and on activation and mediator release from inflammatory cells.[7-9] The identification of at least five families of PDE isozymes with different cell and tissue distribution has opened the prospect for the development of compounds with novel antiasthmatic profiles. On the basis of the presence of the type IV PDE in bronchial smooth muscle and proinflammatory cells, such as eosinophils, and the effects of the PDE IV inhibitor rolipram in experimental studies,[7,8,10] it is generally believed that inhibitors of this isozyme may have useful bronchodilator and antiinflammatory actions. Given the apparent requirement of a 3,4-dialkoxy group in the phenyl ring of rolipram and its analogues for PDE IV activity,[11,12] we have evaluated several series of possible inhibitors in which the pyrrolidinone ring has been replaced. We now report the syntheses and biological activities of potent and selective compounds derived from the benzamide **8a**.



Rolipram



8a

Chemistry

The synthetic strategies used for the preparation of the majority of the 3,4-dialkoxy-*N*-phenylbenzamides (Tables 1 and 2) and the *N*-heterocyclic benzamides (Table 4) are shown in Schemes 1 and 2. As shown in Scheme 1, isovanillin was alkylated to give the requisite aldehyde **1** (methods A, B). Oxidation to the acid **2** was followed by conversion to the acid chloride **3** with thionyl chloride. The final stage reaction between **3** and the anilines to give the benzamides of general structure **4** (compounds **8a–z**, **9a–h**, **15a–v**, and **16a–g**) was accomplished by methods C–F, more forcing conditions being required for the more

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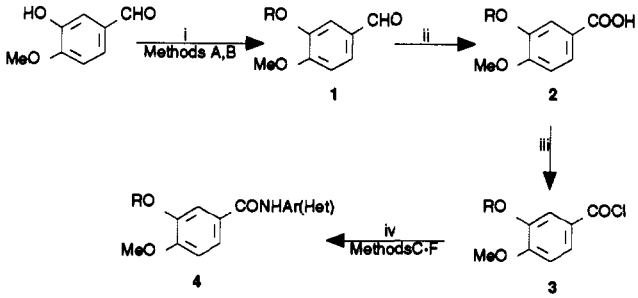
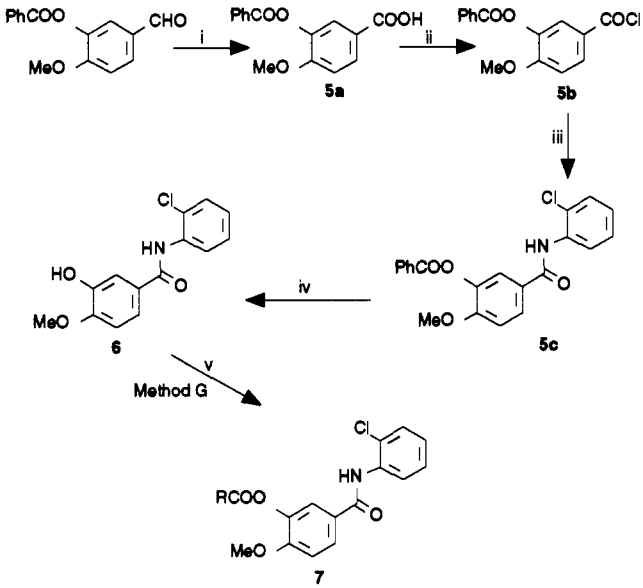
Scheme 1[a]



[a] Reagents: (i) method A, R-Br, K_2CO_3, DMF; method B, R-OH, Ph_3P, DIAD; (ii) $NaClO_2$, H_2NSO_3H, AcOH; (iii) $SOCl_2$, toluene; (iv) Het (Ar) NH_2 under the following conditions, method C, Et_3N, CH_2Cl_2; method D, pyridine up to reflux; method E, melt; method F, NaH, DMF.

Scheme 2[a]



[a] Reagents: (i) $KMnO_4$, acetone; (ii) $SOCl_2$, toluene; (iii) 2-chloroaniline, Et_3N, CH_2Cl_2; (iv) KOH, MeOH; (v) method G, R-COCl, NaH, THF.

sterically hindered or deactivated anilines. In these cases, it was found that pretreatment of the aniline with sodium hydride in DMF to generate the anion (method F) gave the best yields. The desmethoxy compound **11** was synthesized by a similar route (methods A, C). Compounds of general structure **7** in which the cyclopentyl group of the *N*-(2-chlorophenyl)benzamide **8d** has been replaced by acyl groups (compounds **10a–d**) were synthesized as shown in Scheme 2. Isovanillin was protected as the benzoate and converted through to the *N*-(2-chlorophenyl)-benzamide **5c**. Deprotection of **5c** with methanolic KOH gave the intermediate phenol **6** which was acylated (method G) to **7**.

Analogues **12–14**, in which the amide linkage has been modified, are collated in Table 3. The homologous amide **12**, thioamide **13**, and ester **14** were synthetized directly from the acid chloride **3** (R = cyclopentyl) by reaction with 2-chlorobenzylamine, 2-chloroaniline/P_2S_5, and 2,6-dichlorophenol, respectively.

The *N*-heterocyclic benzamides are listed in Table 4. The 3-cyclopentyloxy analogues **15a–v** and the other 3-alkoxy analogues **16a–g** were synthesized as described earlier according to Scheme 1. Oxidation of **15j** with H_2O_2 gave the *N*-oxide **15w**.

Biological Evaluation

In order to assess structure–activity relationships, the compounds were tested for their inhibitory potencies against pig aortic PDE IV in vitro. The kinetic properties of pig aortic PDE IV (K_m = 2 μM) and its susceptibility to inhibition by a range of standards are similar to those displayed by the enzyme from canine airway smooth muscle.[13] Isozyme selectivity was assessed by comparing the IC_{50} values of compounds against PDE IV with their inhibitory activities against Ca^{2+} calmodulin-stimulated PDE (PDE I), cGMP-inhibited PDE (PDE III), and cGMP-specific PDE (PDE V) from the same source. In general, the 4-PDE isozymes were well separated using DEAE-trisacryl anion-exchange chromatography; however, in some preparations, the PDE III and PDE IV peaks overlapped slightly. To eliminate the possibility of isozyme cross-contamination, compounds were tested against PDE III in the presence of rolipram (10 μM), a PDE IV inhibitor, and against PDE IV in the presence of siquozodan (10 μM), a PDE III inhibitor. The most potent compounds were then evaluated in several tests designed to assess antiinflammatory and bronchodilating activities.

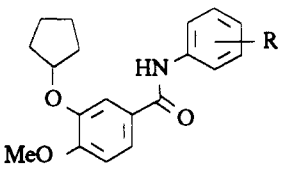
Structure–Activity Relationships

Modification of the type IV isozyme-selective lead compound **8a** by introduction of single substituents into the *N*-phenyl ring gave up to a 20-fold increase in type IV inhibitory potency while retaining good selectivity for the isozyme (when compared to types I, III, and V). The rank order of potency was found to be ortho > meta > para substitution (compounds **8b–d**, **8e–g**, **8h,i** (Table 1)). The 2-bromo, **8j**, and 2-chloro, **8d**, analogues were equipotent, but the 2-fluoro compound **8i** was considerably less potent. 2,6-Disubstitution of the *N*-phenyl ring also led to a marked increase in potency over **8a**; the dichloro compound **8m** was 100-fold more potent than **8a**. The difluoro benzamide **8p** was much more active than the monofluoro compound **8i**, and this may be due to the effect of disubstitution on the torsion angle about the *N*-phenyl bond, which may overcome possible H-bonding between the fluorine atom and the hydrogen on the amide nitrogen. The relative inactivity of the monofluoro compound **8i**, relative to other ortho substituents, could be due to this H-bonding locking the structure in a relatively inactive conformation. The 2,3- and 2,5-dichlorophenyl compounds **8k,l** were less active than the 2,6-dichlorophenyl analogue **8m**. Of the other 2-substituted compounds, **8s–z**, only the 2-nitro analogue **8s** had interesting activity.

Modification of the 3,4-dialkoxy groups was then examined (Table 2). All further compounds were tested on the PDE IV (pig aorta) enzyme only. An increase in the size and lipophilicity of the 3-alkoxy substituent (compounds **8d**, **9b–f**) led to an increase in potency, with the cyclopentyloxy, **8d**, and *exo*-2-norbornyl, **9g**, groups being optimal. The removal of the 3- and 4-alkoxy groups in **9a** and **11** led to a substantial loss in activity. These results agree with earlier findings.[11,12] The hydrophobic requirement at the 3-alkoxy group is reflected by the large loss in activity of the 3-acyloxy compounds **10a–d**. A limited study of replacement groups for the amide linkage was undertaken (Table 3). The homologous amide, **12**, and ester, **14**, linkages led to a large loss of activity; however, the thioamide **13** retained activity.

The activities of the *N*-heterocyclic benzamides are shown in Table 4. The unsubstituted pyridyl, **15a–c**, and

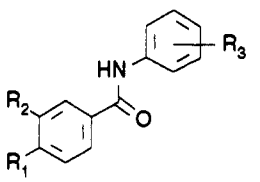
Table 1. *N*-Phenylbenzamides



compd	R	mp, °C	method	formula	analysis[a]	PDE activity (pig aorta) IC_{50}, μM I	III	IV	V
8a	H	169–71	A,C	$C_{19}H_{21}NO_3$	C,H,N	>1000	>1000	2.6	>1000
8b	4-Cl	191	A,C	$C_{19}H_{20}ClNO_3$	C,H,N,Cl	>1000	>1000	>1000	>1000
8c	3-Cl	110–12	A,C	$C_{19}H_{20}ClNO_3$	C,H,[b]N,Cl	>40	>40	1.3	>40
8d	2-Cl	122–4	A,C	$C_{19}H_{20}ClNO_3$	C,H,N,Cl	>200	>200	0.19	>200
8e	4-Me	173–5	A,C	$C_{20}H_{23}NO_3$	C,H,N	>200	>200	>200	>200
8f	3-Me	147–9	A,C	$C_{20}H_{23}NO_3$	C,H,N	22	>200	1	15
8g	2-Me	148–9	A,C	$C_{20}H_{23}NO_3$	C,H,N	>1000	>1000	0.3	>1000
8h	4-F	181–3	A,C	$C_{19}H_{20}FNO_3$	C,[c]H,N,F	>1000	>1000	>1000	>1000
8i	2-F	137–8	A,C	$C_{19}H_{20}FNO_3$	C,H,N,F	>1000	>1000	10.7	>1000
8j	2-Br	126–8	A,C	$C_{19}H_{20}BrNO_3$	C,H,N	>1000	>1000	0.13	>1000
8k	2,3-diCl	167–9	A,C	$C_{19}H_{19}Cl_2NO_3$	C,H,N,Cl	>200	>200	>200	>200
8l	2,5-diCl	117–9	A,C	$C_{19}H_{19}Cl_2NO_3$	C,H,N,Cl	>200	>200	0.5	>200
8m	2,6-diCl	138–40	A,C	$C_{19}H_{19}Cl_2NO_3$	C,H,N,Cl	>1000	>1000	0.023	>1000
8n	2,6-diMe	130–1	A,C	$C_{21}H_{25}NO_3$	C,H,N	250	>200	0.1	>200
8o	2,6-diBr	133–4	A,C	$C_{19}H_{19}Br_2NO_3$	C,H,N,Br	>1000	>1000	0.4	>1000
8p	2,6-diF	158–60	A,C	$C_{19}H_{19}F_2NO_3$	C,[d]H,N	>1000	>1000	0.07	>1000
8q	2-Cl, 6-F	140–2	A,C	$C_{19}H_{19}ClFNO_3$	C,H,N,Cl	>1000	>1000	0.24	>1000
8r	2-Me, 6-Cl	138–40	A,C	$C_{20}H_{22}ClNO_3$	C,[e]H,N	>1000	>1000	0.1	>1000
8s	2-NO_2	130–2	A,C	$C_{19}H_{20}N_2O_5$	C,H,[f]N	400	>200	0.25	65
8t	2-CF_3	127–9	A,C	$C_{20}H_{20}F_3NO_3$	C,H,N	>1000	>1000	3.1	>1000
8u	2-SMe	128–30	A,C	$C_{20}H_{23}NO_3S$	C,H,N,S	>200	>200	1.2	>200
8v	2-COOMe	105–7	A,C	$C_{21}H_{23}NO_5$	C,H,N	>40	>40	3	5.5
8w	2-COPh	106–7	A,C	$C_{26}H_{25}NO_4$	C,H,N	>40	>40	1.7	2.5
8x	2-CN	170–2	A,C	$C_{20}H_{20}N_2O_3$	C,H,N	20	9.5	2.5	35
8y	2-$COCH_3$	126–7	A,C	$C_{21}H_{23}NO_4$	C,H,N	>40	>40	1.2	>40
8z	2-OH	169–71	A,D	$C_{19}H_{21}NO_4$	C,H,N	>200	>200	1.3	175

[a] Unless indicated, all values were within 0.4%. [b] H: calcd, 5.8; found, 6.5. [c] C: calcd, 69.3; found, 68.5. [d] C: calcd, 65.7; found, 65.1. [e] C: calcd, 66.75; found, 66.3. [f] N: calcd, 7.9; found, 7.4.

Table 2. 3,4-Dialkoxy Analogues of *N*-Phenylbenzamides

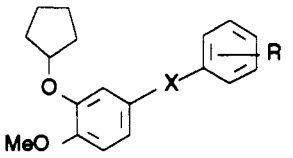


compd	R_1	R_2	R_3	mp, °C	method	formula	analysis[a]	PDE IV (pig aorta) IC_{50}, μM
8d	MeO	cPentO	2-Cl					0.19
9a	MeO	H	2,6-diF	184–6	C	$C_{14}H_{11}F_2NO_2$	C,H,N	37
9b	MeO	MeO	2,6-diF	163–4	D	$C_{15}H_{13}F_2NO_3$	C,H,N	0.9
9f	MeO	MeO	2-Cl	143–5	C	$C_{15}H_{14}ClNO_3$	C,H,Cl,N	0.65
9c	MeO	PrO	2,6-diF	170–2	A,C	$C_{17}H_{17}F_2NO_3$	C,H,N	1.6
9d	MeO	BuO	2,6-diF	150–2	A,C	$C_{18}H_{19}F_2NO_3$	C,H,N	0.3
9e	MeO	cHexO	2,6-diF	60–1	A,C	$C_{20}H_{21}F_2NO_3$	C,H,N[b]	0.4
9g	MeO	(±)-2-*exo*-norbornylO	2,6-diCl	106–7	B,D	$C_{21}H_{21}Cl_2NO_3$	C,H,N	0.01
10a	MeO	cPentCOO	2-Cl	159–60	D,G	$C_{20}H_{20}ClNO_4$	C,H,N	>1000
10b	MeO	*t*-BuCOO	2-Cl	112–4	D,G	$C_{19}H_{20}ClNO_4$	C,H,N	>1000
10c	MeO	PhCOO	2-Cl	127–9	D,G	$C_{21}H_{16}ClNO_4$	C,H,N	>1000
10d	MeO	MeCOO	2-Cl	153–5	D,G	$C_{16}H_{14}ClNO_4$	C,H,Cl,N	70
11	H	cPentO	2,6-diF	oil	A,C	$C_{18}H_{17}F_2NO_2$	C,H,N	15

[a] Unless indicated, all values were within 0.4%. [b] N: calcd, 3.9; found, 3.3.

pyrazine, **15e**, analogues were up to 10 times more active than the phenyl analogue **8a**, the 2-pyrimidyl, **15d**, and thiazolyl, **15f**, analogues being of similar activity to **8a**. The activities of the substituted heterocyclic analogues generally paralleled those of the phenyl series. Substitution in the position ortho to the amide linkage with chlorine in the pyridyl series (compounds **15g–i**) exhibited activities of up to an order of 10 greater than that of the 2-chlorophenyl compound **8d**, the best activity residing in the 4-pyridyl analogue **15i**. However, the monomethyl compound **15p** was less active than the corresponding phenyl analogue **8g**. Activity was enhanced in the *o*-dihalo-substituted 4-pyridyl analogues **15j,k**; further meta substituents (compounds **15l–o**) could be accommodated but with some reduction in activity. Unlike the monomethyl compound **15p**, the *o*-dimethyl compound **15q** was slightly more active than the corresponding phenyl analogue **8n**. Other heterocyclic *o*-disubstituted analogues, **15r–v**, showed reasonable activity, but none reached that of the pyridyl analogues. The most potent analogue was found

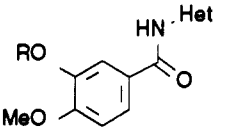
Table 3. Changes to the Amide Linkage of *N*-Phenylbenzamides



compd	X	R	mp, °C	formula	analysis[a]	PDE IV (pig aorta) IC_{50}, μM
12	$CONHCH_2$	2-Cl	152–4	$C_{20}H_{22}ClNO_3$	C,H,Cl,N	>1000
13	CSNH	2-Cl	129–31	$C_{19}H_{20}ClNO_2S$	C,[b]H,N,S	0.2
14	COO	2,6-diCl	98–100	$C_{19}H_{18}Cl_2O_4$	C,H	>1000

[a] Unless indicated, all values were within 0.4%. [b] C: calcd, 63.1; found, 62.6.

Table 4. *N*-Heterocyclic Benzamides



compd	R	Het	mp, °C	method	formula	analysis[a]	PDE IV (pig aorta) IC_{50}, μM
15a	cPent	pyrid-2-yl	92–4	A,D	$C_{18}H_{20}N_2O_3$	C,H,N	1
15b	cPent	pyrid-3-yl	171–2	A,D	$C_{18}H_{20}N_2O_3$	C,H,N	0.55
15c	cPent	pyrid-4-yl	163–5	A,D	$C_{18}H_{20}N_2O_3$	C,H,N	0.8
15d	cPent	pyrimidin-2-yl	108–10	A,D	$C_{17}H_{19}N_3O_3$	C,H,N[b]	3.5
15e	cPent	pyrazin-2-yl	80–2	A,D	$C_{17}H_{19}N_3O_3$	C,H,N	0.25
15f	cPent	thiazol-2-yl	145–6	A,C	$C_{16}H_{18}N_2O_3S$	C,H,N,S	6.5
15g	cPent	3-Cl-pyrid-2-yl	138–40	A,D	$C_{18}H_{19}ClN_2O_3$	C,H,N,Cl	0.36
15h	cPent	4-Cl-pyrid-3-yl	130–2	A,D	$C_{18}H_{19}ClN_2O_3$	C,H,N	0.12
15i	cPent	3-Cl-pyrid-4-yl	124–6	A,D	$C_{18}H_{19}ClN_2O_3$	C,H,N	0.016
15j	cPent	3,5-diCl-pyrid-4-yl	153–5	A,F	$C_{18}H_{18}Cl_2N_2O_3$	C,H,N,Cl	0.001
15k	cPent	3,5-diBr-pyrid-4-yl	160–2	A,F	$C_{18}H_{18}Br_2N_2O_3$	C,H,N	0.007
15l	cPent	2,3,5-triF-pyrid-4-yl	144–6	A,F	$C_{18}H_{17}F_3N_2O_3$	C,H,N	0.085
15m	cPent	2,3,5,6-tetraF-pyrid-4-yl	178–80	A,E	$C_{18}H_{16}F_4N_2O_3$	C,H,N	0.55
15n	cPent	2,3,6-triF-5-Cl-pyrid-4-yl	188–90	A,E	$C_{18}H_{16}ClF_3N_2O_3$	C,H,N,Cl	0.1
15o	cPent	2,6-diF-3,5-diCl-pyrid-4-yl	188–90	A,E	$C_{18}H_{16}Cl_2F_2N_2O_3$	C,H,N,Cl	0.02
15p	cPent	3-Me-pyrid-2-yl	55	A,D	$C_{19}H_{22}N_2O_3$	C,H,N	3.1
15q	cPent	3,5-diMe-pyrid-4-yl	77–80	A,F	$C_{20}H_{24}N_2O_3 \cdot H_2O$	C,H,N	0.035
15r	cPent	2,4-diCl-pyrimidin-3-yl	191–3	A,E	$C_{17}H_{17}Cl_2N_3O_3$	C,H,N,Cl	0.4
15s	cPent	3,5-diMe-isoxazol-3-yl	150–2	A,C	$C_{18}H_{22}N_2O_4$	C,H,N	0.45
15t	cPent	3,5-diMe-isothiazol-4-yl	140–1	A,C	$C_{18}H_{22}N_2O_3S$	C,H,N	0.5
15u	cPent	3-Me-5-Br-isothiazol-4-yl	160–2	A,C	$C_{17}H_{18}BrN_2O_3S$	C,H,N	0.5
15v	cPent	3-Me-5-CN-isothiazol-4-yl	163–4	A,E	$C_{18}H_{19}N_3O_3S$	C,H,N[c]	0.2
15w	cPent	3,5-diCl-pyrid-4-yl *N*-oxide	118–20	Ex[d]	$C_{18}H_{18}Cl_2N_2O_4$	C,H,N	0.002
16a	Me	3,5-diCl-pyrid-4-yl	207–9	F	$C_{14}H_{12}Cl_2N_2O_3$	C,H,N	0.1
16b	Bu	3,5-diCl-pyrid-4-yl	165–7	A,E	$C_{17}H_{18}Cl_2N_2O_3$	C,H,N	0.008
16c	Bu	3,5-diBr-pyrid-4-yl	160–2	A,E	$C_{17}H_{18}Br_2N_2O_3$	C,H,N	0.07
16d	cHex	3,5-diCl-pyrid-4-yl	170	A,F	$C_{19}H_{20}Cl_2N_2O_3$	C,H,N	0.05
16e	(±)-2-*exo*-norbornyl	3,5-diCl-pyrid-4-yl	153–4	B,F	$C_{20}H_{20}Cl_2N_2O_3$	C,H,N	0.001
16f	(−)-2-*exo*-norbornyl	3,5-diCl-pyrid-4-yl	155–6	B,F	$C_{20}H_{20}Cl_2N_2O_3$	C,H,N[e]	0.001
16g	(+)-2-*exo*-norbornyl	3,5-diCl-pyrid-4-yl	156–7	B,F	$C_{20}H_{20}Cl_2N_2O_3$	C,H,N,Cl[f]	0.001

[a] Unless indicated, all values were within 0.4%. [b] C: calcd, 65.2; found, 64.6. [c] C: calcd, 60.5; found, 60.0. [d] Ex = experimental procedure described. [e] $[\alpha]^{25}_D$ (c 1.0, CH_2Cl_2) = −16°. [f] $[\alpha]^{25}_D$ (c 1.0, CH_2Cl_2) = +25°.

to be the *N*-(3,5-dichloro-4-pyridyl) derivative **15j** (IC_{50} = 1 nM), and further analogues of this compound were synthesized and investigated.

The *N*-oxide **15w** retained good activity. The activities of the compounds with changes to the cyclopentyloxy group (compounds **16a–g**) paralleled those in the phenyl series (Table 2), with the highest activity residing in the norbornyloxy analogues, **16e–g**. No difference in activity was observed between the racemate **16e** and the enantiomers **16f,g**.

Antiinflammatory and Bronchodilator Activities

A selected group of the more potent analogues was further tested for their activities against PDE IV from guinea pig peritoneal eosinophils. This cell type was chosen since it is a prominent pathological feature in allergic asthma.[14] The predominant and perhaps only PDE isozyme in guinea pig eosinophils is a tightly membrane-bound PDE IV.[10] Inhibition of this membrane-bound enzyme by a range of compounds and their effects on whole-cell parameters (cAMP accumulation, O_2^- generation) are poorly correlated;[10] however, a closer correlation exists between inhibition of deoxycholate/NaCl-solubilized enzyme and whole-cell parameters for reasons that have been suggested previously.[15] For this reason, compounds were tested against the solubilized eosinophil PDE IV, which was prepared as described previously.[15] As an index of in vitro antiinflammatory activity, the same selected group of compounds was tested for potency in inhibiting superoxide O_2^- generation from guinea pig peritoneal eosinophils.

To assess in vivo bronchodilator activity, the effects of selected compounds on intravenous histamine-induced bronchospasm using a modified Dixon–Brodie method[16]

Table 5. Comparative Antiinflammatory and Bronchodilating Effects of Selected Compounds

compd	PDE IV enzyme activity IC_{50}, nM[a]		eosinophil	histamine bronchoconstriction		
	pig aorta	eosinophil soluble	superoxide IC_{50}, nM[a]	dose, μg	% reduction	duration, min
8a	2566 ± 1718 (3)	145 ± 5 (3)	>10000 (3)	200	32 ± 10	6
8d	186 ± 29 (3)	51 ± 25 (3)	450 ± 280 (3)	100	43 ± 1	27
8n	106 ± 47 (3)	65 ± 25 (3)	170 ± 70 (3)	100	24 ± 13	16
8m	23 ± 10.5 (3)	5 ± 0.9 (3)	530 ± 250 (8)	100	48 ± 3	34
				200	55 ± 11	30
8p	66 ± 20 (3)	18 ± 6 (3)	580 ± 250 (5)	100	29 ± 2	18
				200	54 ± 2	48
15i	16 ± 3 (3)	2 ± 0.7 (3)	30 ± 10 (3)	200	26 ± 12	15
15j	1 ± 0.1 (3)	0.8 ± 0.1 (3)	24 ± 6 (9)	10	36 ± 13	13
				20	56 ± 7	31
				50	78 ± 12	30
15w	2.3 ± 0.3 (3)	2 ± 0.6 (3)	300 ± 230 (5)	50	45 ± 1	18
				100	54 ± 12	39
16b	8.5 ± 1.5 (3)	2.5 ± 0.5 (3)	480 ± 220 (9)	100	52 ± 13	34
				200	78 ± 3	32
16e	1.4 ± 0.3 (3)	0.5 ± 0.1 (3)	22 ± 12 (5)	50	35 ± 5	29
				100	80 ± 6	59
rolipram	1470 ± 90 (3)	20 ± 2 (3)	160 ± 30 (9)	10	15 ± 2	15
				20	48 ± 3	29
				50	58 ± 8	38

[a] Number of experiments indicated in parentheses.

were investigated in anaesthetized guinea pigs. Compounds were administered directly into the lungs as a dry powder, blended with lactose, which was found to be the most effective means of applying these relatively insoluble compounds.

The selected compounds and their activities are collated in Table 5. Compounds in both the *N*-phenyl- and *N*-4-pyridylbenzamide series were chosen, and their activities can be compared to the lead structure **8a**. There is a positive correlation ($r = 0.96, p < 0.001$) between potencies of benzamides in inhibiting PDE IV from pig aorta and eosinophils. This relationship is surprisingly good when viewed in the light of a previous report[20] demonstrating that the IC_{50} values of a range of standard PDE inhibitors against the PDE's from these two sources are very poorly correlated. In the present studies, rolipram was shown to be 70-fold more potent against the solubilized PDE IV compared to the pig aortic enzyme, indicating, perhaps, that the enzymes from the two sources are different. If this contention is correct, then it would appear that many of the novel benzamides reported here differ from rolipram in not discriminating between the two PDE IV's. An alternative explanation is based upon the conjecture that rolipram and many of the benzamides inhibit the enzyme through distinct interactions. Preparation of the pig aortic PDE IV may disrupt the ability of rolipram to influence catalytic activity, whereas the site through which most of the benzamides exert their effects is uninfluenced by rigorous homogenization and subsequent chromatography procedures. Evidence for multiple sites on PDE IV through which inhibitors can interact has recently emerged.[23] As well as the catalytic site, a distinct high-affinity rolipram-binding site has been demonstrated, which may influence enzyme activity and inflammatory cell functions.[20]

A strong positive correlation also exists between the potencies of benzamides in inhibiting solubilized eosinophil PDE IV and reducing O_2^- generation ($r = 0.76, p < 0.01$), suggesting that the two phenomena are causally linked. Eosinophils are thought to play an important role in the pathology of asthma; drugs which dampen their activity would thus be expected to reduce levels of eosinophil-derived products in the lung, thereby reducing the inflammatory response and tissue damage.

Administration of dry powder formulations of the selected benzamides, as well as rolipram, directly into the airways reduces histamine-induced bronchospasm in anaesthetized guinea pigs. As expected, higher activity was observed in the *N*-pyridylbenzamides when compared to the *N*-phenyl series. Within the 4-pyridyl series, the overriding factor for high activity appears to be the presence of *o*-dichloro substitution at the amide linkage. Indeed, the 3-butyloxy analogue **16b** elicited a greater than 50% reduction in the histamine response at 100 μg, which lasted longer than 30 min. The most potent compound tested was **15j** which produced a 78% reduction of the histamine response at 50 μg. This response was greater than that produced by rolipram. No compound produced a greater than 10% change (reduction) in mean arterial blood pressure.

Summary

The chemical syntheses and structure–activity relationships of a series of novel benzamides, as selective PDE IV inhibitors, are described. Within the compounds reported in Tables 1–4, 3,4-dialkoxyphenyl substitution is preferred for PDE IV (from pig aorta) activity with 3-cyclopentyloxy (or *exo*-norbornyloxy) and 4-methoxy groups being optimal. In the *N*-aryl ring, a 4-pyridyl group with disubstitution ortho to the amide is optimal. This aortic PDE IV inhibition correlates well with the eosinophilic PDE IV and superoxide inhibitions. For good bronchodilator activity, dichlorosubstitution in the *N*-aryl ring ortho to the amide is an important factor. With the realization that clinical asthma is a chronic inflammatory disease, it is clear that new drugs must possess inherent antiinflammatory activity if they are to be of therapeutic benefit. These selective PDE IV benzamides offer the exciting possibility of a dual antiinflammatory/bronchodilator mode of action. As such, the *N*-4-pyridylbenzamide analogue **15j** (RP 73401) has been selected for clinical investigation.

Experimental Section

Reagents, starting materials, and solvents were purchased from common commercial suppliers and used as received or distilled from the appropriate drying agent. Reactions requiring anhydrous conditions were performed under an atmosphere of argon.

All organic solutions were dried over magnesium sulfate. Concentration refers to evaporation under aspirator vacuum using a Buchi rotary evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40–63 μm) with the solvent system indicated. Yields are not optimized. Spectroscopic data were recorded on Varian XL-200 and VXR 400 (NMR) instruments and were consistent with the assigned structures. NMR data are reported in ppm downfield relative to external TMS (0 ppm) as standard. Melting points were recorded on a Gallenkamp 595 apparatus and are uncorrected. Elemental analyses were performed by the Analytical Department at Rhône Poulenc Rorer. Where analyses are indicated only by symbols of the elements, results obtained were within 0.4% of the theoretical values.

General Procedures for Alkylation of Isovanillin (Methods A, B). Method A. 3-(Cyclopentyloxy)-4-methoxybenzaldehyde (1; R = cyclopentyl). A suspension of isovanillin (100 g, 0.66 mol), anhydrous K_2CO_3 (136.2 g, 0.99 mol), and KI (3 g) in dry DMF (650 mL) was stirred and heated to 65 °C. Cyclopentyl bromide (127.3 g, 0.85 mol) was added portionwise over 1 h and the stirred mixture heated at 65 °C for a further 21 h. After cooling to room temperature, the reaction mixture was diluted with toluene (2.0 L) and the organic phase extracted with 1 M NaOH (2 × 1.5 L). The combined aqueous washings were extracted with toluene (0.5 L), and the combined organic fractions were washed with water (3 × 500 mL). The organic phase was dried and evaporated to give 1 (R = cyclopentyl) as a light brown oil: yield, 117 g (81%); [17] ^{1}H NMR ($CDCl_3$) 9.84 (s, 1 H), 7.42 (m, 2 H), 6.95 (d, 1 H, J = 9 Hz), 4.87 (m, 1 H), 3.93 (s, 3 H), 2.1–1.6 (m, 8 H).

Method B. (±)3-(*exo*-Bicyclo[2.2.1.]hept-2-yloxy)-4-methoxybenzaldehyde (1; R = *exo*-norbornyl). Isovanillin (10.17 g, 67 mmol), (±)-*endo*-2-norborneol (5 g, 44.6 mmol), and Ph_3P (17.5 g, 67 mmol) were dissolved in dry THF (200 mL), and to this mixture was added dropwise diisopropylazodicarboxylate (13.5 g, 67 mmol). The mixture was heated at reflux for 48 h, cooled, and poured into water (500 mL). The solution was extracted with ether (3 × 100 mL). The combined organic extracts were washed (2 × 100 mL of H_2O, 2 × 100 mL of 1 M NaOH, 100 mL of brine), dried, filtered, and concentrated. The residue was purified by flash chromatography eluting with EtOAc/pentane (15:85) to provide 1 (R = *exo*-norbornyl) as a white solid: yield, 8.2 g (75%); mp 56–58 °C; ^{1}H NMR ($CDCl_3$) 9.83 (s, 1 H), 7.43 (dd, 1 H, J = 9 Hz, J = 1 Hz), 7.35 (d, 1 H, J = 1 Hz), 6.96 (d, 1 H, J = 9 Hz), 4.28 (d, 1 H, J = 6 Hz), 3.93 (s, 3 H), 2.54 (d, 1 H, J = 5 Hz), 2.34 (t, 1 H, J = 3 Hz), 1.88–1.13 (m, 8 H).

The enantiomeric alcohols used for the preparation of **16f,g** were synthesized by literature procedures.[18]

3-(Cyclopentyloxy)-4-methoxybenzoic Acid (2; R = cyclopentyl). A suspension of 1 (R = cyclopentyl; 115 g, 0.52 mol) and sulfamic acid (69 g, 0.7 mol) in 80% CH_3COOH (900 mL) was stirred and a solution of 80% $NaClO_2$ (61 g, 0.54 mol) in water (250 mL) added over 1.25 h. The temperature was maintained at 18–20 °C by external cooling using an ice–water bath. The yellow slurry was stirred at 20 °C for a further 1 h and the reaction mixture diluted by the addition of water (900 mL) over 0.5 h. Filtration and drying gave **2** as a white solid: yield, 113.5 g (92%); mp 166–168 °C; ^{1}H NMR ($CDCl_3$) 7.73 (dd, 1 H, J = 9 Hz, J = 1 Hz), 7.24 (d, 1 H, J = 1 Hz), 6.92 (d, 1 H, J = 9 Hz), 4.84 (m, 1 H), 3.93 (s, 3 H), 2.08–1.6 (m, 8 H).

3-(Cyclopentyloxy)-4-methoxybenzoyl Chloride (3; R = cyclopentyl). A solution of **2** (110 g, 0.47 mol) in toluene (1 L) was dried by azeotropic distillation of some of the toluene. The solution was allowed to cool to 90 °C and DMF (1 mL) added. $SOCl_2$ (45 mL, 0.62 M) was added portionwise over 0.25 h and the solution heated at reflux for 5 h and allowed to cool to ambient temperature. The solution was concentrated to give **3** as a light brown oil which crystallized upon standing: yield, 116 g (98%); ^{1}H NMR ($CDCl_3$) 7.82 (dd, 1 H, J = 9 Hz, J = 1 Hz), 7.53 (d, 1 H, J = 1 Hz), 6.92 (d, 1 H, J = 9 Hz), 4.86 (m, 1 H), 3.87 (s, 3 H), 2.08–1.47 (m, 8 H).

General Procedures for Benzamide Formation (Methods C–F). Method C. 3-(Cyclopentyloxy)-*N*-phenyl-4-methoxybenzamide (8a). To a solution of aniline (0.4 mL, 4.4 mmol) and Et_3N (0.55 mL) in CH_2Cl_2 (50 mL) was added portionwise **3** (1 g, 4 mmol) and the solution stirred and heated at 60 °C for 5 h. After cooling, the solution was washed with water (2 × 50 mL), dried, and concentrated to give a pale yellow solid. Recrystallization from EtOAc gave **8a**: yield, 1.13 g (91%); mp 169–173 °C; ^{1}H NMR ($CDCl_3$) 7.88 (s, 1 H), 7.6 (d, 2 H, J = 5 Hz), 7.46 (d, 1 H, J = 1 Hz), 7.4–7.26 (m, 3 H), 7.1 (t, 1 H, J = 3 Hz), 6.84 (d, 1 H, J = 5 Hz). Anal. ($C_{19}H_{21}NO_3$) C, H, N.

Method D. 3-(Cyclopentyloxy)-*N*-(2-hydroxyphenyl)-4-methoxybenzamide (8z). To a solution of 2-aminophenol (2.15 g, 19.7 mmol) in dry pyridine (50 mL) was added **3** (5 g, 19.6 mmol) and the solution heated at 60 °C for 3 h. Water (10 mL) was added and the solution concentrated to an oil. The oil was treated with water (50 mL) and extracted with CH_2Cl_2 (2 × 75 mL). The extract was dried and concentrated and the residue recrystallized from EtOAc to give **8z** as a fawn solid: yield, 5 g (78%); mp 158–160 °C; ^{1}H NMR ($CDCl_3$) 8.06 (s, 2 H), 7.49 (d, 1 H, J = 2 Hz), 7.43 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.16 (t, 2 H, J = 8 Hz), 7.08 (dd, 1 H, J = 8 Hz, J = 2 Hz), 4.88 (m, 1 H), 3.92 (s, 3 H), 2.06–1.5 (m, 8 H). Anal. ($C_{19}H_{21}NO_4$) C, H, N.

Method E. 3-(Cyclopentyloxy)-*N*-(2,3,5,6-tetrafluoro-4-pyridyl)-4-methoxybenzamide (15m). A mixture of **3** (2.5 g, 9.8 mmol) and 4-amino-2,3,5,6-tetrafluoropyridine (3.3 g, 20 mmol) was intimately mixed and heated gently until molten. The melt was stirred for 2 min and a solid formed. The melt was recrystallized from MeOH to give **15m**: yield, 1.7 g (45%); mp 178–80 °C; ^{1}H NMR ($CDCl_3$) 10.02 (s, 1 H), 7.66 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.6 (d, 1 H, J = 2 Hz), 6.93 (d, 1 H, J = 8 Hz), 4.88 (m, 1 H), 3.93 (s, 3 H), 2.03–1.58 (m, 8 H). Anal. ($C_{18}H_{16}F_4N_2O_3$) C, H, N.

Method F. 3-(Cyclopentyloxy)-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (15j). A solution of 4-amino-3,5-dichloropyridine (16.3 g, 0.1 mol) in dry THF (160 mL) was added dropwise to a stirred suspension of 60% oil dispersion of NaH (9.6 g, 0.24 mol) in dry THF (100 mL), maintaining the temperature below 20 °C. After stirring for a further 0.25 h, a solution of **3** (26.8 g, 0.105 mol) in THF (160 mL) was added dropwise over 1 h, keeping the temperature below 10 °C. After stirring for 0.5 h at room temperature, the solution was treated with 1 M aqueous HCl (200 mL) to destroy excess NaH and liberate the free amide from its salt. The mixture was diluted with CH_2Cl_2 (300 mL) and the organic phase separated and washed successively with water (200 mL), 10% aqueous Na_2CO_3 (200 mL), and again with water (200 mL). Drying and concentration of the organic phase gave a pale brown solid which was recrystallized from *i*-PrOH to give **15j**: yield, 26.4 g (69%); mp 154 °C; ^{1}H NMR ($CDCl_3$) 8.56 (s, 2 H), 7.65 (s, 1 H), 7.53–6.98 (m, 2 H), 6.95 (d, 1 H, J = 8 Hz), 4.87 (m, 1 H), 3.93 (s, 3 H), 2.05–1.55 (m, 8 H). Anal. ($C_{18}H_{18}Cl_2N_2O_3$) C, H, N, Cl.

3-(Benzoyloxy)-*N*-(2-chlorophenyl)-4-methoxybenzamide (5c). 3-Formyl-6-methoxyphenyl benzoate (35.2 g, 0.137 mol) was added to a solution of $KMnO_4$ (28 g, 0.17 mol) in acetone (200 mL). After the solution was stirred at room temperature for 3 min, the vigorous reaction was controlled by ice cooling. The mixture was then stirred at room temperature for 3 h and concentrated. The dark residue was treated with sodium metabisulfite solution, and the resulting white solid was filtered, washed with water, and dried to give 3-carboxy-6-methoxyphenyl benzoate (**5a**): yield, 29.3 g (78%); mp 180–183 °C (lit[19] mp 178 °C); ^{1}H NMR ($CDCl_3$) 8.19 (d, 2 H, J = 8 Hz), 7.99 (dd, 1 H, J = 10 Hz, J = 2 Hz), 7.85 (d, 1 H, J = 3 Hz), 7.66 (t, 1 H, J = 8 Hz), 7.53 (dt, 2 H, J = 9 Hz, J = 1 Hz), 7.06 (d, 1 H, J = 8 Hz), 3.88 (s, 3 H).

The above acid (29.3 g, 0.108 mol) was mixed with $SOCl_2$ (30 mL) in toluene (300 mL) and the mixture stirred and heated at 100 °C for 6 h. After cooling, the solution was concentrated to give 3-(benzoyloxy)-4-methoxybenzoyl chloride (**5b**):[19] yield, 28.7 g (92%); mp 120–122 °C.

A solution of the acid chloride **5b** (28.7 g, 0.1 mol) in CH_2Cl_2 (130 mL) was added dropwise to a stirred solution of 2-chloroaniline (12.63 g, 0.1 mol) and Et_3N (13.78 mL, 0.034 mol) and the solution then stirred at room temperature for 5 h. After standing at room temperature, the solution was washed with water (100 mL) and the organic layer dried and evaporated to give **5c**: yield, 37.7 g (100%); mp 110–114 °C; ^{1}H NMR ($CDCl_3$) 8.54 (dd, 1 H, J = 8 Hz, J = 2 Hz), 8.36 (s, 1 H), 8.24 (dd, 2 H, J = 8 Hz, J = 2 Hz), 7.87 (dd, 1 H, J = 9 Hz, J = 3 Hz), 7.76 (d, 1 H, J = 3 Hz), 7.66 (t, 1 H, J = 8 Hz), 7.54 (t, 2 H, J = 8 Hz),

7.41 (dd, 1 H, J = 9 Hz, J = 1 Hz), 7.34 (dt, 1 H, J = 8 Hz, J = 2 Hz), 7.13 (d, 1 H, J = 9 Hz), 7.08 (dt, 1 H, J = 9 Hz, J = 3 Hz), 3.91 (s, 3 H).

***N*-(2-Chlorophenyl)-3-hydroxy-4-methoxybenzamide (6).** To a stirred solution of KOH (24.42 g, 0.436 mol) in water (76 mL) and MeOH (230 mL) was added **5c** (38.15 g, 0.1 mol) and the mixture heated at reflux for 0.5 h. The solution was cooled, acidified with 1 N HCl, and extracted with CH_2Cl_2 (2 × 200 mL). The organic extract was washed with water (200 mL), dried, and concentrated. The residue was recrystallized from EtOAc to give **6**: yield, 15.8 g (57%); mp 156–158 °C; ^{1}H NMR ($CDCl_3$) 8.54 (dd, 1 H, J = 8 Hz, J = 2 Hz), 8.35 (s, 1 H), 7.52–7.47 (m, 2 H), 7.40 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.32 (dt, J = 8 Hz, J = 2 Hz), 7.06 (dt, 1 H, J = 8 Hz, J = 2 Hz), 6.95 (d, 1 H, J = 9 Hz), 5.88 (s, 1 H), 3.97 (s, 3 H).

General Procedure for Acylation of 6 (Method G). *N*-(2-Chlorophenyl)-3-(cyclopentanoyloxy)-4-methoxybenzamide (10a). To a stirred solution of **6** (1.5 g, 5.4 mmol) in dry DMF (30 mL) was added a 60% oil dispersion of NaH (0.22 g, 5.4 mmol) at room temperature. After stirring for 1 h, cyclopentanoyl chloride (0.72 g, 5.4 mmol) was added dropwise and the reaction mixture stirred at room temperature for 5 h and allowed to stand overnight. The mixture was diluted with water (50 mL) and extracted with EtOAc (50 mL). The organic extract was dried and evaporated to give an oil, which upon trituration with ether gave **10a**: yield, 1.62 g (80%); mp 159–160 °C; ^{1}H NMR ($CDCl_3$) 8.5 (dd, 1 H, J = 8 Hz, J = 2 Hz), 8.33 (s, 1 H), 7.79 (dd, 1 H, J = 8 Hz, J = 3 Hz), 7.63 (d, 1 H, J = 2 Hz), 7.42 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.33 (dt, 1 H, J = 9 Hz, J = 2 Hz), 7.08 (dt, 1 H, J = 8 Hz, J = 2 Hz), 7.06 (d, 1 H, J = 9 Hz), 3.9 (s, 3 H), 3.07 (m, 1 H), 2.05 (m, 4 H), 1.8 (m, 2 H), 1.67 (m, 2 H). Anal. ($C_{20}H_{20}ClNO_4$) C, H, Cl, N.

***N*-(2-Chlorobenzyl)-3-(cyclopentyloxy)-4-methoxybenzamide (12).** The title compound was synthesized from the acid chloride **3** and 2-chlorobenzylamine as in method C: mp 152–54 °C; ^{1}H NMR ($CDCl_3$) 7.45–7.3 (m, 3 H), 7.3–7.16 (m, 3 H), 6.84 (d, 1 H, J = 8 Hz), 6.6 (t, 1 H, J = 6 Hz), 4.82 (m, 1 H), 4.7 (d, 1 H, J = 6 Hz), 3.88 (s, 3 H), 2.06–1.48 (m, 8 H). Anal. ($C_{20}H_{22}ClNO_3$) C, H, N.

***N*-(2-Chlorophenyl)-3-(cyclopentyloxy)-4-methoxythiobenzamide (13).** A solution of **3** (13.3 g, 52 mmol) and 2-chloroaniline (5.5 mL, 52 mmol) in dry pyridine (50 mL) was stirred for 1 h. Then, P_2S_5 (13 g) was added and the mixture heated at 110 °C for 1.5 h, cooled to room temperature, and poured into a cold solution of concentrated HCl (100 mL) and water (400 mL). The mustard-colored solid was collected and subjected to flash chromatography eluting with cyclohexane/EtOAc (3:1) to give **13**: yield, 1.1 g (6%); mp 129–131 °C; ^{1}H NMR ($CDCl_3$) 9.28 (b s, 1 H), 8.7 (b s, 1 H), 7.58 (b s, 1 H), 7.48 (dd, 1 H, J = 8 Hz, J = 2 Hz), 7.45 (dd, 1 H, J = 8 Hz, J = 3 Hz), 7.36 (t, 1 H, J = 6 Hz), 7.23 (dt, 1 H, J = 8 Hz, J = 2 Hz), 6.88 (d, 1 H, J = 9 Hz), 4.87 (m, 1 H), 3.91 (s, 3 H), 2.07–1.5 (m, 8 H). Anal. ($C_{19}H_{20}ClNO_2S$) H, N, S; C: calcd, 63.1; found, 62.6.

(2,6-Dichlorophenyl)-3-(cyclopentyloxy)-4-methoxybenzoate (14). To a stirred solution of 2,6-dichlorophenol (1 g, 6.13 mmol) in DMF (20 mL) was added a 60% oil dispersion of NaH (270 mg, 6.75 mmol) and the solution heated at 80 °C for 0.75 h. The acid chloride **3** (1.54 g, 6.12 mmol) was added and the reaction mixture heated at 80 °C overnight. After cooling to room temperature, the reaction mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water (2 × 100 mL), dried, and concentrated to give a solid. The solid was subjected to flash chromatography eluting with pentane/EtOAc (4:1) to give **14**: yield, 1.56 g (66%); mp 98–100 °C; ^{1}H NMR ($CDCl_3$) 7.86 (dd, 1 H, J = 9 Hz, J = 2 Hz), 7.67 (d, 1 H, J = 2 Hz), 7.36 (d, 2 H, J = 9 Hz), 7.20 (d, 1 H, J = 11 Hz), 7.12 (d, 1 H, J = 8 Hz), 6.92 (d, 1 H, J = 8 Hz), 4.86 (m, 1 H), 3.92 (s, 3 H), 2.1–1.5 (m, 8 H). Anal. ($C_{19}H_{19}Cl_2O_4$) C, H, Cl.

***N*-(3,5-Dichloro-*N*-oxo-4-pyridyl-3-(cyclopentyloxy)-4-methoxybenzamide (15w).** To a suspension of **15j** (2 g, 5.25 mmol) in glacial CH_3COOH (8 mL) was added a 27.5% solution of H_2O_2 (6 mL) and the mixture stirred for 3 h at 70–80 °C. A further quantity of H_2O_2 (4 mL) was added and the solution allowed to stir for a further 12 h. After cooling, the solution was basified with 6 N NaOH and the mixture extracted with CH_2Cl_2 (2 × 30 mL). The extracts were combined, washed with brine, dried, and concentrated. The residue was recrystallized from MeOH to give **15w**: 1.08 g (35%); mp 118–120 °C; ^{1}H NMR ($CDCl_3$) 8.24 (s, 2 H), 7.73 (b s, 1 H), 7.49 (m, 2 H), 6.94 (d, 1 H, J = 8 Hz), 4.87 (m, 1 H), 3.94 (s, 3 H), 2.05–1.58 (m, 8 H). Anal. ($C_{18}H_{18}Cl_2N_2O_4$) C, H, N.

Biological Methods. Partial Purification of Pig Aortic PDE IV. The method of Souness[20] was followed. In brief, pig aortic PDE isozymes (PDE's I, III, IV, and V) were partially purified by DEAE-trisacryl anion-exchange chromatography. For determination of the kinetic constants, the concentration of cyclic AMP was varied while the amount of ^{3}H-labeled cyclic AMP remained constant. The data were analyzed by nonlinear least-squares regression analysis to obtain the K_m value (Enzfitter program, Biosoft).

Preparation of Eosinophil Subcellular Fractions and Measurement of Cyclic Nucleotide Phosphodiesterase. Guinea pig eosinophils were prepared as described previously.[10] Briefly, cells (100–200 × 10^6) suspended in HBSS were centrifuged (250 g, 10 min, 4 °C), the supernatant was removed, and the resulting cell pellet was resuspended in 5 mL of homogenization buffer (20 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM dithiothreitol, 5 mM EDTA, 0.25 sucrose, 20 μM *p*-tosyl-L-lysine chloromethyl ketone, 10 μg/mL leupeptin, and 2000 units/mL aprotinin). Cells were homogenized on ice with a Dounce homogenizer (10 strokes). The homogenate was centrifuged at 105000*g* for 60 min, and the supernatent was collected and the pellet resuspended in an equal volume of homogenization buffer. The membrane-bound cAMP PDE was solubilized by homogenizing freshly prepared membranes with a Dounce homogenizer (10 strokes) in 4 mL of homogenization buffer containing deoxycholate (0.5%) and NaCl (100 mM). The homogenate was centrifuged at 100000*g* for 30 min, and the supernatent containing the solubilized activity was removed and the pellet resuspended in an equal volume of homogenization buffer. Cyclic AMP PDE and protein were measured in the initial homogenate as well as in the cytosolic and particular fractions.

PDE activity was determined by a two-step radioisotope method.[21] The reaction mixture contained 20 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 0.2 mM EGTA, and 0.05 mg/mL of bovine serum albumin. Unless otherwise stated, the concentration of substrate was 2 μM for [^{3}H]cAMP. The IC_{50} values for the compounds examined were determined from concentration–response curves in which concentrations ranged from 0.1 nM to 1 mM. At least three concentration–response curves were generated for each agent.

Measurement of Eosinophil Superoxide Generation. Superoxide anion O_2^- generation was determined as the superoxide dismutase (SOD) inhibitory reduction of *p*-iodonitrotetrazolium violet (INTV).[22] Briefly, cells (10^6/well) were incubated in 96-well microtitre plates in 0.25 mL of HBSS containing INTV (0.5 mg/mL) plus other additions for 45 min at 37 °C. The cells were then centrifuged at 500*g* for 5 min and the supernatent aspirated. The pellet was solubilized by incubation overnight at room temperature in DMSO containing 0.6 M HCl and the absorbance of the reduced dye measured at 492 nM (Titertek Multiskan MCC/340). The results were expressed in absorbance units.

Bronchoconstriction in Anaesthetized Guinea Pigs. This method has been reported before,[16] but briefly, male Dunkin Hartley guinea pigs were anaesthetized and the trachea cannulated to allow ventilation at 60 strokes/min using a constant volume pump. Pulmonary inflation pressure (PIP) was measured using a pressure transducer connected to the tracheal cannula. Mean arterial blood pressure was recorded via a cannula placed in a jugular artery. To induce bronchospasm, histamine solutions were administered through a cannular placed in the right carotid vein.

The maximum ventilation circuit pressure was measured by manually occluding the airflow prior to connecting the tracheal cannula. Guinea pigs were challenged with various doses of iv histamine to establish the dose (typically in the range 5–15 μg/kg) required to produce an increase in PIP to approximately 60% of the maximum ventilation circuit pressure. Histamine was administered at intervals to allow PIP to return spontaneously to base line after each challenge. When responses to histamine

had become stable, a single dose of drug was administered as a dry powder. In some cases, a range of doses of each drug was administered in order to construct dose–response curves. IC_{50} values (the dose producing 50% inhibition of bronchospasm) were calculated by linear interpolation. Drugs administered intratracheally were distributed throughout the entire lung (unpublished observations). Particle size was monitored[16] and was uniform for all compounds tested.

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