# Inhibition of Cyclic Nucleotide Phosphodiesterase by Derivatives of 1,3-Bis(cyclopropylmethyl)xanthine

Derek R. Buckle,* Jonathan R. S. Arch, Brendan J. Connolly,† Ashley E. Fenwick, Keith A. Foster, Kenneth J. Murray,† Simon A. Readshaw, Mark Smallridge, and David G. Smith

*SmithKline Beecham Pharmaceuticals, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, U.K., and SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Hertfordshire, AL6 9AR, U.K*

*Received September 27, 1993*®

Alkylation of the selective type IV phosphodiesterase inhibitor, 8-amino-1,3-bis(cyclopropylmethyl)-xanthine (1, BRL 61063), led exclusively to the N-7 substituted derivatives **2–9**, which showed varying selectivities for the PDE type IV isoenzyme relative to PDE Va. The 4-methoxybenzyl derivative **6** in particular was a highly potent PDE Va inhibitor ($IC_{50}$ 0.14 $\mu$M) and showed a 24-fold selectivity for this isoenzyme relative to PDE IV. Sulfonation of 1 was more complex, with the product profile being highly dependent on the reaction conditions. As with alkylation, sulfonation at N-7 generally increased potency against PDE Va, especially in the aryl-containing moieties lacking strongly electron-withdrawing substituents (**12**, **15-17**, **19**). Bis-arylsulfonation at the exocyclic amino group generally reduced inhibitory potency against both PDE IV and Va. An 8-amidino compound **33**, formed by the unusual reaction of 1 with *N*-methylpyrrolidinone in the presence of benzenesulfonyl chloride, had an $IC_{50}$ value of 0.05 $\mu$M against PDE Va and is believed to be the most potent inhibitor of this isoenzyme reported. No correlation of PDE IV inhibition with displacement of [$^3$H]rolipram from its high-affinity binding site was demonstrated. This suggests that either the catalytic site and the rolipram binding site are not the same or that PDE IV can exist in two conformations, only one of which binds to rolipram with high affinity, and that the compounds described vary in their selectivity for this isoform.

## Introduction

Theophylline preparations have found continuous use as bronchodilators in the treatment of asthma for almost a century, yet evidence has now been provided to suggest that a major part of their benefit may be derived from an antiinflammatory activity.[1,2] Controversy still exists concerning the molecular mechanism by which theophylline exerts its effects, although inhibition of cyclic nucleotide phosphodiesterases may be at least one mechanism by which benefit is mediated.[3] Theophylline is a poor inhibitor of isolated PDEs at those plasma concentrations at which it evokes bronchodilator activity in humans.[4] One possibility, however, is that theophylline acts synergistically with endogenous activators of adenylyl cyclase to suppress the action of inflammatory cells.[5,6]

Despite its widespread use in asthma, theophylline is far from ideal in that it suffers from a relatively low therapeutic index. Headache, nausea, and vomiting are common dose-limiting side effects, and excessive dosing may lead to arrhythmias, convulsions, seizures, and even death.[7,8] While some of the side effects of theophylline may be due to its ability to antagonise adenosine,[2,9] poor selectivity for individual isoenzymes may also play an important contribution[10,11] (see Table 3).

Partly as a result of the efficacy of theophylline in asthma therapy, the inhibition of cyclic nucleotide phosphodiesterases as a target for therapeutic intervention has been recognized for some considerable time, although it is the identification and subsequent characterization of multiple phosphodiesterase isoenzymes that has added greatly to current interest.[12,13] On the basis of their substrate specificity, inhibitor sensitivity, allosteric modulation by cGMP, and calcium/calmodulin sensitivity, at least five classes of isoenzyme have been distinguished, within which over 20 isoforms are known to exist.[13] As a result of the availability of purified isoenzyme preparations, potent and selective inhibitors of the type III, IV, and V phosphodiesterase families have been described, although inhibitors with good selectivity for the phosphodiesterase I and II isoenzymes have not been reported.[14] The realization that distinct populations of particular isoenzymes exist within different tissues, and the availability of selective isoenzyme inhibitors, has also contributed to the current interest by providing a rationale for the design of selective therapeutic agents.[13,15]

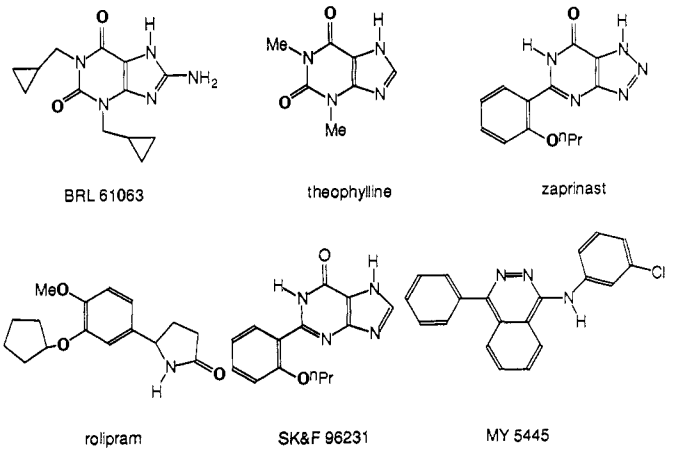
Inhibitors of the low $K_m$, cAMP-specific, type IV isoenzyme (PDE IV) in particular are attractive targets as potential antiasthmatic agents since there is abundant evidence that it is this enzyme which exerts a key role in the regulation of inflammatory cells implicated in the pathology of the disease.[3,16] Thus, studies in human tissues have demonstrated that PDE IV forms the predominant functional isoenzyme present in basophils, neutrophils, eosinophils, monocytes, and mast cells, as well as having a significant presence in other cells such as lymphocytes.[3,16,17] In addition to its role in inflammatory cells, PDE IV is also an important isoenzyme in human bronchi,[18] but in such a heterogeneous tissue the possibility that this enzyme derives from cells other than those from the airway cannot be discounted. Nevertheless, support for the functional relevance of PDE IV was obtained from relaxant studies using selective inhibitors of PDE IV.[18] That selective inhibitors of PDE III also evoked relaxation in human bronchi suggests that dual PDE III/IV inhibitors may be the most effective bronchodilators in humans.[18]

These data combine to suggest that selective inhibitors of PDE IV may address not only asthmatic bronchoconstriction, but also the underlying bronchial inflammation. For this reason we have attempted to identify potent and

† Hertfordshire.
® Abstract published in *Advance ACS Abstracts*, January 1, 1994.

0022-2623/94/1837-0476$04.50/0 

**Chart 1**

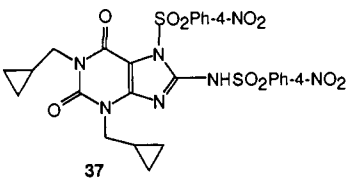



selective inhibitors of PDE IV using the xanthine skeleton from which to design novel compounds. Since 1,3-bis-(cyclopropylmethyl)xanthine (1, BRL 61063; Chart 1)[19] had previously been shown to elicit the desired selectivity profile, this compound was chosen for subsequent modification.

## Chemistry

8-Amino-1,3-bis(cyclopropylmethyl)xanthine (1, BRL 61063), the precursor to all of the derivatives described in this paper, was prepared by nitration and subsequent reduction of 1,3-bis(cyclopropylmethyl)xanthine following the literature procedure.[19] Direct alkylation of 1 in the presence of 1.2 equiv of base at ambient temperature led cleanly to the N-7 substituted compounds **2–9** (Scheme 1, Table 1), which were all isolated in reasonable yield. While alkylation at N-9 or at the C-8 amino group cannot be excluded, no product of these types was isolated. Due to the poor solubility of the anion of 1, reaction in dipolar aprotic solvents such as dimethylformamide (DMF) or dimethoxyethane (DME) was generally the preferred procedure using KOBu$^t$ as base (method A), but NaH was found to be equally suitable. The products derived from sulfonation of 1 were more complex than those arising through alkylation and depended markedly on both the strength and amount of the base utilized. Heating 1 with 1 equiv of base under essentially similar conditions to those used for alkylation (method B) afforded moderate yields of the corresponding 7-sulfonyl derivatives **10–19** (Scheme 1, Table 1). With 2 equiv of strong base (NaH) and 1 equiv of sulfonyl halide (method C), however, only the C-8 derivative, exemplified by **20** (Table 1) was isolated. Formation of the C-8 sulfonamide in this instance was presumably dependent on dianion formation, since this would then result in greater nucleophilicity of the exocyclic anion. The 4-nitrobenzenesulfonyl derivative **21** was isolated during an attempt to prepare an *N,N'*-disulfonyl derivative by reaction of 1 with excess 4-nitrobenzenesulfonyl chloride in THF in the presence of triethylamine (method D). In this instance compound **21** was formed in relatively low yield and was accompanied by similar quantities of the unexpected C-8 *N,N*-bis(4-nitrobenzenesulfonamide) **27** (Table 1). Under these conditions it appears that the nucleophilicity of the endocyclic nitrogen anion and the exocyclic nitrogen atom are approximately similar such that sulfonation proceeds initially at both N-7 and the C-8 amino group. Once sulfonation at the exocyclic position has occurred, however, the remaining hydrogen atom at this position is rendered acidic and is readily abstracted by either residual triethylamine or by anion at N-7, and this center thus becomes the dominant nucleophile. It is interesting that in the presence of excess pyridine (method E) only the C-8 bis-sulfonamide **26** was formed on reaction of 1 with benzenesulfonyl chloride. The exclusive formation of **26** is probably best rationalized by the inability of pyridine ($pK_a$ 5.23) to generate the anion at N-7, whereas the more basic triethylamine ($pK_a$ 10.87) can, yet its basicity is more than sufficient to abstract the acidic proton formed at C-8 after sulfonation at this position.

That **27** had the structure given rather than the 7,8-disubstitution pattern of compound **37** was deduced from NMR spectroscopic studies carried out on **27** itself and on its 7-methyl derivative **29** (prepared by direct alkylation with dimethyl sulfate; method F). The NMR spectra of

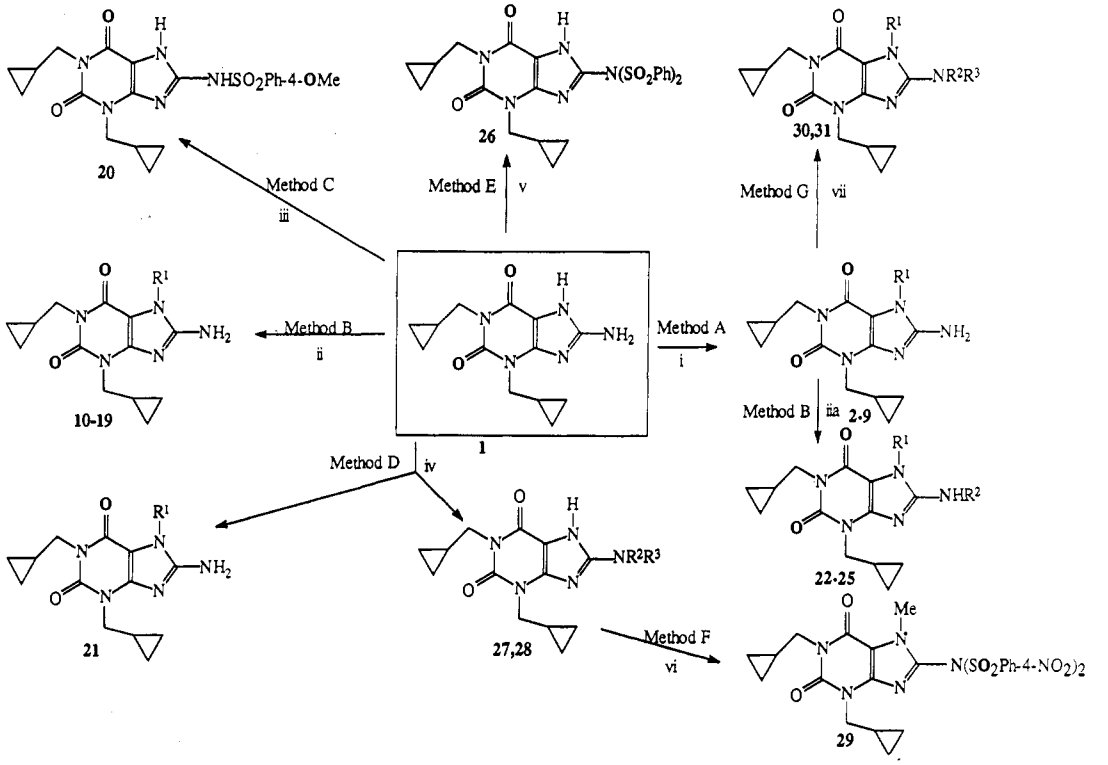



**37**

**27** were inconclusive, although the presence of a single set of resonances for the two 4-nitrobenzenesulfonyl moieties in the $^1$H and $^{13}$C NMR spectra suggested the symmetrical structure **27** rather than **37**. Unambiguous structural assignment was achieved for the 7-methyl derivative **29** through a combination of 1D and 2D NMR techniques. The long-range carbon–proton correlations determined from a 2D $^1$H, $^{13}$C COLOC experiment were particularly conclusive since correlations were observed between the *N*-methyl protons and the carbon atoms resonating at 109.4 and 136.1 ppm (Table 2). Assignment of the 109.4 ppm signal to the C-5 carbon atom is consistent with that expected from other xanthines,[20] and therefore identified the position of the methyl group as N-7. The signal at 136.1 ppm was assigned to C-8 since this carbon would also be 3-bond scalar coupled to the protons of the methyl groups at N-7. The observation of 3-bond carbon–proton coupling from the *N*-methyl to both C-5 and C-8, and not just to C-8 alone, confirmed that the methyl must be at N-7 as depicted in structure **29**. The structure of the unmethylated compound as that of **27** and not **37** is thus established. The bis(4-methoxybenzenesulfonyl) derivative **28** was prepared in a similar manner to **27**, and its structure was deduced by the coincidence of the methoxybenzenesulfonyl proton chemical shifts in the $^1$H NMR spectrum.

Once alkylation at N-7 had taken place, subsequent sulfonation at the C-8 amino group was simply effected and compounds **22–25** were prepared in moderate to good yields following the procedure of method B (Table 1). When a similar reaction was carried out in the presence of a small excess of both base and sulfonyl halide (method G), compound **6** furnished the bis-sulfonamides **30** and **31**, albeit in rather poor yield (Table 1). Since considerable amounts of **6** were recovered during these reactions it is expected that improved yields would be achieved using optimal proportions of both base and sulfonyl chloride. Attempts to benzenesulfonate the methoxybenzyl derivative **6** in DMF rather than DME (method H), however, did not lead to the expected sulfonamide, and nucleophilic attack at the formamide carbonyl group, leading to the intermediate **38** (Scheme 2), appears to have taken preference. Subsequent elimination of benzenesulfonic

**Scheme 1**[a]




[a] Reagents: (i) $R^1X$, KOBu$^t$, DMF or DME, room temperature; (ii) $R^1X$, KOBu$^t$, DME, 80 °C; (iia) $R^2X$, KOBu$^t$, DME, 80 °C; (iii) 4-MeOPhSO$_2$Cl, NaH, THF, room temperature; (iv) R-PhSO$_2$Cl, NEt$_3$, THF, room temperature; (v) PhSO$_2$Cl, pyridine, room temperature; (vi) Me$_2$SO$_4$, K$_2$CO$_3$; (vii) RSO$_2$Cl, KOBu$^t$, DME, reflux, 24 h.

acid from **38** then leads to the isolated amidine **32**. In a similar manner reaction of either **6** or **7** with *N*-methylpyrrolidinone afforded the corresponding amidines **33** and **34** (Scheme 2; single isomer of undefined stereochemistry isolated).

Similar products to those found with sulfonyl chlorides in amidic solvents were also observed on reaction of compound **1** with *N'-tert*-butyl-*N*,*N*-dimethylformamidine in toluene at reflux (method I, Scheme 3). The presumed intermediate **39** is able to eliminate an alkylamine to generate either compound **35** (via route a) or compound **36** (via route b). That almost identical yields of **35** and **36** were formed in this reaction, which proceeded almost quantitatively, suggest that there is little kinetic preference for either compound under the conditions prevailing.

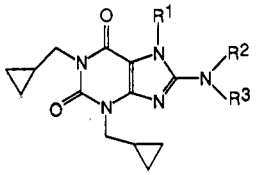
## Results and Discussion

Starting with BRL 61063 (**1**), a xanthine ultimately derived from theophylline,[19] we have endeavoured to refine the structure in order to identify other potent and selective PDE IV inhibitors which, unlike theophylline, show a reduced affinity for adenosine receptors.

**Phosphodiesterase Inhibition.** In contrast to theophylline, which elicits a low level of inhibitory activity against the PDE I, III, IV, and Va isoenzymes at 100 $\mu$M concentrations, BRL 61063 (**1**) is a potent and selective inhibitor of PDE IV ($IC_{50}$ 1.9 $\mu$M) (Table 3). Most derivatives of BRL 61063 described here, on the other hand, generally showed variable amounts of PDE IV and/or Va inhibition, but like BRL 61063 showed little if any tendency to attenuate the action of the other PDE isoenzymes at concentrations <100 $\mu$M. Compounds **9** and **29**, however, were unusual in showing potent inhibitory activity against PDE III and PDE II, respectively. Substitution at N-7 by small alkyl groups, **2–4**, generally reduced potency against PDE IV, causing a 5–10-fold drop, but evoked a 4–6-fold increase in inhibitory potency against PDE Va. Potency against PDE Va was further enhanced by benzylation at N-7 (compound **5**), while providing little change against PDE IV. The introduction of the 4-methoxybenzyl substituent at N-7 (compound **6**) provided one of the most potent PDE Va inhibitors so far reported ($IC_{50}$ 0.14 $\mu$M), while maintaining potency against PDE IV relative to that of compound **1**. Other substitutions on the benzyl moiety (**7–9**) generally reduced potency against PDE IV relative to that of the isobutyl compound **4**, and since the introduction of these bulky groups at N-7 resulted in a reduction in selectivity for PDE IV over PDE Va, no attempt was made to optimize the substituent present.

The introduction of small alkyl sulfonyl substituents at N-7 (**10** and **11**) also had a tendency to reduce potency against PDE IV relative to compound **1**, while at the same time marginally increasing that against PDE Va. The benzenesulfonyl derivative **12**, however, showed a considerably enhanced PDE Va inhibitory potency, thus following a similar pattern to that of benzylation (cf. **5**), although in this instance the effects of PDE IV and Va were approximately equal. The effect of aromatic substitution on the potency of compound **12** was somewhat variable. Potency against PDE IV was maintained with 4-chloro-, 4-methyl-, 4-methoxy-, and 3,4-dimethoxybenzenesulfonamides (**15–17** and **19**, respectively), where it paralleled a simultaneous potency increase against the Va isoenzyme, but was disfavoured by strongly electron-withdrawing substituents (**13**, **14**, and **21**) and by the alternative 2,5-disposition of the methoxy groups (**18**). Positioning of 4-methoxybenzenesulfonyl on the exocyclic amino group (compound **20**) rather than at N-7 as in **17** was also detrimental. Indeed, with the exception of **24**, sulfonation at the 8-amino group in those compounds

**Table 1**



| no. | $R^1$ | $R^2$ | $R^3$ | method of prepn[a] | yield, % | recryst solvent | mp, °C | formula | anal. |
|---|---|---|---|---|---|---|---|---|---|
| 2 | Me | H | H | A | 52 | | 204–5 | $C_{14}H_{19}N_5O_2$ | C,H,N |
| 3 | $Pr^n$ | H | H | A | 59 | | 172–3 | $C_{16}H_{23}N_5O_2$ | C,H,N |
| 4 | $Bu^i$ | H | H | A | 30 | ethyl acetate–hexane | 156–7 | $C_{17}H_{25}N_5O_2$ | M+ |
| 5 | $CH_2Ph$ | H | H | A | 84 | | 158 | $C_{20}H_{23}N_5O_2$ | C,H,N |
| 6 | $CH_2Ph$-4-OMe | H | H | A | 64 | | 176 | $C_{21}H_{25}N_5O_3$ | C,H,N |
| 7 | $CH_2Ph$-3,4,5-$(OMe)_3$ | H | H | A | 84 | acetone–hexane | 173–4 | $C_{23}H_{29}N_5O_5$ | C,H,N |
| 8 | $CH_2Ph$-4-$NO_2$ | H | H | A[b] | 69 | ethyl acetate–hexane | 200–1 | $C_{20}H_{22}N_6O_4$ | C,H,N |
| 9 | $CH_2$-1-naphthyl | H | H | A[c] | 54 | | 215–6 | $C_{24}H_{25}N_5O_2$ | C,H,N |
| 10 | $SO_2Me$ | H | H | B[d] | 19 | acetone–hexane | 201–2 | $C_{14}H_{19}N_5O_4S$ | C,H,N |
| 11 | $SO_2Pr^i$ | H | H | B[d] | 45 | ethyl acetate–hexane | 119–20 | $C_{16}H_{23}N_5O_4S$ | M+ |
| 12 | $SO_2Ph$ | H | H | B | 43 | | 170–1 | $C_{18}H_{21}N_5O_4S$ | C,H,N |
| 13 | $SO_2Ph$-3-$CF_3$ | H | H | B | 37 | ethyl acetate–hexane | 170 dec | $C_{20}H_{20}F_3N_5O_4S$ | C,H,N |
| 14 | $SO_2Ph$-4-$CF_3$ | H | H | B | 35 | acetone–hexane | 207–8 | $C_{20}H_{20}F_3N_5O_4S$ | C,H,N |
| 15 | $SO_2Ph$-4-Cl | H | H | B | 29 | acetone–hexane | 181–3 | $C_{18}H_{20}ClN_5O_4S$ | C,H,N |
| 16 | $SO_2Ph$-4-Me | H | H | B | 30 | acetone–hexane | 182–3 | $C_{20}H_{23}N_5O_4S$ | C,H,N |
| 17 | $SO_2Ph$-4-OMe | H | H | B | 37 | acetone–hexane | 177-8 | $C_{20}H_{23}N_5O_5S$ | N[e],C,H |
| 18 | $SO_2Ph$-2,5-$(OMe)_2$ | H | H | B | 33 | acetone–hexane | 200 dec | $C_{21}H_{25}N_5O_6S$ | C,H,N |
| 19 | $SO_2Ph$-3,4-$(OMe)_2$ | H | H | B | 31 | acetone–hexane | 183–4 | $C_{21}H_{25}N_5O_6S$ | C,H,N |
| 20 | H | $SO_2Ph$-4-OMe | H | C | 31 | | >240 dec | $C_{20}H_{23}N_5O_5S$ | M+ |
| 21 | $SO_2Ph$-4-$NO_2$ | H | H | D | 17 | | 188–9 | $C_{19}H_{20}N_6O_6S$ | C,H,N |
| 22 | Me | $SO_2Ph$ | H | B | 42 | acetone–hexane | 163 | $C_{20}H_{23}N_5O_4S$ | C,H,N |
| 23 | Me | $SO_2Ph$-4-OMe | H | B | 35 | acetone–hexane | 208–9 | $C_{21}H_{25}N_5O_5S$ | C,H,N |
| 24 | $CH_2Ph$-4-OMe | $SO_2Ph$-4-OMe | H | B | 64 | acetone–hexane | 187 | $C_{28}H_{31}N_5O_6S$ | C,H,N |
| 25 | $CH_2Ph$-3,4,5-$(OMe)_3$ | $SO_2Ph$-4-OMe | H | B | 64 | | 200–1 | $C_{30}H_{35}N_5O_8S$ | C,H,N |
| 26 | H | $SO_2Ph$ | $SO_2Ph$ | E | 59 | | 238 dec | $C_{25}H_{25}N_5O_6S_2$ | C,H,N |
| 27 | H | $SO_2Ph$-4-$NO_2$ | $SO_2Ph$-4-$NO_2$ | D | 23 | | 198 | $C_{25}H_{23}N_7O_{10}S_2$ | C,H,N |
| 28 | H | $SO_2Ph$-4-OMe | $SO_2Ph$-4-OMe | D | 15[f] | | 220–5 | $C_{27}H_{29}N_5O_8S_2$ | C,H,N |
| 29 | Me | $SO_2Ph$-4-$NO_2$ | $SO_2Ph$-4-$NO_2$ | F | 67 | ethyl acetate–hexane | 181–183 | $C_{26}H_{25}N_7O_{10}S_2$ | C,H,N |
| 30 | $CH_2Ph$-4-OMe | $SO_2Ph$ | $SO_2Ph$ | G | 19 | | 183 | $C_{33}H_{33}N_5O_7S_2$ | C,H,N |
| 31 | $CH_2Ph$-4-OMe | $SO_2Ph$-4-OMe | $SO_2Ph$-4-OMe | G | 20[g] | | 182–3 | $C_{35}H_{37}N_5O_8S_2$ | C,H,N |
| 32 | $CH_2Ph$-4-OMe | =$CHNMe_2$ | | H | 56[h] | acetone–hexane | 142–3 | $C_{24}H_{30}N_6O_3$ | C[i],H,N |
| 33 | $CH_2Ph$-4-OMe |  | | H | 67 | | 122 | $C_{28}H_{32}N_6O_3$ | C,H,N |
| 34 | $CH_2Ph$-3,4,5-$(OMe)_3$ |  | | H | 46[j] | | 127–8 | $C_{28}H_{36}N_6O_5$ | C[k],H,N |
| 35 | H | =$CHNMe_2$ | | I | 49[l] | | 248 | $C_{16}H_{22}N_6O_2$ | C,H,N |
| 36 | H | =$CHNBu^t$ | | I | 50[l] | acetone–hexane | >265 | $C_{18}H_{26}N_6O_2$ | C,H,N |

[a] See text and Experimental Section. [b] NaH/DMSO/RT. [c] DME used as a solvent. [d] NaH used as base. [e] N found 15.28, requires 15.72. [f] Formed with 6.7% of 17 and 41% of 1. [g] 44% of 6 recovered. [h] 18% of 6 recovered. [i] C found 63.45, requires 63.98. [j] 35% of 7 recovered. [k] C found 62.18, requires 62.67. [l] From same reaction.

alkylated at N-7 usually resulted in a lower inhibitory activity (**22**, **23**, and **25**). Whether the retention of potency with **24** simply reflects the greater activity of the parent compound **6** is not known. Those compounds having bissulfonyl substitution at the 8-amino group (**26–31**) were generally of poor potency against either PDE IV or Va, although the bis(4-nitrobenzenesulfonyl) compound, **27**, suggests that with suitable substitution compounds having adequate potency as inhibitors of PDE IV may be achievable.

Of the amidino compounds prepared (**32–36**), those substituted with 4-methoxybenzyl groups at N-7 were the most interesting. Thus, while **32** and **33** were the only compounds of this type that were effective inhibitors of the PDE IV isoenzyme, compound **33** in particular was a highly potent inhibitor of PDE Va ($IC_{50}$ 0.05 $\mu$M), comparing favourably with zaprinast ($IC_{50}$ 0.9 $\mu$M), SK&F 96,231 ($IC_{50}$ 1 $\mu$M), and MY-5445 ($IC_{50}$ 0.5 $\mu$M) (Chart 1)[3] and is to our knowledge the most potent inhibitor of this isoenzyme reported so far. However, the role of PDE Va, a cyclic GMP-specific PDE, in asthma is not clear.[14] Inhibitors of PDE Va have shown bronchodilatory activity in the guinea pig, although the lack of beneficial effects on bovine airways suggests that such effects are species dependent.[21] More importantly, however, zaprinast has shown mixed efficacy in humans,[22,23] leading to the conclusion that cyclic GMP phosphodiesterases are not important in the control of airway caliber in asthma.

Since zaprinast is the only PDE Va inhibitor that has been evaluated in humans, it is possible that its poor performance may not adequately reflect the potential of PDE Va inhibitors as a class. Moreover, *in vitro* studies indicate a diverse spectrum of properties for inhibitors of this isoenzyme, ranging from smooth muscle relaxation[21] to inhibitory effects on mast cell degranulation[24] and platelet aggregation.[25] No beneficial effects have been observed on other inflammatory cells such as neutrophils[26] and eosinophils.[27] On the basis of available evidence it seems likely that smooth muscle relaxation is the most promising of the potential uses of PDE Va inhibitors, but

a full assessment of their value in humans will require the evaluation of other selective inhibitors of this class.

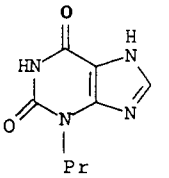
Compound **6**, one of the more potent PDE inhibitors identified from this series, showed competitive inhibition of PDE IV with respect to cAMP and also displayed competitive inhibition of PDE Va with respect to cGMP; the respective $K_i$ values being 21 and 7.5 $\mu$M (see Figure 1). These data indicate that compound **6** binds to the active, hydrolytic sites of PDE IV and Va in a manner similar to that of the natural substrate nucleotides.

**High-Affinity Rolipram Binding.** Rolipram, a selective inhibitor of PDE IV which is structurally distinct from the xanthines (Chart 1), has been shown to bind to specific high-affinity binding sites in the brain,[28] although there is no evidence to suggest that this site and the catalytic site are the same. Nevertheless, studies with human recombinant PDE IV indicate that binding and PDE IV catalytic activity are properties of the same protein.[29] Attempts to relate displacement of [$^3$H]rolipram binding with PDE IV inhibitory activity have shown a good correlation ($r = 0.84$) for a series of close analogues of rolipram,[30] although with more diverse compounds the correlation was relatively poor.[29,31,32]

While the importance of this central rolipram binding site is not known, all those compounds showing low micromolar $IC_{50}$ values against PDE IV have been routinely evaluated for their ability to displace [$^3$H]rolipram from its high-affinity binding site in order to establish whether any correlation between potency at this site and enzyme inhibitory potency within the current series existed. Although structurally very different from rolipram, BRL 61063 has high affinity ($IC_{50}$ 0.006 $\mu$M) for the rolipram binding site, but it is evident that a number of modifications leading to potent inhibitors of the PDE IV isoenzyme do not simultaneously result in high potency at displacing rolipram binding (Table 3). Particularly significant deviations arise in the case of the N-7 4-methoxybenzyl derivatives **6** and **24** which have virtually identical $IC_{50}$ values on PDE IV to that of BRL 61063 yet display greater than a 250-fold lower affinity for the rolipram binding site. In addition to the inhibition of PDE IV, both **6** and **24** are potent inhibitors of PDE Va (having $IC_{50}$ values in the same range against each isoenzyme), but it is unlikely that this factor alone is responsible for the divergence seen since other inhibitors of PDE Va such as **15** and **16** are also potent displacers of rolipram binding. Moreover, there are other PDE IV inhibitors such as **11** with potencies not too different from that of BRL 61063 that are poor inhibitors of both PDE Va and rolipram binding.

It is not clear which factors control the relative potencies of this series of compounds for affinity at the rolipram binding site, but by empirical observation it is evident that its relationship with that of PDE inhibitory activity is not irrevocably linked. These findings therefore support those of others who have observed a similar divergence in compounds unrelated structurally to rolipram[29,31,32] and argue against the notion that the PDE IV catalytic site and the rolipram binding site are the same. However, our binding and inhibition studies were carried out using different protein sources, and it has not yet been proved that rolipram will bind with high affinity to the guinea pig cardiac ventricle PDE IV used to evaluate catalytic activity. It is possible, therefore, that alternative explanations for the differences shown may exist. One such possibility is that PDE IV exists in two conformations, the catalytic site of one having a much higher affinity for rolipram than the catalytic site of the other.[29] Compounds other than rolipram might then differ in their selectivities for these conformations.

**Adenosine Antagonism.** The central stimulant and diuretic actions of adenosine antagonists such as caffeine and theophylline are familiar phenomena, but it is the more proconvulsant and cardiotonic actions of adenosine antagonists that have precluded their successful development as cognition enhancers.[33] More recently, however, selective adenosine $A_1$ receptor antagonists have been identified which appear to lack the side effect liabilities of the earlier nonselective antagonists.[34] Theophylline itself is a relatively nonselective adenosine antagonist, acting at both the $A_1$ and the $A_2$ sites,[33] but since adenosine antagonism is perceived as a disadvantage of the use of theophylline in asthma,[35] a potent inhibitor of PDE IV with a reduced affinity at adenosine receptors may offer significant advantages. Thus, enprofylline, **38**, a xanthine derivative claimed to be devoid of adenosine antagonistic activity in animal models,[35] was shown to be more potent than theophylline as a bronchodilator in man,[36,37] yet not to elicit the more severe side effects associated with theophylline.[35]



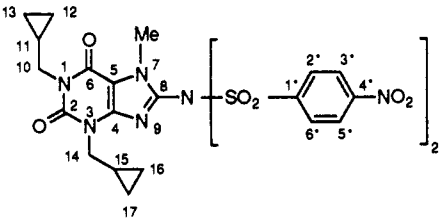

**38**

In order to evaluate compounds for their relative effects on PDEs and at adenosine receptors, we have arbitrarily chosen affinity for the $A_1$ site as generally representative of adenosine antagonism. In contrast to its weak potency as an inhibitor of PDE IV ($IC_{50}$ 179 $\mu$M),[10] theophylline has an $IC_{50}$ of 19 $\mu$M at adenosine $A_1$ receptors (Table 3). BRL 61063, which is approximately 100 times more potent as an adenosine antagonist, is also >100 times more potent than theophylline as an inhibitor of PDE IV (Table 3) and therefore has a similar selectivity for PDE inhibition. By contrast, however, most of those derivatives of BRL 61063 evaluated for $A_1$ binding affinity had a noticeably reduced ratio of PDE IV inhibition/$A_1$ binding relative to theophylline. Thus, whereas the ratio of the $IC_{50}$ values for PDE IV inhibition/adenosine binding was 9.3 for theophylline, for compounds **4**, **5**, **10**, **11**, **15**, **16**, and **24** this ratio lay in the range of 1–2.9, whereas compound **3** was marginally less selective (ratio 6.4). Only **20** and **33**, with ratios of 25 and 16, respectively, offered no improvement relative to theophylline. Insufficient data exist to allow a full SAR discussion, but these preliminary results indicate that potent xanthine-based PDE IV inhibitors can be synthesized which show a reduced relative affinity for adenosine receptors when compared with theophylline.

## Conclusions

This study on derivatives of the selective PDE IV inhibitor BRL 61063 has identified a number of other potent PDE IV inhibitors which vary in their selectivity with respect to the simultaneous inhibition of PDE Va. In addition, one compound, **33**, has been identified which is believed to be the most potent PDE Va inhibitor reported. It is also evident that some xanthines show high affinity for the rolipram binding site, although there is no obvious

Table 2. NMR Data for 29 in $CDCl_3$



29

| atom | $\delta_C$/ppm[a] | $\delta_H$/ppm[a] | COLOC correlations[b] |
|---|---|---|---|
| 2 | 151.0 | | H10, H14 |
| 4 | 146.3 | | H14 |
| 5 | 109.4 | | N7-Me |
| 6 | 155.3 | | H10 |
| 8 | 136.1 | | N7-Me |
| 10/14 | 46.0 and 48.1 | 3.92 and 3.87 | H12/H13/H16/H17 |
| 11/15 | 10.0 | 1.19–1.33 | H10/H14 |
| 12/13/16/17 | 3.8 and 3.9 | 0.36–0.54 | |
| 1′ | 143.1[c] | | H2′/H6′ |
| 2′/6′ | 130.6[c] | 8.24 | H3′/H5′ |
| 3′/5′ | 124.5[c] | 8.45 | H2′/H6′ |
| 4′ | 151.4[c] | | H3′/H5′ |
| N7-Me | 32.5 | 3.72 | |

[a] $\delta_C$ relative to $\delta_{CDCl_3}$ = 77.0 ppm; $\delta_H$ relative to $\delta_{TMS}$ = 0 ppm. [b] COLOC correlations arising from the long-range scalar coupling between carbon n and the protons tabulated. [c] Assignments based on chemical shifts calculated using reported substituent chemical shifts.[45]

link between this property and inhibitory activity at the PDE IV catalytic site in another tissue. Whether the difference in *in vitro* profile demonstated within this closely defined chemical series confer advantages over existing phosphodiesterase inhibitors remains to be proven by detailed *in vivo* pharmacological studies, but ample literature exists to suggest that improved selectivity for PDE IV *vis à vis* adenosine receptors may well lead to a reduction in adverse effects.

The lead compounds generated within this study have led to a more specific investigation of other related xanthines which show more pronounced differences to those described here and which are the subject of a subsequent publication.

## Experimental Section

Melting points were determined with a Buchi melting point apparatus and were recorded uncorrected. The structures of all compounds were consistent with their IR and $^1H$ NMR spectra, which were determined with a Perkin-Elmer 298 spectrophotometer and a Varian EM390, a JEOL GX270, or a Bruker AMX400 spectrometer, respectively. All 2D NMR experiments were conducted on a Bruker AMX400 spectrometer using standard software. The 2D $^1H$, $^{13}C$ COSY NMR spectrum[38] was aquired with $^1H$ decoupling in both dimensions and was tuned for $^1J_{CH}$ = 140 Hz with 32 scans for each of 128 × 4K FIDs. The 2D $^1H$, $^{13}C$ COLOC experiment[38,39] was tuned for $^nJ_{CH}$ = 8.5 Hz and aquired with 128 scans for each of 256 × 4K FIDs. The sweep widths for all 2D NMR experiments were optimized prior to aquisition. Mass spectra were recorded with a JEOL SX102 spectrometer using electron-impact or chemical ionization techniques. Where represented by elemental symbols, the analyses of these elements fall within ±0.4% of the calculated value. All organic extracts were dried over $MgSO_4$, and samples were chromatographed on silica in all instances.

**8-Amino-1,3-bis(cyclopropylmethyl)-7-(4-methoxybenzyl)xanthine (6). Method A.** Potassium *tert*-butoxide (1.34 g, 12 mmol) was added to a solution of 8-amino-1,3-bis(cyclopropylmethyl)xanthine (1; 2.7 g, 10 mmol) in DMF (25 mL), and the resulting mixture was stirred for 0.5 h at ambient temperature. 4-Methoxybenzyl chloride (1.56 g, 1.35 mL, 10 mmol) was added to the red solution, and after being stirred for 1 h at ambient temperature the mixture was added to ethyl acetate, washed with hydrochloric acid (2 M) and water, and dried. Removal of the solvent under reduced pressure gave a solid which was chromatographed (hexane–acetone, gradient) to give **6** (2.55 g, 64%): mp 176 °C; $^1H$ NMR ($CDCl_3$) δ 0.43–0.53 (8 H, m, cyclic $CH_2$), 1.26–1.35 (2 H, m, cyclic CH), 3.79 (3 H, s, OMe), 3.89 (4 H, t, $J$ = 7 Hz, $NCH_2$), 4.55 (2 H, br s, $NH_2$), 5.32 (2 H, s, $CH_2Ar$), 6.90 (2 H, d, $J$ = 9 Hz, C-3′, C-5′ H), 7.30 (2 H, d, $J$ = 9 H, C-2′, C-6′ H). Anal. ($C_{21}H_{25}N_5O_3$) C, H, N.

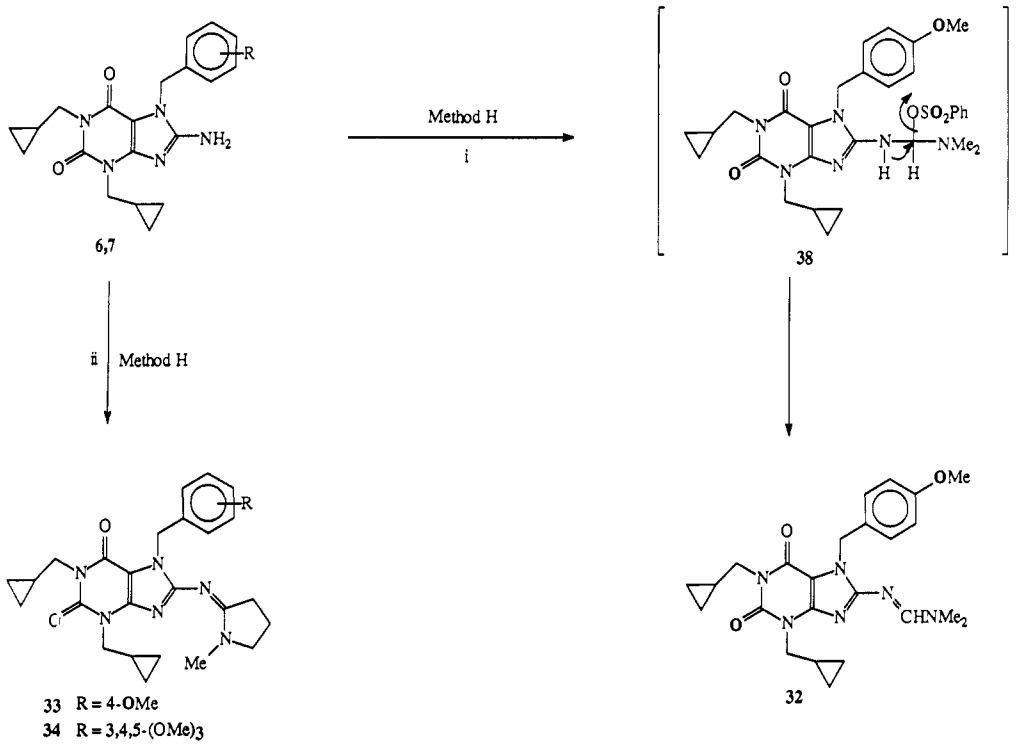
**8-Amino-7-(phenylsulfonyl)-1,3-bis(cyclopropylmethyl)xanthine (12). Method B.** Potassium *tert*-butoxide (0.12 g, 1.1 mmol) was added to a suspension of 8-amino-1,3-bis(cyclopropylmethyl)xanthine (1; 0.27 g, 1 mmol) in dimethoxyethane (4 mL), and the resulting mixture was stirred at ambient temperature for 2 h. Benzenesulfonyl chloride (0.35 g, 2 mmol) was added, and the mixture was stirred at ambient temperature for 18 h and then at 80 °C for 6 h. After cooling, the reaction mixture was added to ethyl acetate, washed with water, and dried. Removal of the solvent at reduced pressure gave a solid (0.5 g) which was chromatographed (acetone–hexane, 1:3) to give **12** (0.18 g, 43%): mp 170–171 °C; $^1H$ NMR ($CDCl_3$) δ 0.34–0.52 (8 H, m, cyclic $CH_2$), 1.16–1.31 (2 H, m, cyclic CH), 3.82 (4 H, d, $J$ = 7.2 Hz, $NCH_2$), 6.36 (2 H, br s, $NH_2$), 7.58 (2 H, t, $J$ = 8 Hz, C-3′, C-5′ H), 7.69 (1 H, t, $J$ = 7 Hz, C-4′ H), 8.21 (2 H, d, $J$ = 7 Hz, C-2′, C-6′ H). Anal. ($C_{19}H_{21}N_5O_4S$) C, H, N, S.

**1,3-Bis(cyclopropylmethyl)-8-(((4-methoxyphenyl)sulfonyl)amino)xanthine (20). Method C.** Sodium hydride (0.31 g, 2.1 equiv of a 60% dispersion in mineral oil) was added to a suspension of 8-amino-1,3-bis(cyclopropylmethyl)xanthine (1; 1.00 g, 3.64 mmol) in tetrahydrofuran (25 mL). After 30 min, 4-methoxybenzenesulfonyl chloride (0.82 g, 1.1 equiv) was added, and stirring was continued for 48 h. The reaction mixture was poured into water, acidified with hydrochloric acid (2 M), and extracted with ethyl acetate. The combined organic solutions were dried, filtered, and concentrated, and the residue was chromatographed (2–5% methanol–dichloromethane) to afford **20** (0.50 g, 31%) as a white solid: mp >240 °C; $^1H$ NMR ($CDCl_3$/DMSO-$d_6$) δ 0.21–0.43 (8 H, m, cyclic $CH_2$), 1.06–1.23 (2 H, m, cyclic CH), 3.70 (2 H, d, $J$ = 6.9 Hz, $NCH_2$), 3.74 (2 H, d, $J$ = 7.3 Hz, $NCH_2$), 3.82 (3 H, s, OMe), 7.10 (2 H, d, $J$ = 9 Hz, C-3′, C-5′ H), 7.81 (2 H, d, $J$ = 9 Hz, C-2′, C-6′ H), 11.50 (1 H, br s, C-7 H), 12.98 (1 H, br s, $NHSO_2Ar$); MS found $MH^+$ 445.1420 ($C_{20}H_{23}N_5O_5$ requires 445.1420).

**8-Amino-1,3-bis(cyclopropylmethyl)-7-((4-nitrophenyl)sulfonyl)xanthine (21) and 1,3-Bis(Cyclopropylmethyl)-8-[bis((4-nitrophenyl)sulfonyl)amino]xanthine 27. Method D.** 8-Amino-1,3-bis(cyclopropylmethyl)xanthine (1; 2.7 g, 10 mmol), 4-nitrobenzenesulfonyl chloride (5.5 g, 25 mmol), and triethylamine (3 mL, 20 mmol) were stirred in tetrahydrofuran (40 mL) at ambient temperature for 24 h. After addition of water the mixture was extracted with ethyl acetate, and the organic solution was dried and evaporated. Chromatography (hexane/acetone gradient) of the residue gave **21** (0.77 g, 17%): mp 188–9 °C; $^1H$ NMR ($CDCl_3$) δ 0.34–0.52 (8 H, m, cyclic $CH_2$), 1.15–1.25 (2 H, m, cyclic CH), 3.81 (4 H, t, $J$ = 7.5 Hz, $NCH_2$), 6.35 (2 H, br S, $NH_2$), 8.42 (4 H, ABq, $J$ = 9.6 Hz, Ar). Anal. ($C_{19}H_{20}N_6O_6S$) C, H, N, S. Continued elution provided **27** (1.5 g, 23%): mp 198 °C; $^1H$ NMR ($CDCl_3$/DMSO-$d_6$) δ 0.35–0.57 (8 H, m, cyclic $CH_2$), 1.20–1.40 (2 H, m, cyclic CH), 3.67 (2 H, d, $J$ = 7 Hz, $NCH_2$), 3.95 (2 H, d, $J$ = 7 Hz, $NCH_2$), 8.38 (8 H, AB q, $J$ = 8.5 Hz, Ar), 10.13 (1 H, br, N-7 H); $^{13}C$ NMR ($CDCl_3$, see Table 2 for numbering) δ 155.0, 151.2 (C2 or C4′), 151.1 (C2 or C4′), 146.8 (C4), 143.5 (C1′), 135.5 (C8), 130.5 (C2′ and C6′), 124.2 (C3′ and C5′), 109.3 (C5), 48.2 (C10 or C14), 45.8 (C10 or C14), 10.0 (C11 and C15), 3.7 (C12, C13, C16, and C17). Anal. ($C_{25}H_{23}N_7O_{10}S_2$), C, H, N.

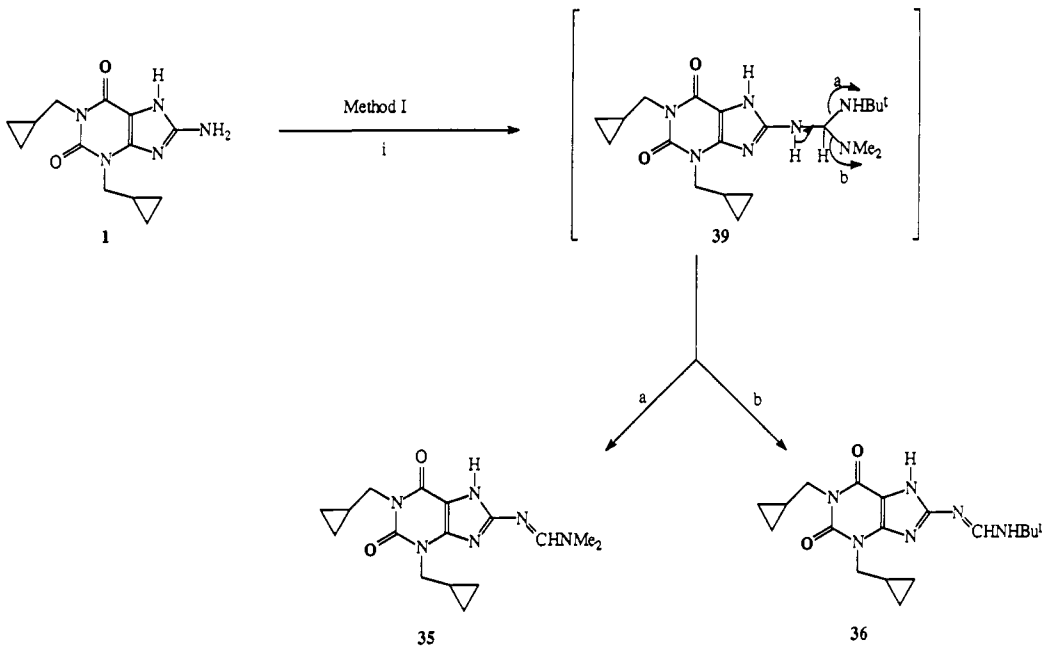
**1,3-Bis(cyclopropylmethyl)-8-bis[(phenylsulfonyl)amino]xanthine (26). Method E.** 8-Amino-1,3-bis(cyclopropylmethyl)xanthine (1; 0.27 g, 1 mmol) and benzenesulfonyl chloride (0.34 g, 2 mmol) were stirred in pyridine (4 mL) at ambient temperature overnight. The mixture was extracted with ethyl acetate, and the extracts were washed with hydrochloric acid (2 M) and water and dried. Removal of the solvent under reduced pressure gave a solid (0.48 g) which was chromatographed (acetone/hexane gradient) to give **26** (0.33 g, 59%): mp 238 °C; $^1H$ NMR ($CDCl_3$) δ 0.41–0.58 (8 H, cyclic $CH_2$), 1.17–1.43 (2 H, m, cyclic CH), 3.80 (2 H, d, $J$ = 7.15 Hz, $NCH_2$), 3.98 (2 H, d, $J$ = 7.15 Hz, $NCH_2$), 7.48–7.68 (6 H, m, C-3′, C-4′, C-5′H), 7.99 (4

**Scheme 2**[a]




[a] Reagents: (i) $PhSO_2Cl$, DMF, $KOBu^t$, 80 °C; (ii) $PhSO_2Cl$, *N*-methylpyrrolidinone, $KOBu^t$, 80 °C.

**Scheme 3**[a]




[a] Reagents: (i) $Me_2NCH{=}NBu^t$, toluene, reflux.

H, d, $J$ = 7.4 Hz, C-2′, C-6′H), 13.25 (1 H, br, N-H). Anal. ($C_{25}H_{25}N_5O_6S_2$) C, H, N.

**1,3-Bis(cyclopropylmethyl)-8-[bis((4-nitrophenyl)sulfonyl)amino]-7-methylxanthine 29. Method F.** A mixture of 1,3-bis(cyclopropylmethyl)-8-[bis((4-nitrophenyl)sulfonyl)amino]xanthine (**27**; 0.5 g, 0.77 mmol), potassium carbonate (0.21 g, 1.52 mmol), and dimethyl sulfate (0.07 mL, 0.74 mmol) was stirred in acetone (10 mL) for 16 h. The reaction mixture was poured into water and extracted with ethyl acetate. Concentration of the dried organic extracts gave a residue which was chromatographed (ethyl acetate–hexane, 1:1) to give **29** (0.342 g, 67%) as an off-white solid: mp 181–183 °C (ethyl acetate–hexane); $^1H$ NMR ($CDCl_3$) $\delta$ 0.36–0.54 (8 H, m, cyclic $CH_2$), 1.19–1.33 (2 H, m, cyclic CH), 3.72 (3 H, s, Me), 3.87 (2 H, d, $J$ = 7.1 Hz, $NCH_2$), 3.92 (2 H, d, $J$ = 7.1 Hz, $NCH_2$), 8.24 (4 H, d, $J$ = 9 Hz), 8.45 (4 H, d, $J$ = 9 Hz). For $^{13}C$ data see Table 2. Anal. ($C_{26}H_{25}N_7O_{10}S_2$) C, H, N.

**1,3-Bis(cyclopropylmethyl)-8-[bis((4-methoxyphenyl)sulfonyl)amino]-7-(4-methoxybenzyl)xanthine (31). Method G.** Potassium *tert*-butoxide (0.34 g, 3 mmol) was added to a solution of 8-amino-1,3-bis(cyclopropylmethyl)-7-(4-methoxybenzyl)xanthine (**6**; 0.99 g, 2.5 mmol) in dimethoxyethane (10 mL), and the resulting solution was stirred for 3 h at ambient temperature. A solution of 4-methoxybenzenesulfonyl chloride (0.77 g, 3.75 mmol) in dimethoxyethane (3 mL) was slowly added over 5 min, and the reaction mixture was stirred for a further 48 h at ambient temperature. The mixture was extracted with ethyl acetate, and the extracts were washed with water and dried. Removal of the solvent at reduced pressure gave a solid which

**Table 3.** *In Vitro* Profile of 1,3-Bis(cyclopropylmethyl)xanthine Derivatives

| no. | PDE inhibition, $IC_{50}$ ($\mu$M) or % inhibition at 100 $\mu$M | | | | | | rolipram binding $IC_{50}$ ($\mu$M) | adenosine $A_1$ binding $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|---|---|---|
| | 1b | 1c | II | III | IV | Va | | |
| 1 | 5% | 21% | 60% | 17% | 1.9 ± 0.4 | 71 ± 15 | 0.006 | 0.17 |
| 2 | 30% | 14% | 66% | 19% | 17 ± 3 | 20 ± 4 | 0.81 | |
| 3 | 18% | 51% | 46% | 21% | 14 ± 1 | 14 ± 2 | 1.3 | 2.2 |
| 4 | 12% | 45% | 39% | 14% | 10 ± 2 | 11 ± 2 | 6.3 | 3.5 |
| 5 | 27% | 30% | 66% | 24% | 7.1 ± 0.76 | 0.84 ± 0.17 | 0.92 | 2.8 |
| 6 | 60% | 42% | 78% | 30% | 3.3 ± 0.66 | 0.14 ± 0.02 | 1.7 | |
| 7 | 46% | 45% | 41% | 12% | 18 ± 3 | 13 ± 3 | >10 | |
| 8 | 30% | 55% | 32 | 9% | 14 ± 3 | 8.3 ± 1.8 | | |
| 9 | 0% | 40% | 32% | 3 | 24 ± 8 | 9.8 ± 2.6 | >10 | |
| 10 | | 44% | 31% | 12% | 8.1 ± 1.5 | 32 ± 4.0 | 0.19 | 2.9 |
| 11 | | 37% | 7% | 10% | 6.9 ± 1.2 | 38 ± 10.0 | 1.85 | 2.7 |
| 12 | 13% | 27% | 51% | 11% | 3.3 ± 0.7 | 3.6 ± 0.8 | 0.092 | |
| 13 | | 14% | 0% | 6% | 45 ± 28 | 37 ± 15 | 0.028 | |
| 14 | 0% | 41% | 19% | 12% | 54% | 49% | | |
| 15 | 1% | 71% | 12% | 21% | 3.7 ± 1.1 | 0.9 ± 0.1 | 0.054 | 4.1 |
| 16 | 9% | 70% | 9% | 16% | 7.1 ± 3.1 | 1.2 ± 0.4 | 0.086 | 3.8 |
| 17 | 25% | 18% | 31% | 12% | 3.0 ± 0.65 | 0.44 ± 0.07 | 0.3 | |
| 18 | 31% | 57% | 30% | 22% | 24 ± 6 | 40 ± 6 | 2.1 | |
| 19 | 56% | 65% | 52% | 38% | 3.7 ± 0.3 | 0.30 ± 0.3 | 0.16 | |
| 20 | | 30 | 43 | 31% | 37 ± 10 | 68 ± 10 | | 1.5 |
| 21 | 14% | 12% | 25% | 0% | 9.7 ± 2.5 | 38% | 0.016 | |
| 22 | 47% | 44% | 43% | 25% | 52 ± 8 | 53% | | |
| 23 | 32% | 44% | 52% | 44% | 44 ± 7 | 54% | | |
| 24 | 48% | 58 ± 17 | 63% | 43% | 1.5 ± 0.3 | 2.2 ± 0.1 | 5.1 | 1 |
| 25 | 52% | 50% | 58% | 10% | 56% | 37 ± 5 | | |
| 26 | 43% | 50% | 27% | 17% | 23% | 20% | | |
| 27 | 30 ± 8 | 15% | 26 | 50% | 5.4 ± 0.5 | 24 ± 3 | 0.24 | 2.4 |
| 28 | 20% | 20% | 2% | 7% | 42 ± 12 | 9% | | |
| 29 | 23% | 50% | 5.4 ± 2.0 | 21% | 20 ± 3 | 34 ± 6 | | |
| 30 | 5% | 23% | 13% | 0% | 42% | 6.5 ± 1.2 | | |
| 31 | 14% | 0% | 4% | 2% | 48% | 33% | | |
| 32 | 29% | 59% | 47% | 46% | 6.5 ± 2.0 | 0.24 ± 0.03 | >10 | |
| 33 | 9% | 28% | 25% | 10% | 9.5 ± 3.4 | 0.05 ± 0.01 | | 0.61 |
| 34 | 48 | 15 | 103 | | 44 | 3 | >10 | |
| 35 | 7% | 6% | 31% | 11% | 41 ± 13 | 56 ± 7 | | |
| 36 | 11% | 9% | 0% | 14% | 37% | 22% | | |
| theophylline | 46% | 13% | 0% | 30% | 26% | 13% | | 19 |
| (±)-rolipram | 20% | 1% | 8% | 3% | 0.6 ± 0.1 | 14% | 0.007 | >10 |
| zaprinast | 22% | 13 ± 3 | 20% | 14% | 53 ± 7 | 0.9 ± 0.2 | >10 | |

was chromatographed (acetone–hexane, gradient) to give **31** (0.36 g, 20%): mp 182–3 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.35–0.51 (8 H, m, cyclic $CH_2$), 1.25–1.28 (2 H, m, cyclic CH), 3.78 (3 H, s, N-7 ArOMe), 3.88 (6 H, s, N-8 ArOMe), 3.88 (4 H, t, $J$ = 7.0 Hz, $NCH_2$), 5.50 (2 H, s, $ArCH_2$), 6.82 (4 H, d, $J$ = 9.0 Hz, N-8 Ar C-3′, C-5′ H), 6.88 (2 H, d, $J$ = 9.0 Hz, N-7 Ar C-3′, C-5′ H), 7.27 (2 H, d, $J$ = 9.0 Hz, N-7 Ar C-2′, C-6′H), 7.69 (4 H, d, $J$ = 9.0 Hz, N-8 Ar C-2′, C-6′ H). Anal. ($C_{35}H_{37}N_5O_9S_2$) C, H, N, S. Continued elution gave unchanged **6** (0.43 g, 44%).

**1,3-Bis(cyclopropylmethyl)-7-(4-methoxybenzyl)-8-[*N*-(1-methyl-2-propylidene)amino]xanthine (33). Method H.** Potassium *tert*-butoxide (0.34 g, 3 mmol) was added to a solution of 8-amino-1,3-bis(cyclopropylmethyl)-7-(4-methoxybenzyl)xanthine (**6**; 0.79 g, 2 mmol) in *N*-methylpyrrolidinone (4 mL) at 80 °C. After 2 h benzenesulfonyl chloride (0.53 g, 3 mmol) was added and stirring continued for 2 h. After cooling, the mixture was added to ethyl acetate and the organic solution was washed with water and dried. Removal of the solvent under reduced pressure gave a residue (1.39 g) which was chromatographed (hexane–acetone gradient) to give **33** (0.64 g, 67%): mp 122 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.43–0.47 (8 H, m, cyclic $CH_2$), 1.27–1.34 (2 H, m, cyclic CH), 2.09 (2 H, m, $NCH_2CH_2CH_2$), 3.06 (2 H, t, $J$ = 8 Hz, $NCH_2CH_2CH_2$), 3.11 (3H, s, NMe), 3.49 (2H, t, $J$ = 7.2 Hz, $NCH_2CH_2CH_2$), 3.76 (3H, s, OMe), 3.90 (2 H, d, $J$ = 7.2 Hz, $NCH_2$), 3.91 (2 H, d, $J$ = 7.2 Hz, $NCH_2$), 5.39 (2 H, s, $NCH_2Ar$), 6.82 (2 H, d, $J$ = 8.8 Hz, C-3′, C-5′H), 7.50 (2 H, d, $J$ = 8.8 Hz, C-2′, C-6′H). Anal. ($C_{28}H_{32}N_6O_3$) C, H, N.

**1,3-Bis(cyclopropylmethyl)-8-(((dimethylamino)methylene)amino)xanthine (35) and 1,3-Bis(cyclopropylmethyl)-8-(((*tert*-butylamino)methylene)amino)xanthine (36). Method I.** 8-Amino-1,3-bis(cyclopropylmethyl)xanthine (**1**; 0.27 g, 1 mmol), and *N′-tert*-butyl-*N*,*N*-dimethylformamidine (0.39 g, 3 mmol) were stirred in toluene (5 mL) at reflux for 18 h. After cooling, **35** (0.16 g, 49%), mp 248 °C, was collected by filtration: $^1$H NMR ($CDCl_3$) $\delta$ 0.43–0.50 (8 H, m, cyclic $CH_2$), 1.31–1.44 (2 H, m, cyclic CH), 3.13 (3 H, s, Me), 3.19 (3 H, s, Me), 3.95 (4 H, t, $J$ = 6.6 Hz, $NCH_2$), 8.52 (1 H, s, N=CH), 11.47 (1 H, br s, N-7 H). Anal. ($C_{16}H_{22}N_6O_2$) C, H, N. The filtrate was evaporated and the residue was chromatographed (hexane–acetone gradient) to give **36** (0.18 g, 50%): mp >265 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.43–0.52 (8 H, m, cyclic $CH_2$), 1.25–1.43 (2 H, m, cyclic CH), 1.47 (9 H, s, $Bu^t$), 3.94 (2 H, d, $J$ = 7.2 Hz, $NCH_2$), 4.00 (2 H, d, $J$ = 7.2 Hz, $NCH_2$), 8.77 (1 H, d, $J$ = 13.7 Hz, N=CH), 9.72 (1 H, d, $J$ = 13.5 Hz, $Bu^tNH$), 14.35 (1 H, br s, N-7 H). Anal. ($C_{18}H_{26}N_6O_2$) C, H, N.

**Phosphodiesterase Inhibition: Isolation of Phosphodiesterases.** The $Ca^{2+}$/calmodulin-stimulated PDE (PDE I, see ref 13 for nomenclature) was prepared from bovine cardiac ventricle. Following chromatography on a Mono Q column, the fractions showing stimulation of PDE activity by $Ca^{2+}$ and calmodulin were pooled and further purified on a calmodulin-affinity column. cGMP-stimulated PDE (PDE II) was prepared from bovine heart by use of a cGMP affinity column.[40] cGMP-inhibited PDE (PDE III) was also isolated from bovine heart by the method of Harrison et al.[41] cAMP-specific PDE (PDE IV) was obtained from a bovine lung supernatant fraction by chromatography on DEAE cellulose. Fractions with rolipram-inhibited cAMP-PDE activity were pooled and further purified by chromatography on a Mono Q column. cGMP-selective PDE (PDE Va) was obtained from a porcine lung supernatant fraction using chromatography on DEAE-cellulose. Fractions exhibiting zaprinast-inhibitable cGMP-PDE activity were pooled and rechromatographed on a Mono Q column. Finally a calmodulin-affinity column was used to remove residual PDE I activity and the flow-through fraction was used as PDE Va.

**Characteristics of Phosphodiesterase Isoenzymes.** With the exception of PDE II, which displayed positive cooperativity, all the preparations showed simple Michaelis-Menton kinetics. The activity of the preparations responded in a predictable manner to physiological modulators (e.g., only PDE I was

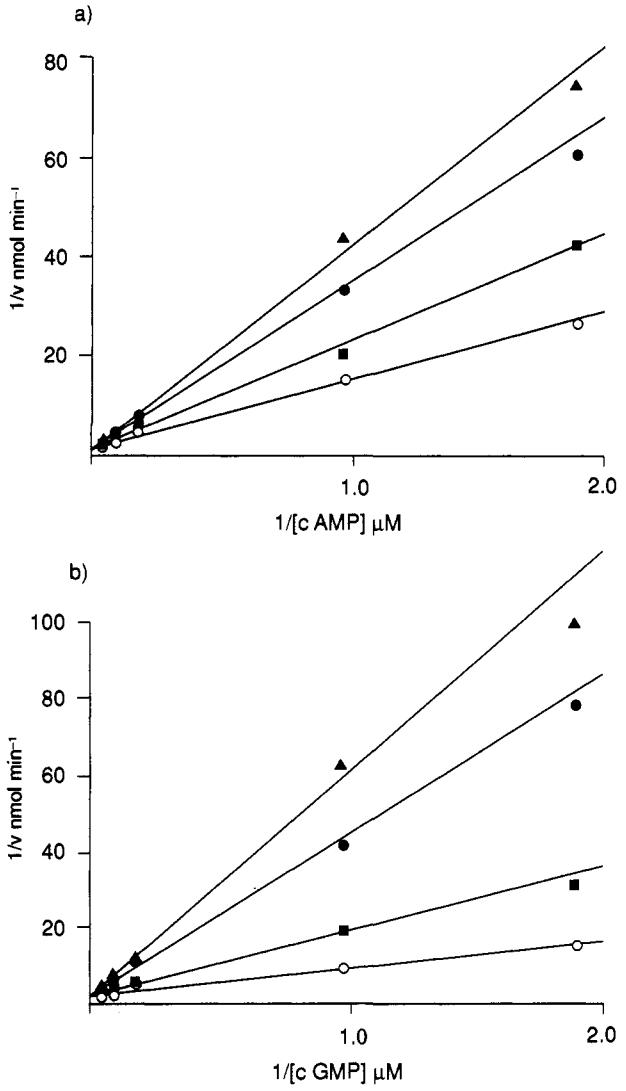



**Figure 1.** Lineweaver–Burke plot for compound 6 against (a) PDE IV; (▲) 10 μM, (●) 5 μM, (■)2.5 μM; (○) control and (b) PDE Va; (▲) 1 μM, (●) 0.5 μM, (■) 0.1 μM; (○) control.

stimulated by $Ca^{2+}$/calmodulin) and to pharmacological agents (e.g., known selective inhibitors). PDE I: the activity of only this isoenzyme was stimulated by the $Ca^{2+}$/calmodulin complex. The isoenzyme could hydrolyse both cAMP and cGMP, although the latter was the preferred substrate ($K_m$: cAMP = 36 μM, cGMP = 5 μM; $V_{max}$ cAMP/$V_{max}$ cGMP = 5). PDE II: the activity of this isoenzyme with cAMP as a substrate was stimulated by cGMP. The isoenzyme could hydrolyze both cAMP and cGMP, the latter being the preferred substrate under basal conditions ($K_m$: cAMP = 45 μM, cGMP = 14 μM; $V_{max}$ cAMP/$V_{max}$ cGMP = 1). PDE III: the activity of this isoenzyme with cAMP as a substrate was inhibited by cGMP. The isoenzyme could hydrolyze both cAMP and cGMP; the former was the preferred substrate ($K_m$: cAMP = 0.5 μM, cGMP = 0.1 μM; $V_{max}$ cAMP/$V_{max}$ cGMP = 5). PDE IV: this isoenzyme had high affinity for cAMP, the hydrolysis of which was not inhibited by cGMP ($K_m$: cAMP = 2 μM, cGMP > 100 μM; $V_{max}$ cAMP/$V_{max}$ cGMP not determined). PDE Va: this isoenzyme had high affinity for cGMP ($K_m$: cAMP > 100 μM, cGMP = 1 μM; $V_{max}$ cAMP/$V_{max}$ cGMP not determined).

**Assay of Phosphodiesterase Activity.** PDE activity was assayed by the boronate column method as previously described.[42] The enzymes were incubated at 37 °C for 5 min in 50 mM Tris, 5 mM $MgCl_2$, pH 7.5 with $^3$H-labeled cyclic nucleotide ($4 \times 10^5$ disintegrations $min^{-1}$) and $^{14}$C-labeled nucleotide 5′-monophosphate ($3 \times 10^3$ disintegrations $min^{-1}$). The assay was stopped by boiling and the $^3$H-labeled 5′-monophosphate product was separated from substrate on boronate columns. The reaction mixture was diluted with 0.5 mL of 100 mM HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid], 100 mM NaCl, pH 8.5, and applied to the column. The column was extensively washed with the same buffer, and the 5′-nucleotide, was eluted with 6 mL of 0.25 M acetic acid. The recovery of product, as judged by $^{14}$C-recovery, was approximately 80%. All assays were linear with time of incubation and concentration of enzyme over the range used in these experiments. $IC_{50}$ values (the concentration of inhibitor required for 50% inhibition of activity) were obtained by incubation of the isoenzyme with 1 μM cGMP as a substrate for PDE I (in the absence of $Ca^{2+}$ and calmodulin), PDE II, and PDE Va and with 1 μM cAMP as a substrate for PDE III and PDE IV and various concentrations of the inhibitor; curves were fitted to the logistic equation by the program ALLFIT.[43]

**High-Affinity Rolipram Binding.** Rat forebrain was homogenized in buffer (50 mM Tris-HCl, pH 7.5; $MgCl_2$ [5 mM]; benzamidine [2 mM]; bacitracin [100 μg $mL^{-1}$]; trypsin inhibitor [20 μg $mL^{-1}$]; *N*-α-tosyl-L-lysyl chloromethyl ketone [100 μg $mL^{-1}$]; PMSF [50 μM]; leupeptin [100 nM]) and sedimented at 3000*g* for 10 min at 4 °C. The supernatant and pellet were decanted into separate clean tubes, and both were resedimented at 100000*g* for 60 min at 4 °C. The supernatants from this were combined and again centrifuged at 100000*g* for 20 min. The supernatant from this final step was stored in aliquots at −80 °C until required for binding assay.

Binding was performed at 30 °C in 50 mM Tris-HCl, pH 7.5 containing $MgCl_2$ [5 mM] and 5′-AMP [50 μM]. To tubes containing the desired concentration of drug or vehicle in buffer was added 1 pmol of [$^3$H]rolipram (25.6 Ci $mmol^{-1}$). Each concentration was tested in duplicate. The reaction was started by addition of sufficient rat forebrain cytosol to bind 10% of the [$^3$H]rolipram (determined by a preliminary experiment). The total assay volume was 0.5 mL. Following a 1-h incubation with shaking at 30 °C, the tubes were placed in an ice bath. Bound [$^3$H]rolipram was separated from free material by filtration through Whatman GF/B filters which had been presoaked in 0.3% (v/v) polyethylenimine, and the filters were washed rapidly four times with ice-cold buffer. Bound [$^3$H]rolipram was measured by counting the filters in a liquid scintillation counter, and the $IC_{50}$ value was determined from a $B/B_o$ plot using the vehicle control as $B_o$. Nonspecific binding was determined in the presence of rolipram and was subtracted from all other bound counts. The $K_D$ for rolipram binding was 1.5 nM.

**Adenosine ($A_1$) Antagonism.** The method used was based on that of Bruns *et al.*[44] Whole brain (without cerebellum or brainstem) from male Sprague–Dawley rats was homogenized in 10 volumes of 50 mM Tris-HCl, pH 7.7. The homogenate was sedimented at 50000*g* for 10 min at 4 °C, and the supernatant was discarded. The pellet was resuspended, with homogenization, in 10 volumes of buffer, and the process was repeated twice more. The pellet from the third sedimentation step was again resuspended in 10 volumes of Tris-HCl buffer at pH 7.7 and incubated with 2 units $mL^{-1}$ of adenosine deaminase at 37 °C for 20 min. Following this incubation, the membrane preparation was again sedimented at 50000*g* for 10 min at 10 °C. The pellet was then suspended at 1 g 5 $mL^{-1}$ in 50 mM Tris-HCl at pH 7.7 and stored in aliquots at −80 °C until required.

Binding was performed in duplicate for 60 min at 25 °C in 1 mL of 50 mM Tris-HCl at pH 7.7 containing 0.1 unit $mL^{-1}$ adenosine deaminase. To each tube was added 0.3 nM [$^3$H]PD 116,948 [120 Ci $mmol^{-1}$] and the desired concentration of drug or vehicle. The reaction was started by the addition of 2.5-mg original wet weight of membranes. Incubations were terminated by filtration through Whatman GF/B filters followed by rapid washing of the filters three times with ice-cold buffer. Filters were counted by liquid scintillation spectrometry. Nonspecific binding was defined as that in the presence of 100 μM $N^6$-cyclopentyladenosine, and this was subtracted from the total binding to give specific binding. $IC_{50}$ values were extrapolated from $B/B_o$ plots using the vehicle control binding as $B_o$. The $K_D$ for [$^3$H]PD 116,948 was 0.4 nM.

**Acknowledgment.** The authors acknowledge the helpful discussions with Dr. T. Torphy and his assistance with the establishment of the rolipram binding assay.

## References


(1) Persson, C. G. A. Overview of effects of theophylline. *J. Allergy Clin. Immunol.* **1986**, *78*, 780–787.
(2) Persson, C. G. A.; Erjefalt, I.; Gustafsson, B. Xanthines - symptommatic or prophylactic in asthma? *Agents Actions (Suppl)*, **1988**, *23*, S137–155.
(3) Torphy, T. J.; Undem, B. J. Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. *Thorax* **1991**, *46*, 512–523.
(4) Polson, J. B.; Krzanowski, J. J.; Goldman, A. L.; Szentivanyi, A. Inhibition of human pulmonary phosphodiesterase activity by therapeutic levels of theophylline. *Clin. Exp. Pharmacol. Physiol.* **1978**, *8*, 535–539.
(5) Kuehl, F. A.; Zaneti, M. E.; Soderman, D. D. Miller, D. K.; Ham, E. A. Cyclic AMP-dependent regulation of lipid mediators in white cells. A unifying concept for explaining the efficacy of theophylline in asthma. *Am. Rev. Resp. Dis.* **1987**, *136*, 210–213.
(6) Torphy, T. J.; Livi, G. P.; Balcarek, J. M.; White, J. R.; Chilton, F. H.; Undem, B. J. Therapeutic potential of isozyme-selective phosphodiesterase inhibitors in the treatment of asthma. *Adv. Sec. Mess. Phosphoprotein Res.* **1992**, *25*, 289–305.
(7) Hendeles, L.; Weinberger, M. Avoidance of adverse effects during chronic therapy with theophylline. *Eur. J. Resp. Dis. Suppl. 109*, **1980**, *61*, 103–119.
(8) Tsiu, S. J.; Self, T. H.; Burns, R.; Theophylline toxicity: update. *Ann. Allergy* **1990**, *64* (part 2), 241–257.
(9) Church, M. K.; Featherstone, R. L.; Cushley, M. J.; Mann, J. S.; Holgate, S. T. Relationships between adenosine, cyclic nucleotides, and xanthines in asthma. *J. Allergy Clin. Immunol.* **1986**, *78*, 670–675.
(10) Nicholson, C. D.; Jackman, S. A.; Wilke, R. The ability of denbufylline to inhibit cyclic nucleotide phosphodiesterase and its affinity for adenosine receptors and the adenosine re-uptake site. *Br. J. Pharmacol.* **1989**, *97*, 889–897.
(11) Howell, R. E.; Museham, W. T.; Kinnier, W. J. Mechanism for the emetic side effect of xanthine bronchodilators. *Life Sci.* **1990**, *46*, 563–568.
(12) Beavo, J. A. Multiple isozymes of cyclic nucleotide phosphodiesterase. *Adv. Sec. Mess. Phosphoprotein Res.* **1988**, *22*, 1–38.
(13) Beavo, J. A.; Reifsnyder, D. H. Primary sequence of cyclic nucleotide phosphodiesterease isozymes and the design of selective inhibitors. *Trends Pharmacol. Sci.* **1990**, *11*, 150–155.
(14) Murray, K. J.; England, P. J.; Inhibitors of cyclic nucleotide phosphodiesterases as therapeutic agents. *Biochem. Soc. Trans.* **1992**, *20*, 460–464.
(15) Weishaar, R. E.; Cain, M. H.; Bristol, J. A. A new generation of phosphodiesterase inhibitors: multiple molecular forms of phosphodiesterase and the potential for drug selectivity. *J. Med. Chem* **1985**, *28*, 537–545.
(16) Giembycz, M. A. Could isozyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of asthma? *Biochem. Pharmacol.* **1992**, *43*, 2041–2051.
(17) Dent, G.; Rabe, K.; Giembycz, M. A.; Barnes, P. J. Inhibition of eosinophil respiratory burst activity by type IV-, but not type III-selective phosphodiesterase inhibitors. *Am. Rev. Resp. Dis.* **1990**, *141*, A878.
(18) de Boer, J.; Philpott, A. J.; van Amsterdam, R. G. M.; Shahid, M.; Zaagsma, J.; Nicholson, C. D. Human bronchial cyclic nucleotide phosphodiesterase isoenzymes: biochemical and pharmacological analysis using selective inhibitors. *Br. J. Pharmacol.* **1992**, *106*, 1028–1034. Torphy, T. J.; Undem, B. J.; Cieslinski, L. B.; Luttmann, M. A.; Reeves, M. L.; Hay, D. W. P. Identification, characterization and functional role of phosphodiesterase isozymes in human airway smooth muscle. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 1213–1223.
(19) Beecham Wulfing Patent; EP 0,389,282, 1990.
(20) Pouchert, C. J.; Behnke, J. *The Aldrich Library of $^{13}C$ and $^{1}H$ FT NMR spectra*; Aldrich Chemical Company: Milwaukee, WI, 1993; Vol. III, p 214.
(21) Murray, K. J. Phosphodiesterase Va inhibitors. *Drug News Perspect.* **1993**, *6*, 150–156.
(22) Rudd, R. M.; Gellert, A. R.; Studdy, P. R.; Geddes, D. M. Inhibition of exercise-induced asthma by an orally absorbed mast cell stabilizer (M&B 22,948). *Br. J. Dis. Chest.* **1983**, *77*, 78–86.
(23) Reiser, J.; Yeang, Y.; Warner, J. O. The effect of zaprinast (M&B 22,948, an orally absorbed mast cell stabilizer) on exercise-induced asthma in children. *Br. J. Dis. Chest.* **1986**, *80*, 157–163.
(24) Frossard, N.; Landry, Y.; Pauli, G.; Ruckstuhl, M. Effects of cyclic AMP- and cyclic GMP-phosphodiesterase inhibitors on immunological release of histamine and on lung contraction. *Br. J. Pharmacol.* **1981**, *73*, 933–938.
(25) Hagiwara, M.; Endo, T.; Kanayama, T.; Hidaka, H. Effect of 1-(3-chloroanilino)-4-phenylphthalazine (MY-5445), a specific inhibitor of cyclic GMP phosphodiesterase, on human platelet aggregation. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 467–471.
(26) Wright, C. D.; Kuipers, P. J.; Kobylarz-Singer, D.; Devall, L. J.; Klinkefus, B. A.; Weishaar, R. E. Differential inhibition of human neutrophil functions. Role of cyclic AMP-specific, cyclic GMP-sensitive phosphodiesterase. *Biochem. Pharmacol.* **1990**, *40*, 699–707.
(27) Souness, J. E.; Carter, C. M.; Diocee, B. K.; Hassal, G. A.; Wood, L. J.; Turner, N. C. Characterization of guinea-pig eosinophil phosphodiesterase activity. Assessment of its involvement in regulating superoxide generation. *Biochem. Pharmacol.* **1991**, *42*, 937–945.
(28) Schneider, H. H.; Schmiechen, R.; Brezinski, M.; Siedler, J. Stereospecific binding of the antidepressant rolipram to brain protein structures. *Eur. J. Pharmacol.* **1986**, *127*, 105–115.
(29) Torphy, T. J.; Stadel, J. M.; Burman, M.; Cieslinski, L. B.; McLaughlin, M. M.; White, J. R.; Livi, G. P. Coexpression of human cAMP-specific phosphodiesterase activity and high affinity rolipram binding in yeast. *J. Biol. Chem.* **1992**, *267*, 1798–1804.
(30) Saccomano, N. A.; Vinick, F. J.; Koe, B. K.; Nielsen, J. A.; Whalen, W. M.; Meltz, M.; Phillips, D.; Thadieo, P. F.; Jung, S.; Chapin, D. S.; Lebel, L. A.; Russo, L. L.; Helweg, D. A.; Johnson, J. L.; Ives, J. L.; Williams, I. H. Calcium-independent phosphodiesterase inhibitors as putative antidepressants: [3-(Bicycloalkyloxy)-4-Methoxyphenyl]-2-imidazolidinones. *J. Med. Chem.* **1991**, *34*, 291–298.
(31) Koe, B. K.; Lebel, L. A.; Nielsen, J. A.; Russo, L. L.; Saccomano, N. A.; Vinick, F. J.; Williams, I. H. Effects of novel catechol ether imidazolidinones on calcium-independent phosphodiesterase activity (H-3) rolipram binding, and reserpine-induced hypothermia in mice. *Drug. Develop. Res.* **1990**, *21*, 135–142.
(32) Lowe, J. A., III; Archer, R. L.; Chapin, D. S.; Cheng, J. B.; Helweg, D.; Johnson, J. L.; Koe, B. K.; Lebel, L. A.; Moore, P. F.; Nielsen, J. A.; Russo, L. L.; Shirley, J. T. Structure-activity relationships of quinazolinedione inhibitors of calcium-independent phosphodiesterase. *J. Med. Chem.* **1991**, *34*, 624–628.
(33) Jacobson, K. A. van Galen, P. J. M.; Williams, M. Adenosine receptors: pharmacology, structure-activity relationships, and therapeutic potential. *J. Med. Chem.* **1992**, *35*, 407–422.
(34) Schingnitz, G.; Küfner-Mühl, U.; Ensinger, H.; Lehr, E.; Kuhn, F. J. Selective $A_1$-antagonists for treatment of cognitive deficits. *Nucleosides Nucleotides* **1991**, *10*, 1067–1076.
(35) Persson, C. G. A.; Erjefalt, I.; Karlsson, J.-A. Adenosine antagonism, a less desirable characteristic of xanthine asthma drugs? *Acta Pharmacol. Toxicol.* **1981**, *49*, 317–320.
(36) Lunell, E.; Svedmyr, N.; Andersson, K.-P.; Persson, C. G. A. A novel bronchodilator xanthine apparantly without adenosine receptor antagonism and tremorogenic effect. *Eur. J. Resp. Dis.* **1984**, *64*, 333–339.
(37) Chapman, B. J.; McDonald, C.; Capewell, S.; Frame, M. H.; Crompton, G. K. Enprofylline in chronic asthma. *Br. J. Dis. Chest.* **1988**, *82*, 354–359.
(38) Derome, A. *Modern NMR Techniques for Chemical Research*; Pergammon Press: Oxford, 1987.
(39) Kessler, H.; Griesinger, C.; Lautz, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 444–445.
(40) Martins, T. J.; Mumby, M. C.; Beavo, J. A. Purification and characterization of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine tissues. *J. Biol. Chem.* **1982**, *257*, 1973–1979.
(41) Harrison, S. A.; Beier, N.; Martins, T. J.; Beavo, J. A. Isolation and comparison of bovine heart cGMP-inhibited and cGMP-stimulated phosphodiesterases. In *Methods in Enzymology*; Initiation and Termination of cyclic nucleotide action; Corbin, J. D., Johnson, R. A., Eds. Academic Press: London, 1988; Vol. 159, pp 685–702.
(42) Reeves, M. L.; Leigh, B. K.; England, P. J. The identification of a new cyclic nucleotide phosphodiesterase activity in human and guinea-pig cardiac ventricle. *Biochem. J.* **1987**, *241*, 535–541.
(43) De Lean, A.; Munson, T. J.; Rodbard, D. Simultaneous analysis of families of sigmoid curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.* **1978**, *253*, E97-E102.
(44) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the $A_1$-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59–63.
(45) Ewing, D. F. $^{13}C$ Substituent effects in monosubstituted benzenes. *Org. Magn. Reson.* **1979**, *12*, 499–524.