8-Substituted Analogues of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-isopropyl-adenine: Highly Potent and Selective PDE4 Inhibitors

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3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-isopropyl-adenine V11294 (**1**) has been identified as a lead structure, which selectively inhibits human lung PDE4 (436 nM) and is also active in a number of in vitro and in vivo models of inflammation. Here we describe the synthesis and pharmacology of a series of highly potent 8-[(benzyloxy)methyl]-substituted analogues, with potencies in the range 10−300 nM. In addition, several compounds showed interesting PDE4 subtype specificities, for example, the 3-thienyl derivative **5v**, which showed 6−10 nM potency at PDE4B, D3, and D5 and a 20- to 200-fold selectivity over A and D2, respectively.

Introduction

Asthma is a chronic inflammatory disease characterized by reversible narrowing and inflammation of the airways, accompanied by hyper-reactivity of the respiratory tract to external stimuli. The development of highly effective drugs is urgently needed, because the mortality and morbidity due to asthma are increasing despite extensive treatment.[1−3]

The central role of cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), in regulating the function of airway smooth muscle, inflammatory cells, and immune cells is well established.[4−8] Cyclic nucleotide phosphodiesterases (PDEs) play a key role in the metabolism of cAMP and cGMP. PDEs have been characterized into 11 distinct families of isozymes that differ in their selectivity and specificity for the hydrolysis of cAMP and cGMP to their inactive 5′-nucleotide products.[9,10] The PDE4 subtype consisting of four gene products (PDE4A to PDE4D) is characterized by selective high-affinity hydrolysis of cAMP. It is predominantly found within airway smooth muscle and proinflammatory cells, making it an attractive target for novel antiasthma and antiinflammatory therapy.[11−14] Inhibition of this enzyme results in increased cellular levels of cAMP, which contribute to both the relaxation of airway smooth muscle and the prevention of proinflammatory cell activation and recruitment to the lungs and airways.

The prototype PDE4 inhibitor is rolipram, originally developed as an antidepressant.[15] This compound is however highly emetic and therefore precluded from use as a human therapeutic. First generation derivatives also suffered from this side effect and in general had low oral bioavailability. A new generation of highly potent and orally available PDE4 inhibitors are looking more promising in late stage clinical trials for both asthma and chronic obstructive pulmonary disease and are expected to reach the market in 2005. The most advanced of these are GlaxoSmithKline's cilomilast (Ariflo)[16] and Byk Gulden's roflumilast,[17] currently in phase 2/3 trials for asthma and chronic obstructive pulmonary disease (COPD). Other PDE4 compounds currently undergoing phase 2 trials include Celltech/Schering-Plough's D4396[18] and Almirall's arofylline.[19]

1 has been identified as a lead structure, which selectively inhibits human lung hPDE4 (K_i = 436 nM) and is also active in a number of in vitro and in vivo models of inflammation.[20−22] In addition, it is orally active at doses that are not emetogenic,[22] a side effect that has proved dose limiting for some earlier PDE4 inhibitors. Considerable SAR studies around the purine ring skeleton particularly at N-3, C-8, and the amino group had led to the identification of **1** as a lead structure, in particular the "rolipram-like" disubstituted catechol moiety at N-3.[21] During the synthesis of V11689 (**2**),[21,23] the 8-hydroxy human metabolite of **1**, the 8-benzyloxy analogue **5a** was also prepared[21] and was found to be 3 times more potent than its parent in the mixed PDE4 assay and over 30 times as potent in PDE4B and 4D 3/5 assays. These interesting results coincided with a publication, which described a series of highly potent PDE4 inhibitors.[24] These compounds, hydroxamic acid analogues of rolipram, were suggested to bind at the divalent metal-ion binding pocket of the PDE4 enzyme active site.[24] We speculated that the increased potency of **5a** could be due to binding of the electron-rich benzylic moiety at this binding site. This paper describes the synthesis and pharmacology of a range of 8-benzyloxy analogues of **5a**, a hitherto unexplored region of the purine skeleton.

Chemistry

2-(6-Amino-9*H*-purin-8-yl)-2-propanol **3** and 1-(6-amino-9*H*-purin-8-yl)ethanol **6** were synthesized according to the literature method[25] starting from 6-chloropurine. These adenines **3** and **6** were then reacted with 3-cyclopentyloxy-4-methoxy-benzyl chloride[21] in acetonitrile under reflux to give the 3-alkylated products **4** and **7**. Carrying out this reaction in the absence of base, ensures selective 3-alkylation with none of the

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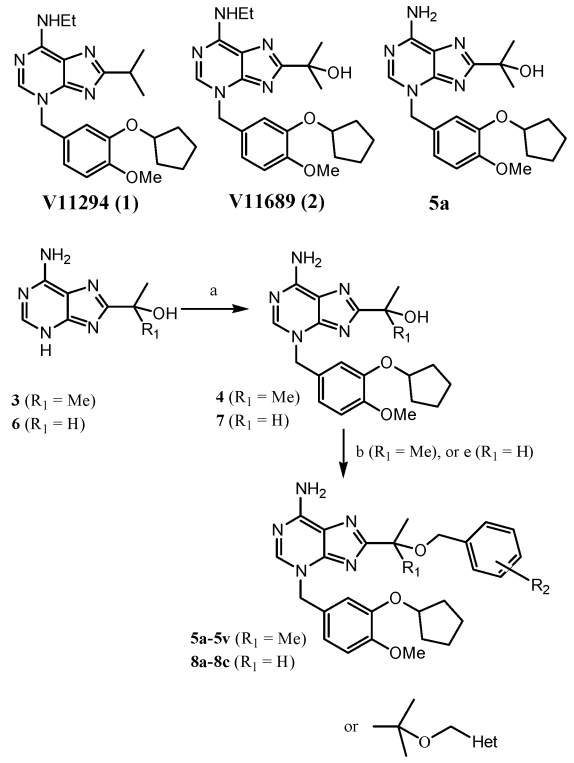
Scheme 1[a]



[a] Reagents and conditions: (a) 3-cyclopentyloxy-4-methoxybenzyl chloride, DMF, 100 °C; (b) KH, THF, 50 °C → 0 °C, then benzyl or aryl chloride, 0 °C → 25 °C; (c) NH_4OH, EtOH, reflux, 18 h; (d) NaOH, EtOH, room temperature, 4 h; (e) NaH, DMF, 50 °C → 0 °C, then benzyl chloride, 0 °C → 25 °C, 5 h.

9-alkylated regioisomer being formed as has been observed in the literature.[26]

The hydroxy-alkyl side chain was then alkylated with a range of benzyl and heteroaryl bromides and chlorides to give the O-benzylated derivatives **5a**−**v** and **8a**−**c** (Scheme 1). O-Alkylation was found to be accompanied by O,N-dialkylation. The optimum conditions to minimize this were NaH in DMF for the alkylation of **7** and KH in THF for alkylations of the more sterically hindered **4**. The reactions were monitored by TLC and stopped when the O,N-dialkylated product began to appear. Yields were typically in the range 10−40%. The carboxamide **5r** and carboxylic acid **5s** derivatives were synthesized from the methyl ester **5q** by hydrolysis using aqueous ammonia and sodium hydroxide, respectively. The carboxylic acid **5s** was isolated as its sodium salt (Scheme 1).

In Vitro Pharmacology

The two series of compounds **5a**−**v** and **8a**−**c** were tested first against human PDE4 enzymes from human lung tissue. They were then tested in human PDE4A, B, D2, D3, and D5 subtypes, which had been cloned and expressed in a baculovirus system.[28] The hPDE4AL and hPDE4BL are the long form of the cloned enzymes. The PDE4C enzyme is an unstable clone, and so we were not able to test our compounds in this enzyme subtype. The PDE4C enzyme is mainly found in testis, skeletal muscle, the CNS, and human fetal lung, while the PDE4A, B, and D subtypes are expressed in many inflammatory cells in man and are therefore of more relevance to the asthmatic disease state (Table 1).[29] The compounds were then tested against human PDE3 and PDE5 enzymes from human blood platelets. With the exception of **8b** and **8c**, which showed low-micromolar PDE5 activity of 1.44 and 4.02 μM, respectively, the remaining compounds were inactive with both PDE3 and PDE5 enzymes at 30 μM concentration.

Results and Discussion

With the exception of the carboxylic acid **5s** (K_i = 5230 nM), all the gem-dimethyl analogues **5a**−**v** were more potent at PDE4 (K_i = 9.7−191 nM) than **1** (K_i = 436 nM). The most potent compound was the methyl ester **5q** (K_i = 9.3 nM), a hydrogen bond acceptor, which is 47-fold more potent than the parent **1**.

The carboxylic acid **5s** is the most weakly active compound in this series at all PDE4 subtype enzymes. This cannot be due to steric requirements as the similarly sized carboxamide **5r** (K_i = 25 nM) is one of the most potent analogues. Rather, it must be due either to the polarity of the carboxylate or to the entropically unfavorable desolvation of the carboxylate anion required before binding can occur. The dimethyl series **5a**−**v** were 10- to 20-fold more potent than their monomethyl analogues **8a**−**c** at all PDE4 subtype enzymes. This may be due either to extra lipophilic binding of the former or to the dimethyl analogue having a greater conformational preference for the desired bioactive conformation. A number of trends were seen in the SAR of the dimethyl series **5a**−**v**. At the 4-position most substituents are well-tolerated. They showed high potency at PDE4B, D3, and D5 subtypes (5−30 nM) and approximately 10-fold lower potency at PDE4A and D2 subtypes (100−600 nM). The 3,4-dimethoxy **5i** and 3,5-dimethoxy **5j** analogues are of interest in that at PDE4A, B, D3, and D5 the 3,5-dimethoxy compound is approximately twice as potent as the 3,4-dimethoxy derivative. However at the D2 subtype, the 3,5-analogue is 8 times as potent as the 3,4-analogue. Similarly, the 2-thienyl **5u** and 3-thienyl **5v** derivatives showed similar potencies at PDE4A, D3, and D5, but at PDE4B, the 3-thienyl **5v** was 5 times more potent than the 2-thienyl **5u**. However at PDE4D2, the potencies were reversed with the 2-thienyl **5u** being 6.5 times more potent than the 3-thienyl **5v**.

The 4-cyano derivative **5m** shows an interesting gradation of tolerance for the PDE4 subtype enzymes being very potent at PDE4D5 (K_i = 19.4 nM) and D3 (K_i = 33.4 nM) but only weakly active against PDE4A (K_i = 1190 nM) and D2 (K_i = 1840 nM). The trend is D5 > D3 > B > A > D2. These observations taken together could be used in the design of third generation subtype selective PDE4 inhibitors which could lead to increased tissue and cell selectivity, to decreased side effects, and improved therapeutic index.[33−35] Recent literature supports this hypothesis; for instance, dual PDE4A/B inhibition has been found to significantly correlate with inhibition of LPS-stimulated TNF-α secretion and with T-cell proliferation in peripheral blood monocytes, while no correlation was found for PDE4D inhibition. The PDE4C[36] subtype is not generally found in proinflammatory cells, but it is highly expressed in the CNS[37] where PDE4 inhibitors are

Table 1. PDE4 Subtype Enzyme Potencies (nM)

compd	R_2 (or het)	PDE4 (mix) mean ± SEM (*n*)	hPDE4AL mean ± SEM (*n*)	hPDE4BL mean ± SEM (*n*)	hPDE4D2 mean ± SEM (*n*)	hPDE4D3 mean ± SEM (*n*)	hPDE4D5 mean ± SEM (*n*)
rolipram			7940 ± 1840 (6)	1470 ± 820 (5)	8460 ± 1260 (7)	382 ± 42 (5)	375 ± 80 (4)
CI-930			>50000 (6)	>50000 (5)	>50000 (5)	>50000 (5)	>50000 (6)
zaprinast			>50000 (4)	>50000 (3)	>50000 (5)	>50000 (4)	8760 ± 3300 (5)
1		436 ± 23 (3)	3900 ± 1290 (3)	270 ± 42.1 (3)	2890 ± 805 (5)	172 ± 72.9 (3)	141 ± 17 (3)
5a	H	191 ± 21 (3)	179 ± 65 (4)	8.9 ± 3.0 (3)	413 ± 81 (5)	5.7 ± 0.7 (3)	4.1 ± 0.6 (3)
5b	2-OMe	38 ± 2.1 (3)	137 ± 25.1 (3)	11.0 ± 3.8 (3)	255 ± 53.5 (3)	7.1 ± 3.7 (3)	5.1 ± 0.6 (3)
5c	3-OMe	30.3 ± 3.2 (3)	175 ± 32 (3)	13.0 ± 4.8 (3)	296 ± 39 (3)	6.0 ± 3.3 (3)	5.1 ± 0.9 (3)
5d	4-OMe	22.6 ± 2.2 (3)	113 ± 27 (3)	10.3 ± 3.1(3)	347 ± 52 (3)	5.6 ± 2.9 (3)	8.1 ± 5.5 (3)
5e	4-Me	19.7 ± 2.0 (3)	206 ± 26 (3)	9.8 ± 3.9 (3)	347 ± 50 (3)	6.6 ± 3.0 (3)	7.6 ± 3.5 (3)
5f	4-Cl	25.3 ± 2.6 (3)	126 ± 43 (3)	11.0 ± 2.0 (3)	205 ± 34 (3)	5.8 ± 0.9 (3)	4.8 ± 1.8 (3)
5g	2-F	45.3 ± 7.4 (3)	271 ± 76 (3)	18.9 ± 1.9 (3)	427 ± 45 (3)	11.0 ± 6.1 (3)	6.8 ± 3.2 (3)
5h	3-F	60.0 ± 6.4 (3)	208 ± 21 (3)	11.6 ± 3.4 (3)	369 ± 74 (3)	7.4 ± 2.9 (3)	8.4 ± 4.6 (3)
5i	3,4-OMe	91.7 ± 23.2 (3)	405 ± 8(3)	42.8 ± 19.3 (4)	2640 ± 530 (3)	15.0 ± 4.5 (3)	7.8 ± 1.8 (3)
5j	3,5-OMe	44.0 ± 2.1(3)	225 ± 29 (3)	21.9 ± 8.1 (3)	340 ± 84 (3)	9.0 ± 1.8 (3)	7.9 ± 1.2 (3)
5k	3,4-F	49.0 ± 1.5 (3)	244 ± 47.0 (3)	17.3 ± 2.8 (3)	459 ± 63 (3)	13.0 ± 1.6 (3)	13.3 ± 0.7 (3)
5l	3,4-OCH_2CH_2O	35.0 ± 8.3 (3)	147 ± 27 (3)	21.4 ± 11.3 (3)	218 ± 41 (3)	6.6 ± 1.9 (3)	3.7 ± 1.4 (3)
5m	4-CN	58.0 ± 2.0 (3)	1190 ± 170 (3)	71.3 ± 18.2 (3)	1840 ± 290 (3)	33.4 ± 8.0(3)	19.4 ± 9.6(3)
5n	3,4-Cl	26.0 ± 3.0 (3)	166 ± 22 (3)	9.7 ± 5.8 (3)	355 ± 30 (3)	10.6 ± 4.8 (3)	6.6 ± 2.7 (3)
5o	4-*t*-Bu	15.0 ± 3.0 (3)	175 ± 41 (3)	5.8 ± 1.3 (3)	255 ± 8 (3)	5.0 ± 0.6 (3)	4.3 ± 1.4 (3)
5p	4-OCF_3	27.0 ± 3.5 (3)	415 ± 95 (3)	26.6 ± 10.0 (3)	782 ± 225 (5)	6.5 ± 3.6 (3)	7.2 ± 1.1 (3)
5q	4-CO_2Me	9.3 ± 1.0 (3)	103 ± 13 (3)	5.2 ± 2.1 (3)	246 ± 68 (3)	3.0 ± 0.1 (3)	5.5 ± 1.7 (3)
5r	4-$CONH_2$	25.0 ± 2.5 (3)	155 ± 34 (3)	7.6 ± 3.2 (3)	312 ± 32 (3)	8.0 ± 2.1 (3)	4.9 ± 1.7 (3)
5s	4-CO_2H	5230 ± 60 (3)	>50000 (3)	>50000 (3)	>50000 (3)	4300 ± 2420 (3)	>50000 (3)
5t	4-CF_3	23.5 ± 1.0 (3)	309 ± 34 (3)	33 ± 13 (3)	580 ± 192 (4)	9.9 ± 4.7 (3)	10.3 ± 4.3 (3)
5u	2-thienyl	53.3 ± 11.7 (3)	236 ± 65 (4)	46.9 ± 20.0 (3)	412 ± 100 (4)	7.0 ± 1.3 (3)	6.3 ± 1.5 (3)
5v	3-thienyl	83.3 ± 12.7 (3)	223 ± 72 (3)	9.5 ± 3.1 (3)	2680 ± 870 (3)	9.0 ± 4.7 (3)	6.6 ± 3.6 (3)
8a	H	1210 ± 140 (3)	15200 ± 9900 (3)	533 ± 164(3)	4450 ± 1560 (4)	640 ± 150 (4)	590 ± 107 (3)
8b	3-OMe	1470 ± 170 (3)	5200 ± 930 (3)	430 ± 40 (3)	5050 ± 620 (3)	493 ± 133 (4)	322 ± 83 (3)
8c	3-F	204 ± 69 (3)	3390 ± 1350 (3)	269 ± 95 (4)	4300 ± 580 (3)	114.7 ± 24.3 (3)	231 ± 112 (3)

believed to promote many of their side effects such as emesis. Third generation inhibitors could target minimizing PDE4C inhibition as a drug design strategy. Interestingly, cilomilast (Ariflo)[16] currently in clinical phase trials shows 10-fold selectivity for PDE4D versus other PDE4 subtypes.

Conclusions

The synthesis of the 8-benzyloxy analogues of **1** has led to the discovery of highly potent and selective PDE4 inhibitors that are 10- to 20-fold more potent than the parent compound. The 4-methylester derivative **5q** was the most potent compound in the series showing single digit nanomolar potency at the B, D3, and D5 subtypes. In addition, a number of compounds in particular the 4-cyano **5m**, the 2- and 3-thienyl **5u** and **5v**, and the 3,4- and 3,5-dimethoxy **5i** and **5j** analogues showed interesting PDE4 subtype specificities which could be further exploited to design third generation PDE4 inhibitors with reduced side effect liability.

Experimental Section

Anhydrous diethyl ether (ether) and tetrahydrofuran (THF) were obtained by distillation from sodium hydride/benzophenone ketyl under argon immediately prior to use. All other reagents were used as obtained from commercial sources or purified by standard methods. *N*,*N*-Dimethylformamide is abbreviated to DMF and ethyl acetate to EtOAc. All reactions were performed under an inert atmosphere unless otherwise stated. Thin-layer chromatography was performed on glass plates precoated with Merck silica gel 60 F_{254} 0.2 mm, which were visualized by quenching of UV fluorescence (λ_{max} 254 nM) and by staining with iodine vapor or Dragendorff's reagent. R_f values are quoted to the nearest 0.05. Flash chromatography was performed on YMC 60A I-400/230 mesh silica. Melting points (mp) were determined using an Electrothermal IA9200 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX400 or AMX500 spectrometer. The spectra were referenced to residual protonated solvent residues as an internal standard, and chemical shifts are quoted in parts per million (ppm) downfield from tetramethylsilane in the solvent indicated in parentheses. Values are quoted to 0.01 ppm. Signal multiplicities are described as s (singlet), d (doublet), t (triplet), and q (quartet) with *J* being reported to the nearest 0.1 Hz. The symbol br indicates that the peak was broad. Low-resolution mass spectra (MS) were recorded on a Micromass Platform LC system with electrospray (ES) mode of ionization. Intensities were recorded as percentages of the largest signal, and only major peaks were recorded, as the mass to charge (*m*/*z*) ratio.

Preparation of 2-(6-Amino-9*H*-purin-8-yl)-2-propanol (3). 2-(6-Amino-9*H*-purin-8-yl)-2-propanol **3** was prepared according to the literature method. [25]

Preparation of 2-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}-2-propanol (4). 2-(6-Amino-9*H*-purin-8-yl)-2-propanol **3** (1.0 g, 5.18 mmol) was dissolved in dry DMF (25 mL) with heating and stirring (45−50 °C) under nitrogen. To the clear solution was added freshly prepared 3-cyclopentyloxy-4-methoxy-benzyl chloride[21] (1.3 g, 5.4 mmol), and the resulting mixture was heated to 100 °C with stirring for 4 h. The cooled solution was evaporated to dryness in vacuo, the residue was partitioned between saturated sodium bicarbonate solution (400 mL) and dichloromethane (400 mL), the organic phase was separated and dried ($MgSO_4$), and the solvent was evaporated to dryness in vacuo. The residue was chromatographed over flash silica, eluting with dichloromethane/methanol (8:1) to give a yellow gum. Trituration with ice-cold acetone (50 mL) gave **4** as a white solid (0.63 g, 31%): mp = 207.2−209 °C. TLC (SiO_2, CH_2Cl_2/MeOH, 8:1) R_f = 0.22; detection, UV and Dragendorff's reagent. δ_H {400 MHz, DMSO-d_6}: 1.50 (6H, s), 1.55−1.96 (8H, m), 3.66 (3H, s), 4.66 (1H, m), 4.75 (1H, s,), 5.36 (2H, s), 6.86 (1H, d, *J* = 8.3 Hz), 7.04 (1H, dd, *J* = 8.2, 1.8 Hz), 7.24 (1H, d, *J* = 1.8 Hz), 7.73 (1H, bs), 8.46 (1H, s).

Preparation of 1-(6-Amino-9*H*-purin-8-yl) Ethanol (6). 1-(6-Chloro-9*H*-purin-8-yl) ethanol (prepared from 6-chloro-9-tetrahydropyran-2-yl-purine[27] using *n*-butyllithium, in tet-

rahydrofuran at −78 °C, followed by treatment with acetaldehyde, using the literature method[25]) (5.7 g, 28.7 mmol) was dissolved in concentrated ammonia $\rho = 0.88$ (30 mL), and the solution was heated to 150 °C with stirring in a sealed tube for 48 h. The cooled mixture was evaporated to dryness in vacuo. The residue was chromatographed over flash silica eluting with dichloromethane/methanol (5:1) to give **6** as a white solid (4.27 g, 83%): mp 240.1−247 °C. TLC, SiO_2 (CH_2Cl_2/MeOH, 5:1) $R_f = 0.41$; detection, UV. δ_H {400 MHz, DMSO-d_6}: 1.32 (3H, d, $J = 6.6$ Hz), 4.87 (1H, q, $J = 6.6$ Hz), 5.8 (1H, bs), 7.05 (2H, bs), 8.11 (1H, s), 12.5−13.0 (1H, bs).

Preparation of 1-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}ethanol (7). 3-Cyclopentyloxy-4-methoxybenzyl chloride[21] (5.91 g, 24.55 mmol) and 1-(6-amino-9*H*-purin-8-yl) ethanol **6** (4.0 g, 22.3 mmol) were heated together in dry DMF (35 mL) with stirring at 100 °C under nitrogen for 4 h. The solvent was evaporated to dryness in vacuo, and the residue was partitioned between CH_2Cl_2/MeOH (5:1) (400 mL) and saturated sodium bicarbonate solution (400 mL). The organic phase was separated and dried ($MgSO_4$), and the solvent was evaporated in vacuo to leave a yellow gum. Flash chromatography (SiO_2, CH_2Cl_2/MeOH, 12:1) gave a yellow foam which was triturated with cold acetone (0 °C) to give **7** (2.64 g, 31%) as a white solid: mp = 199−202 °C. $MH^+ = 384.05$ (100%). TLC (SiO_2, CH_2Cl_2/MeOH, 12:1) $R_f = 0.25$; detection, UV and Dragendorff's reagent. δ_H {400 MHz, DMSO-d_6}: 1.42 (2H, d, $J = 6.5$ Hz), 1.48−1.70 (6H, m), 1.77−1.88 (2H, m), 3.67 (3H, s), 4.67 (1H, m), 4.73 (1H, d, $J = 4.6$ Hz), 4.79 (1H, m), 6.85 (1H, d, $J = 8.3$ Hz), 6.99 (1H, dd, $J = 8.2$, 1.6 Hz), 7.24 (1H, d, $J = 1.6$ Hz), 7.70 (2H, bs), 8.43 (1H, s). Anal. ($C_{20}H_{25}N_5O_5 \cdot 0.3H_2O$) C, H, N.

General Procedure B for the Alkylation of 4. Potassium hydride (2.2 equiv, 35 wt % dispersion in mineral oil) under a constant flow of argon was transferred to a flame dried round-bottom flask. The potassium hydride was washed with pentane (2 mL, dried over NaH). Compound **4** (1 equiv, 1 mmol), then THF (15 mL) was added, and the solution was heated to 50 °C for 1 h. The resulting bright yellow solution was cooled to 0 °C, and the substituted benzyl halide (1.2 equiv) in THF (5 mL) was added dropwise over 20 min. The reaction was maintained at 0 °C for 1 h and then allowed to warm to room temperature. The reaction was stirred for 5 h at room temperature or until visualization of the bis-benzylated material by TLC. The reaction was recooled to 0 ° C, and water (10 mL) was added dropwise. EtOAc (10 mL) was added, and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organics were dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was triturated with cold ether (10 mL) to give recovered starting material **4** (40−70%). Evaporation of the ether filtrate under reduced pressure gave the crude product. Purification by flash column chromatography [SiO_2, EtOAc (500 mL), and then EtOAc/MeOH/NH_3, 60:10:1 (460 mL)] gave the product (6−35%). This was converted to its crystalline fumarate salt in most cases.

General Procedure E for the Alkylation of 7. Sodium hydride (1.2 equiv, 60% dispersion in mineral oil) under a constant flow of argon was transferred to a flame-dried round-bottom flask. The sodium hydride was washed with pentane (2 mL, dried over NaH). DMF (40 mL) and then compound **7** (1 mmol, 1.0 equiv) were added, and the solution was heated to 50 °C for 30 min. The resulting bright yellow solution was cooled to 0 °C, and the substituted benzyl halide (1.2 equiv) in DMF (3 mL) was added dropwise. The reaction was maintained at 0 °C for 1 h and then allowed to warm to room temperature. The reaction was recooled to 0 °C, and water (10 mL) was added dropwise. EtOAc (20 mL) was added, and the layers were separated. The organic layer was washed with brine (3 × 10 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give the crude product. Flash chromatography (SiO_2, EtOAc/MeOH/NH_3, 100:10:1 to 40:10:1; gradient elution) gave the product (3−35%) which if not crystalline was converted to its fumarate salt.

General Procedure for the Preparation of Fumarate Salts. Fumaric acid (1.05 equiv) was dissolved in the minimum quantity of anhydrous ether/methanol (10:1). This solution was added dropwise to the adenines **5a**−**v** or **8a**−**c** (1 equiv) in a minimum volume of ether, immediately forming a precipitate. Filtration and washing with cold anhydrous ether (3 × 2 mL) gave the desired salt as a crystalline solid. Evaporation of the solvent and trituration with cold ether gave a second crop of the salt.

Preparation of 8-(1-Benzyloxy-1-methyl-ethyl)-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-6-ylamine Hydrochloride (5a). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5a** (205 mg, 35% yield) as a white solid. It was dissolved in ether (40 mL) and treated with hydrogen chloride 1 M in ether (0.5 mL, 0.5 mmol). The mixture was filtered, washed with ether (20 mL), and dried in vacuo to give the hydrochloride salt as a white solid: mp = 203 °C. δ_H {500 MHz, DMSO-d_6}: 1.53−1.69 (2H, m), 1.74 (6H, s), 1.80−1.97 (6H, m), 3.70 (3H, s), 4.48 (2H, s), 4.73 (1H, m), 5.45 (2H, s), 6.91 (1H, d, $J = 8.3$ Hz), 7.11 (1H, dd, $J = 8.3$, 2.0 Hz), 7.20 (1H, d, $J = 2.0$ Hz), 7.24−7.37 (5H, m), 8.99 (1H, s), 9.34 (1H, bs), 14.04 (2H, bs). Anal. ($C_{28}H_{33}N_5O_3 \cdot HCl$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(2-methoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine (5b). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5b** (10% yield): mp = 202.3−202.5 °C. δ_H {400 MHz, $CDCl_3$}: 1.49−1.92 (14H, m), 3.81 (3H, s), 3.86 (3H, s), 4.65−4.68 (1H, m), 4.81 (2H, s), 5.41 (2H, s), 6.78−6.95 (4H, m), 7.06 (1H, s), 7.20−7.32 (2H, m), 7.97 (1H, s). Anal. ($C_{29}H_{35}N_5O_3 \cdot 1.2H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3-methoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate Hemihydrate (5c). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5c** (10% yield). The fumarate salt was formed using the general procedure above: mp = 171.1−171.3 °C. δ_H {500 MHz, $CDCl_3$}: 1.54−1.59 (2H, m), 1.73−1.86 (12H, m), 3.77 (3H, s), 3.82 (3H, s), 4.44 (2H, s), 4.66−4.68 (1H, m), 5.45 (2H, s), 6.63 (1H, dd, $J = 8.0$, 2.0 Hz), 6.81 (1H, d, $J = 8.0$ Hz), 6.94−7.01 (5H, m), 7.17 (1H, t, $J = 8.0$ Hz), 8.06 (1H, s). Anal. ($C_{29}H_{35}N_5O_4 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-methoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5d). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5d** (40% yield). It was converted to the fumarate salt using the general procedure above: mp = 199.8−200.2 ° C. δ_H {500 MHz, DMSO-d_6}: 1.48−1.55 (2H, m), 1.63−1.68 (4H, m), 1.71 (6H, s), 1.77−1.85 (2H, s), 3.70 (3H, s), 3.74 (3H, s), 4.17 (2H, s), 4.65−4.70 (1H, m), 5.45 (2H, s), 6.63 (1H, s), 6.83 (2H, d, $J = 8$ Hz), 6.86 (2H, d, $J = 8.5$ Hz), 7.10 (1H, dd, $J = 8.15$, 1.5 Hz), 7.18 (2H, d, $J = 8$ Hz), 7.25 (1H, d, $J = 1.5$ Hz), 7.80−7.98 (2H, brs), 8.55 (1H, s). Anal. $C_{29}H_{35}N_5O_4 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-methyl-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5e). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5e** (35% yield). This was converted to the fumarate salt using the general procedure above: mp = 196.9−197.2 °C. δ_H {500 MHz, $CDCl_3$}: 1.55−1.57 (2H, m), 1.73−1.86 (12H, m), 2.28 (3H, s), 3.83 (3H, s), 4.42 (2H, s), 4.65−4.68 (1H, m), 5.46 (2H, s), 6.81 (1H, d, $J = 8.0$ Hz), 6.94−6.98 (3H, m), 7.08 (2H, d, $J = 8.0$ Hz), 7.29 (2H, $J = 8.0$ Hz), 8.06 (1H, s), 10.74 (1H, brs). Anal. ($C_{29}H_{35}N_5O_3 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 8-{1-[(4-Chlorobenzyl)oxy]-1-methyl-ethyl}-3-[3-cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-6-amine Hemifumarate Hydrate (5f). Using the general procedure b, we obtained the 3*H*-purin-6-ylamine **5f** (10% yield). It was converted to the fumarate salt using the general procedure above: mp = 170.8−171.3 °C. δ_H {500 MHz, $CDCl_3$}: 1.56−1.58 (2H, m), 1.73−1.88 (12H, m), 3.83 (3H, s), 4.43 (2H, s), 4.66−4.69 (1H, m), 5.45 (2H, s), 6.81 (1H, d, $J = 8.0$ Hz), 6.92−6.96 (4H, m), 7.23 (2H, d, $J = 8.5$ Hz), 7.34 (2H,

d, J = 8.5 Hz), 8.08 (1H, s). Anal. ($C_{28}H_{32}ClN_5O_3 \cdot 0.5C_4H_4O_4 \cdot H_2O$) C, N. H analysis was 0.41% lower than theory.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(2-fluoro-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine (5g). 2-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}-2-propanol **4** (400 mg, 1.006 mmol) was alkylated with 2-fluorobenzyl chloride (0.145 mL, 1.2 mmol) using the general method b to give a colorless gum (120.9 mg, 0.239 mmol) after flash column chromatography (SiO_2, CH_2Cl_2/MeOH, 20:1): R_f = 0.15; detection, UV and Dragendorff's reagent. This was dissolved in ether (20 mL); fumaric acid (27.8 mg, 0.239 mmol) in methanol(0.5 mL) was added, and the mixture was allowed to crystallize slowly. The mixture was filtered to give **5g** (103.5 mg, 17%) as a white solid: mp = 202.5−203 °C. δ_H {400 MHz, DMSO-d_6}: 1.47−1.52 (2H, m), 1.58−1.60 (4H, m), 1.70 (6H, s), 1.74−1.79 (2H, m), 3.66 (3H, s), 4.30 (2H, s), 4.65−4.63 (1H, m), 5.40 (2H, s), 6.60 (1H, s,), 6.80 (1H, d, J = 8.3 Hz), 7.06−7.14 (3H, m), 7.29 (1H, d, J = 7.5 Hz), 7.22−7.29 (1H, m), 7.42 (1H, t, J = 6.7 Hz), 7.85−8.00 (2H, bd), 8.51 (1H, s). Anal. ($C_{28}H_{32}FN_5O_3 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3-fluoro-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate Hemihydrate (5h). 2-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}-2-propanol **4** (400 mg, 1.006 mmol) was alkylated with 3-fluorobenzyl chloride (0.145 mL, 1.2 mmol) using method b to give a white solid (126.9 mg, 0.25 mmol) after flash column chromatography (SiO_2, CH_2Cl_2/MeOH, 25:1): R_f = 0.22; detection, UV and Dragendorff's reagent. This was dissolved in ether (15 mL); fumaric acid (29.1 mg, 0.25 mmol) in methanol (0.4 mL) was added, and the resulting mixture was allowed to crystallize slowly with cooling to 0 °C. The mixture was filtered and dried in vacuo at 50 °C to give **5 h** (102.1 mg, 16%) as a white solid: mp = 197.5−198 °C. δ_H {400 MHz, DMSO-d_6}: 1.45−1.65 (6H, m), 1.69 (6H, s), 1.75−1.85 (2H, m), 3.69 (1H, s), 4.27 (2H, s), 4.62−4.66 (1H, m), 5.39 (2H, s), 6.61 (1H, s), 6.79 (1H, d, J = 8.3 Hz), 7.00−7.16 (4H, m), 7.22 (1H, d, J = 1.67 Hz), 7.30 (1H, m), 7.75−7.95 (2H, m), 8.51 (1H, s). Anal. ($C_{28}H_{32}FN_5O_3 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3,4-dimethoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Fumarate Hemihydrate (5i). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5i** (11% yield). This was converted to the fumarate salt using the general method above: mp = 160.2−160.8 °C. δ_H (400 MHz, $CDCl_3$): 1.58−1.59 (2H, m), 1.71 (6H, s), 1.78−1.88 (6H, m), 3.82 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 4.66−4.70 (1H, m), 4.79 (2H, s), 5.40 (2H, s), 6.73−7.00 (8H, m), 8.10 (1H, s), 10.70 (1H, brs). Anal. ($C_{30}H_{37}N_5O_5 \cdot C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3,5-dimethoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Fumarate (5j). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5j** (21% yield). This was converted to the fumarate salt using the general procedure above: mp = 165.3−165.8 °C. δ_H {400 MHz, $CDCl_3$}: 1.52−1.59 (2H, m), 1.73−1.85 (12H, m), 3.73 (6H, s), 3.91 (3H, s), 4.38 (2H, s), 4.64−4.68 (1H, m), 5.43 (2H, s), 6.28 (1H, t, J = 2.5 Hz), 6.57 (2H, d, J = 2.5 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.93−6.99 (4H, m), 8.03 (1H, s). Anal. ($C_{30}H_{37}N_5O_6 \cdot C_4H_4O_4$) H, N. C analysis was 0.42% higher than theory.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3,4-difluoro-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Fumarate (5k). Using the general procedure b, we obtained the3*H*-purin-6ylamine **5k** (21% yield). This was converted to the fumarate salt using the general procedure above: mp = 169.1−169.4 °C. δ_H {400 MHz, $CDCl_3$ (1% DMSO-d_6)}: 1.38−1.42 (2H, m), 1.63−1.75 (12H, m), 3.66 (3H, s), 4.20 (2H, s), 4.51−4.54 (1H, m), 5.32 (2H, s), 6.64−6.66 (3H, m), 6.81−6.99 (4H, m), 7.14−7.19 (1H, m), 8.10 (1H, s). Anal. ($C_{28}H_{31}F_2N_5O_3 \cdot C_4H_4O_4$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3,4-methylenedioxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5l). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5l** (13% yield). This was converted to the fumarate salt using the general procedure above: mp = 164.1−164.5 °C. δ_H {400 MHz, $CDCl_3$}: 1.53−1.58 (2H, m), 1.71−1.88 (12H, m), 3.82 (3H, s), 4.31 (2H, s), 4.64−4.65 (1H, m), 5.46 (2H, s), 5.87 (2H, s), 6.69 (1H, d, J = 8.0 Hz), 6.81 (2H, m), 6.90−7.01 (4H, m), 8.02 (1H, s). Anal. ($C_{29}H_{33}N_5O_5 \cdot 0.5C_4H_4O_4 \cdot 0.3H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-cyano-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine (5m). Using the general procedure b, we obtained the *3H*-purin-6-yl-amine **5m** (10% yield). This was converted to the fumarate salt using the general procedure above: mp = 170 °C. δ_H {400 MHz, $CDCl_3$}: 1.55−1.62 (2H, m), 1.73 (6H, s), 1.78−1.90 (8H, m), 3.83 (3H, s), 4.65−4.73 (1H, m), 4.91 (2H, s), 5.42 (2H, s), 6.80−6.85 (2H, m), 6.92−7.00 (2H, m), 7.50−7.59 (4H, m), 8.08 (1H, s), 11.26 (1H, brs).

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(3,4-dichloro-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate Hemihydrate (5n). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5n** (29% yield). This was converted to the fumarate salt using the general procedure above: mp = 215 °C. δ_H {400 MHz, $CDCl_3$}: 1.32−1.42 (2H, m), 1.56−1.67 (8H, m), 3.62 (3H, s), 4.16 (2H, s), 4.49 (1H, m), 5.30 (2H, s), 6.60 (1H, d, J = 8.3 Hz), 6.63 (1H, s), 6.78 (1H, dd, J = 8.3, 2.0 Hz), 6.85 (1H, d, J = 2.0 Hz), 7.02 (1H, dd, J = 8.25, 1.9 Hz), 7.15 (1H, d, J = 8.2 Hz), 7.37 (1H, d, J = 1.83 Hz), 7.95 (1H, s). Anal. ($C_{28}H_{31}Cl_2N_5O_3 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-*tert*-butyl-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate Hydrate (5o). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5o** (29% yield). It was converted to the fumarate salt using the general procedure above: mp = 167 °C. δ_H {400 MHz, $CDCl_3$}: 1.23 (9H, s), 1.50 (2H, m), 1.70−1.78 (6H, m), 1.79 (6H, s), 3.77 (3H, s), 4.38 (2H, s), 4.63 (1H, m), 5.43 (2H, s), 6.76 (1H, d, J = 8.2 Hz), 6.79 (1H, s), 6.97 (1H, dd, J = 8.2, 2.0 Hz), 7.01 (1H, d, J = 2.0 Hz), 7.23−7.28 (4H, m), 7.99 (1H, s). Anal. ($C_{32}H_{41}N_5O_3 \cdot 0.5C_4H_4O_4 \cdot H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-trifluoromethoxy-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5p). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5p** (19% yield). It was converted to the fumarate salt using the general procedure above: mp = 210 °C. δ_H {400 MHz, $CDCl_3$}: 1.33−1.37 (8H, m), 2.15 (6H, s), 3.36 (3H, s), 3.95 (2H, s), 4.24 (1H, brs), 5.06 (2H, s), 6.31 (1H, s), 6.36−6.38 (1H, s), 5.59−5.61 (1H, s), 6.67−6.69 (2H, m), 6.96−6.98 (1H, s), 7.79 (1H, s). Anal. ($C_{29}H_{32}F_3N_5O_4 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 4-{2-[6-Amino-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-8-yl]-2-methyl-propoxy}-benzoic Acid Methyl Ester Hemifumarate Hemihydrate (5q). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5q** (36% yield). It was converted to the fumarate salt using the general method above: mp = 188.5−189 °C. δ_H {400 MHz, DMSO-d_6}: 1.45−1.51 (2H, m), 1.56−1.65 (4H, m), 1.69 (6H, s), 1.72−1.79 (2H, m), 3.65 (3H, s), 3.82 (3H, s), 4.32 (2H, s), 4.63 (1H, m), 5.38 (2H, s), 6.60 (1H, s), 6.75 (1H, d, J = 8.3 Hz), 7.02 (1H, dd, J = 8.3, 1.7 Hz), 7.19 (1H, d, J = 1.7 Hz), 7.40 (2H, d, J = 8.2 Hz), 7.85 (2H, d J = 8.2 Hz), 7.90−7.95 (2H, brs), 8.50 (1H, s), 13.15 (1H, bs). Anal. ($C_{30}H_{35}N_5O_5 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

Preparation of 4-{2-[6-Amino-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-8-yl]-2-methyl-propoxy}-benzamide Fumarate Hemihydrate (5r). The methyl ester **5q** (200 mg, 0.367 mmol) was dissolved in ethanol (5 mL). Concentrated ammonia ρ = 0.88 (2 mL) was added, and the mixture was heated to 100 °C in a sealed tube for 18 h. The cooled reaction mixture was evaporated to dryness in vacuo, and the residue was chromatographed over flash silica (SiO_2, CH_2Cl_2/MeOH, 20:1) to give the amide **5r** (151 mg, 75%) as a white solid: mp = 167.3−168.2 °C; R_f = 0.07; detection, UV and Dragendorff's reagent. δ_H {400 MHz, DMSO-d_6}: 1.45−1.52 (2H, m), 1.57−1.67 (4H, m), 1.70 (6H, s), 1.72−1.80 (2H,

s), 3.66 (3H, s), 4.31 (2H, s), 4.61−4.65 (1H, m), 5.40 (2H,s), 6.60 (2H, s), 6.79 (1H, d, J = 8.3 Hz), 7.05(1H, dd, J = 8.3, 1.8 Hz), 7.18 (1H, d, J = 1.8 Hz), 7.32 (2H, d, J = 8.1 Hz), 7.75 (2H, d, J = 8.1 Hz), 8.45 (1H, s). Anal. ($C_{29}H_{34}N_6O_4 \cdot C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

Preparation of 4-{2-[6-Amino-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-8-yl]-2-methyl-propoxy}-benzoic Acid Sodium Salt Trihydrate (5s). The methyl ester **5q** (200 mg, 0.367 mmol) was dissolved in ethanol (5 mL). Sodium hydroxide (58.6 mg, 1.47 mmol) in water (0.2 mL) was added, and the mixture was stirred at room temperature for 5 h, after which time a white precipitate formed. The mixture was filtered, washed with cold ethanol (2 mL), and dried in vacuo to give the sodium salt **5s** (125 mg, 60%) as a white solid: mp = 317.8−319.6 °C. δ_H {400 MHz, DMSO-d_6}: 1.46−1.53 (2H, m), 1.55 (6H, s), 1.57−1.67 (4H, m), 1.78−1.85 (2H, m), 3.66 (3H, s), 4.65−4.69 (1H, m), 5.47 (2H, m), 6.88 (1H, d, J = 8.3 Hz), 7.07 (1H, d, J = 8.3 Hz), 7.17 (1H, s), 7.48 (2H, d, J = 8.1 Hz), 7.88 (2H, d, J = 8.1 Hz), 9.05 (1H, s). Anal. ($C_{29}H_{32}N_5O_5Na \cdot 3H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-trifluoromethyl-benzyloxy)-1-methyl-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5t). Using the general procedure b, we obtained the 3*H*-purin-6ylamine **5t** (22% yield). It was converted to the fumarate salt using the procedure given: mp = 196 °C. δ_H {500 MHz, $CDCl_3$}: 1.25 (6H, s), 1.50−1.91 (8H, m), 3.80 (3H, s), 4.43 (2H, s), 4.68 (1H, m), 5.47 (2H, s), 6.76 (1H, d, J = 8.3 Hz), 6.96 (1H, dd, J = 8.3, 2 Hz), 7.02 (1H, d, J = 2 Hz), 7.45−7.55 (4H, m), 7.95 (1H, s). Anal. ($C_{29}H_{32}F_3N_5O_3 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1,1-dimethyl-2-(thiophen-2-yloxy)-ethyl]-3*H*-purin-6-ylamine (5u). Potassium hydride 35% dispersion in mineral oil (301.9 mg, 2.64 mmol) was washed with dry pentane (3 × 10 mL) and suspended in dry THF (60 mL) under argon. 2-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}-2-propanol **4** (500 mg, 1.26 mmol) was added, and the resulting suspension was heated to 45−50 °C for 1 h. The mixture was cooled to −10 °C; 2-chloromethylthiophene (183.8 mg, 1.39 mmol) in dry THF (20 mL) was added, and the mixture was allowed to warm to room temperature with stirring for 48 h. The mixture was quenched with brine (250 mL) and extracted with EtOAc (2 × 250 mL); the organic phase was dried ($MgSO_4$), and the solvent was evaporated to dryness in vacuo to leave a yellow gum. Flash chromatography(SiO_2, CH_2Cl_2/MeOH, 20:1) gave a yellow gum (67.9 mg, 0.137 mmol), which was dissolved in ether/methanol (10:1, 2 mL), and fumaric acid (16 mg, 0.137 mmol) in methanol (0.4 mL) was added. The mixture was cooled in ice for 1 h and filtered to give **5u** as a white solid (35 mg, 6%): mp = 162−165.9 °C. TLC (SiO_2, CH_2Cl_2/MeOH, 20:1) R_f = 0.21; detection, UV and Dragendorff's reagent. δ_H {400 MHz, DMSO-d_6}: 1.48−1.53 (2H, m), 1.58 (4H, m), 1.67 (6H, s), 1.75−1.79 (2H, m), 3.60 (3H, s), 4.40 (2H, s), 4.65 (2H, s), 5.41 (2H, s), 6.55 (2H, s), 6.83−6.86 (2H, m), 6.90 (1H, dd, J = 8.4, 3.48 Hz), 7.09 (1H, dd, J = 8.2, 1.4 Hz), 7.23 (1H, d, J = 1.4 Hz), 7.75−7.95 (2H, bd), 8.51 (1H, s). Anal. ($C_{26}H_{31}N_5O_3S \cdot C_4H_4O_4 \cdot$ 0.5 H_2O) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1,1-dimethyl-3-(thiophen-3-yloxy)-ethyl]-3*H*-purin-6-ylamine Hemifumarate (5v). 2-{6-Amino-3-[3-(cyclopentyloxy)-4-methoxybenzyl]-3*H*-purin-8-yl}-2-propanol **4** (500 mg, 1.26 mmol) was alkylated with 3-chloromethylthiophene (183.8 mg, 1.39 mmol) using the general method b to give a white solid (22 mg, 44.56 μmol) after flash column chromatography (CH_2Cl_2/MeOH, 25:1) and trituration with EtOAc (20 mL). TLC (SiO_2, CH_2Cl_2/MeOH, 25:1) R_f = 0.24; detection, UV and Dragendorff's reagent. The solid was dissolved in methanol (1 mL); fumaric acid (5.2 mg, 44.56 μmol) in methanol (1 mL) was added, and the mixture was evaporated to dryness in vacuo. The residue was dissolved in hot EtOAc (10 mL) and allowed to crystallize slowly at 0 °C. The mixture was filtered to give **5v** as a white solid: mp = 191−193 ° C. δ_H {400 MHz, DMSO-d_6}: 1.49−1.57 (2H, m), 1.62−1.69 (4H, m), 1.71 (6H, s), 1.79−1.88 (2H, m), 3.70 (3H, s), 4.42 (1H, m), 5.48 (2H, s), 6.69 (2H, s), 6.91 (1H, m), 7.09 (2H, m), 7.19 (1H, s), 7.37−7.48 (2H, m), 8.84 (1H, bs), 8.98 (2H, bs), 13.85 (1H, bs). HRMS: $[M + 1]^+$ calcd for $C_{26}H_{32}N_5O_3S$, 494.2226; found, 494.2232. Anal. ($C_{26}H_{31}N_5O_3 \cdot 0.5C_4H_4O_4$) C, H, N.

Preparation of 8-(1-Benzyloxy-ethyl)-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-6-ylamine (8a). Using the general procedure e, we obtained the 3*H*-purin-6ylamine **8a** (16% yield): R_f = 0.6 (EtOAc/MeOH/NH_3, 10:1:1), mp = 210 °C. δ_H {400 MHz, $CDCl_3$}: 1.56−1.61(2H, m), 1.78−1.91 (6H, m), 1.63 (3H, d, J = 6.5), 3.84 (3H, s), 4.68−4.71 (1H, m), 4.85 (2H, d, J = 15.5 Hz), 5.11 (1H, q, J = 6.5 Hz), 5.44 (2H, brs), 6.83 (1H, d, J = 8 Hz), 6.94 (1H, dd, J = 8, 2 Hz), 7.04 (1H, d, J = 2 Hz), 7.24−7.41 (5H, m), 8.03 (1H, s). MS $(ES)^+$ *m/z:* 496 (MNa^+, 36%), 474 (MH^+, 100%). Anal. ($C_{27}H_{31}N_5O_3 \cdot 0.25H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-methoxy-benzyloxy)-ethyl]-3*H*-purin-6-ylamine (8b). Using the general procedure e, we isolated the 3*H*-purin-6ylamine **8b** (5% yield): R_f = 0.2 (EtOAc/MeOH/NH_3, 20:1:1), mp = 204 °C. δ_H {400 MHz, $CDCl_3$}: 1.56−1.67, 1.76−1.92 (11H, m), 3.80 (3H, s), 3.84 (3H, s), 4.68−4.72 (1H, m), 4.77 (2H, brs), 5.09 (1H, q, J = 6.5 Hz), 5.45 (2H, brs), 6.83−6.89 (4H, m), 6.95 (1H, dd, J = 8, 2 Hz), 7.06 (1H, d, J = 2 Hz), 7.30 (1H, bs), 8.00 (1H, brs). MS (ES^+) *m/z*: 526 (MNa^+, 28%), 504 (MH^+, 100%). Anal. ($C_{28}H_{33}N_5O_4 \cdot 0.25H_2O$) C, H, N.

Preparation of 3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-[1-(4-fluoro-benzyloxy)-ethyl]-3*H*-purin-6-ylamine (8c). Using the general procedure e, we isolated the 3*H*-purin-6-ylamine **8c** (3% yield): R_f = 0.2 (EtOAc/MeOH/NH_3, 20:1:1), mp = 217 °C. δ_H {400 MHz, $CDCl_3$}: 1.60−1.69, 1.80−1.92 (8H, m), 1.72 (3H, d, J = 7 Hz), 3.85 (3H, s), 4.69−4.73 (1H, m), 4.84 (2H, brs), 5.16 (1H, q, J = 7 Hz), 5.44 (2H, brs), 6.86 (1H, d, J = 8 Hz), 6.94 (1H, dd, J = 8, 2 Hz), 6.99−7.03 (3H, m), 7.36−7.53 (2H, m), 8.18 (1H, s). MS (ES^+) *m/z*: 514 (MNa^+, 41%), 492 (MH^+, 100%). Anal. ($C_{27}H_{30}FN_5O_3 \cdot 0.25H_2O$) C, H. N analysis was 0.42% lower than theory.

PDE Binding Assay. The PDE4 enzyme was isolated from cadaver human lung (8 h post-mortem); PDE3 and PDE5 were isolated from freshly collected human platelets. The PDE4 isoform assays were obtained using recombinant hPDE4AL, hPDE4BL, hPDE4D2, hPDE4D3, and hPDE4D5 from the baculovirus system. They were obtained from Professor Conti at Stanford University Medical Center. Assays were conducted using [^{3}H] cAMP SPA kit 40 μCi for PDE3 and PDE4; for the PDE5 assay, [^{3}H] cGMP SPA kit 40 μCi was used, both from Amersham Biosciences Corp.[31,32] In each assay, substrate concentration was 200 nM, near the K_m values for the enzymes under the conditions of the assays.

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Supporting Information Available: Elemental analysis data of compounds **5a**−**v**, **6**, and **8a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Flemming, D. H.; Crombie, D. L. Prevalence of asthma and hay fever in England and Wales. *Br. Med. J.* **1987**, *294*, 279−83.
(2) Sly, R. M. Increases in death from asthma. *Ann. Allergy* **1984**, *53*, 2−25.
(3) Barnes, P. J. Asthma deaths a continuing problem. *Advanced Medicine*; Sheppard, M., Ed.; Bailliere Tindall: London, 1988; pp 53−61.
(4) Beavo, J. A. Multiple isozymes of cyclic nucleotide phosphodiesterase. In A*dvances in Second Messenger and Phosphorylation Research*; Greengard, P., Robinson, G. A., Eds.; Raven Press: New York, 1988; Vol. 22, pp 1−38.
(5) Beavo, J. A.; Reifsnyder, D. H. Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol. Sci*. **1990**, *11*, 150−155.
(6) Torphy, T. J.; Hay, D. W. P. Biochemical regulation of airway smooth muscle tone: An overview. In *Airway Smooth Muscle*: *Modulation of Receptors and Response*; CRC Press: Boca Raton, FL, 1990; pp 39−68.

(7) Kramer, G. M. The adenylate cyclase-cAMP-protein kinase pathway and regulation of immune response. *Immunol. Today* **1988**, *9*, 222−229.
(8) Torphy, T. J.; Undem, B. J. Phosphodiesterase inhibitors: New opportunities for the treatment of asthma. *Thorax* **1991**, *12*, 19−27.
(9) Torphy, T. J. Phosphodiesterase enzymes: Molecular targets for novel anti-asthma agents. *Am. J. Respir. Crit. Care Med*. **1998**, *157*, 351−370.
(10) Soderling, S. H.; Beavo, J. A. Regulation of cAMP and cGMP signalling: New phosphodiesterases and new functions. *Curr. Opin. Cell Biol*. **2000**, *12*, 174−179.
(11) Torphy, T. J.; Livi, G. P.; Christensen, S. B. Novel phosphodiesterase inhibitors for the therapy of asthma. *Drug News Perspect*. **1993**, *6*, 537−545.
(12) Palfreyman, M. N. Phosphodiesterase inhibitors as antiasthmatic agents. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1994; Vol. *29*, pp 185−194.
(13) Norman, P. PDE4 Inhibitors 1998. *Expert Opin. Ther. Pat.* **1998**, *8* (7), 771−784.
(14) Teixeira, M. M.; Gristwood, R. W.; Cooper, N.; Hallewell, P. G. Phosphodiesterase (PDE)4 Inhibitors: Anti-inflammatory drugs of the future? *TIPS* **1997**, *18* (5), 164−170.
(15) Hebenstreit, G. F.; Fellerer, K.; Fichte, K.; Fischer, G.; Geifer, N.; Meya, U.; Sastre-y-Hernandez, M.; Schany, W.; Schratzer, M.; Soukop, W. Rolipram in major depressive disorder. Results of a double blind comparative study with imipramine. *Pharmacopsychiatry* **1989**, *22* (4), 156−160.
(16) Barnette, M. S.; Christensen, S. B.; Essayan, D. M. SB207499 (Ariflo), a potent and selective second-generation phosphodiesterase 4 inhibitor: in vitro anti-inflammatory actions. *J. Pharmacol. Exp. Ther*. **1988**, *284*, 420−426.
(17) IDDB Drug Report for roflumilast (BY-217).
(18) IDDB Drug Report for D-4396 (Sch-351591).
(19) LAS-31025. *Clin. Trials Monitor* **1997**, *69*, 25−26.
(20) Cavalla, D.; Gale, D. A case history of successful virtual research. *Drug News Perspect*. **1997**, *10*, 470−476.
(21) Cavalla, D.; Chasin, M.; Hofer, P.; Gehrig, A.; Wintergerst, P. Preparation of purine derivatives having phosphodiesterase IV inhibiting activity. WO 9931102A1, 1999; 44 pp.
(22) Gale, D. D.; Hofer, P.; Spina, D.; Seeds, E. A.; Banner, K. H.; Harrison, S.; Douglas, G.; Matsumoto, T.; Page, C. P.; Wong, R. H.; Jordan, S.; Smith, F.; Banik, N.; Halushka, P. V.; Cavalla, D.; Rotshteyn, Y.; Kyle, D. J.; Burch, R. M.; Chasin, M. Pharmacology of a new cyclic nucleotide phosphodiesterase type 4 inhibitor, V11294. *Pulm. Pharmacol. Ther.* **2003**, *16*, 97−104.
(23) Subrahmanyam, V.; Renwick, A. B.; Walters, D. G.; Price, R. J.; Tonelli, A. P.; Lake, B. G. Metabolism of a novel phosphodiesterase-IV inhibitor (V11294) by human hepatic cytochrome P450 forms. *Xenobiotica* **2002**, *32* (6), 521−534.
(24) Kleinman, E. F.; Campbel, E.; Giordano, L. A.; Cohan, V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, R. E.; Salter, E. D.; Hibbs, T. A.; DiCapua, F. M.; Bordner, J. Striking effects of hydroxamic acid substitution on the phosphodiesterase type 4 (PDE4) and TNFα inhibitory activity of two series of rolipram analogues; implications for a new active site model of PDE4. *J. Med. Chem*. **1998**, *41*, 266−270.
(25) Leonard, N. J.; Bryant, J. D. Regioselective electrophilic reactions on substituted purines. Predominant intermediacy of 6- or 8-purinyl carbanions. *J. Org. Chem*. **1979**, *44* (25), 4612−4617.
(26) Leonard, N. J.; Fujii, T. The synthesis of 7-substituted adenines through the use of a blocking group at the 3-position. Site of alkylation of 7-substituted adenines. *J. Am. Chem. Soc*. **1963**, *85*, 3719.
(27) Press, J. B.; Falotico, R.; Hajos, Z. G.; Sawyers, R. A.; Kanojia, R. M.; Williams, L.; Haertlein, B.; Kauffman, J. A.; Lakas-Weiss, C.; Salata, J. Synthesis and structure−activity relationship of 6-substituted purine derivatives as novel selective positive inotropes. *J. Med. Chem*. **1992**, *35* (24), 4509−4515.
(28) Pollok, B. A.; Fuog, E. D.; Robbins, M. D.; Fisher, D. A.; Umland, J. P.; Pillar, J. S.; Cheng, J. B. Baculovirus expression of human phosphodiesterase (PDE) IV enzymes: Comparison with rPDE-IV produced in mammalian and bacterial expression systems. Presented at the Baculovirus and Insect Cell Expression Symposium, Pinehurst, NC, March 26−30, 1995.
(29) Spina, D. The potential of PDE4 inhibitors in asthma or COPD. *Curr. Opin. Invest. Drugs* **2000**, *1* (2), 204−213.
(30) Xu, R. X.; Hassal, A. M.; Vanderwall, D.; Lambert, H. M.; Holmes, W. D.; Luther, M. A.; Rocque, W. J.; Milburn, M. V.; Zhao, Y.; Ke, H.; Nolte, R. T. Atomic structure of PDE4. Insights into phosphodiesterase mechanism and specificity. *Science* **2000**, *288*, 1822−25.
(31) Weishaar, R. E.; Burrows, S. D.; Kobylarz, D. C.; Quade, M. M.; Evans, D. B. Multiple molecular forms of cyclic nucleotide phosphodiesterase in cardiac and smooth muscle and in platelets. Isolation, characterization, and effects of various reference phosphodiesterase inhibitors and cardiotonic agents. *Biochem. Pharmacol*. **1986**, *35*, 787−800.
(32) Weishaar, R. E.; Kobylarz-Singer, D. C.; Keiser, J.; Haleen, S. J.; Major, T. C.; Rapundalo, S.; Peterson, J. T.; Panek, R. Subclass of cyclic GMP-specific phosphodiesterase and their role in regulating the effects of atrial natriuretic factor. *Hypertension* **1990**, *15*, 528−40.
(33) Houslay, M. D.; Sullivan, M.; Bolger, G. B. The multienzyme PDE4 cyclic adenosine monophosphate-specific phosphodiesterase family: Intracellular targeting, regulation, and selective inhibition by compounds exerting anti-inflammatory and antidepressant actions. *Adv. Pharmacol*. **1998**, *44*, 225−342.
(34) Bolger, G.; Michaeli, T.; Martins, T.; Souness, J. E.; Rao, S. Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterases. *Cell. Signalling* **1997**, *9* (3-4), 227−36.
(35) Muller, T.; Engel, P.; Fozard, J. R. Subtypes of the type 4 cAMP phosphodiesterases: Structure, regulation and selective inhibition. *TIPS* **1996**, *17*, 294−298.
(36) Obernolte, R.; Ratzliff, J.; Baecker, P. A. Multiple splice variants of phosphodiesterase PDE4C cloned from human lung and testis. *Biochim. Biophys. Acta* **1997**, *1353*, 287−297.
(37) Engels, P.; Adbel' Al, S.; Hulley, P. Brain distribution of four rat homologues of the Drosophila dunce cAMP phosphodiesterase. *J. Neurosci. Res*. **1995**, *41*, 169−78.

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