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Perspective

Recent Advances on Phosphodiesterase 4 Inhibitors for the Treatment of Asthma and Chronic Obstructive Pulmonary Disease

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1. Introduction: Chronic Obstructive Pulmonary Diseases and Asthma. A Major Public Health Burden

Bronchial asthma, a complex multifactorial disease related to the respiratory system, is characterized by hyperactivity of the respiratory tract to external stimuli such as cold or warm or moist air, exercise, exertion, and emotional stress. Under this condition the airway occasionally constricts, becomes inflamed, and is lined with excessive amounts of mucus. This airway narrowing and inflammation lead to a number of severe lung diseases including asthma and chronic obstructive pulmonary diseases (or COPD, also known as chronic obstructive airway disease, chronic obstructive lung disease, or chronic airflow limitation and chronic airflow obstruction). The airflow limitation is usually progressive and associated with abnormal inflammatory response of the lungs to noxious particles or gases. COPD and asthma, a major public health burden worldwide, are reported to be causing the deaths of more than 250 000 people every year according to the latest WHO statistics (2007). COPD is projected to be the third leading cause of death by 2020. The estimation that 300 million people worldwide have asthma and 210 million people have COPD highlights the continued need for improved therapies. Bronchodilator drugs are currently the preferred choice for the symptomatic management of COPD, as they are more effective and have fewer side effects. However, these agents do not address the underlying chronic inflammation or the changes in airway structure. While the introduction of more effective treatments and the use of nonpharmacological interventions, such as pulmonary rehabilitation and noninvasive ventilation (NIV), have improved the management of COPD considerably,[1] no existing therapies have been shown to reduce the disease progression (via limiting or preventing the progressive and destructive changes of airways observed in COPD). Notably, among the new anti-inflammatory agents currently being developed, phosphodiesterase 4 (PDE4) inhibitors proved to be very effective in attenuating the responses of various inflammatory cells through their ability to elevate cyclic 3′,5′-adenosine monophosphate (cAMP) levels. This review highlights the design and structure−activity relationships of the PDE4 inhibitors for oral and inhaled delivery reported over the past 10 years along with the status of advanced clinical candidates. Also, an attempt was made to mainly cover the PDE4 inhibitors reported in various scientific journals rather than in patent literature.

2. Phosphodiesterases: Their Functional Significances

Many biological responses (e.g., regulation of important cell functions such as secretion, contraction, metabolism, and growth)[2] are mediated by levels of cyclic nucleotides, mainly cAMP and cyclic 3′,5′-guanosine monophosphate (cGMP). The presence of these cyclic nucleotides or intracellular messenger molecules has regulatory effects on protein kinase A (PKA) and protein kinase G (PKG), the guanine−nucleotide exchange factors (GEFs), and the cyclic-nucleotide gated (CNG) sodium and calcium channels. Manipulation of cAMP and cGMP levels in the cell represents a powerful mechanism for controlling cellular physiology. To maintain their intracellular levels, cAMP and cGMP are synthesized by adenylyl cyclases (ACs) and guanylate cyclases (GCs) whereas their degradation (hydrolysis) is mediated by a variety of phosphodiesterases (PDEs) present in the cells (Figure 1). PDEs hydrolyze the phosphodiester bond of cAMP and cGMP to provide the inactive products, e.g., 5′-

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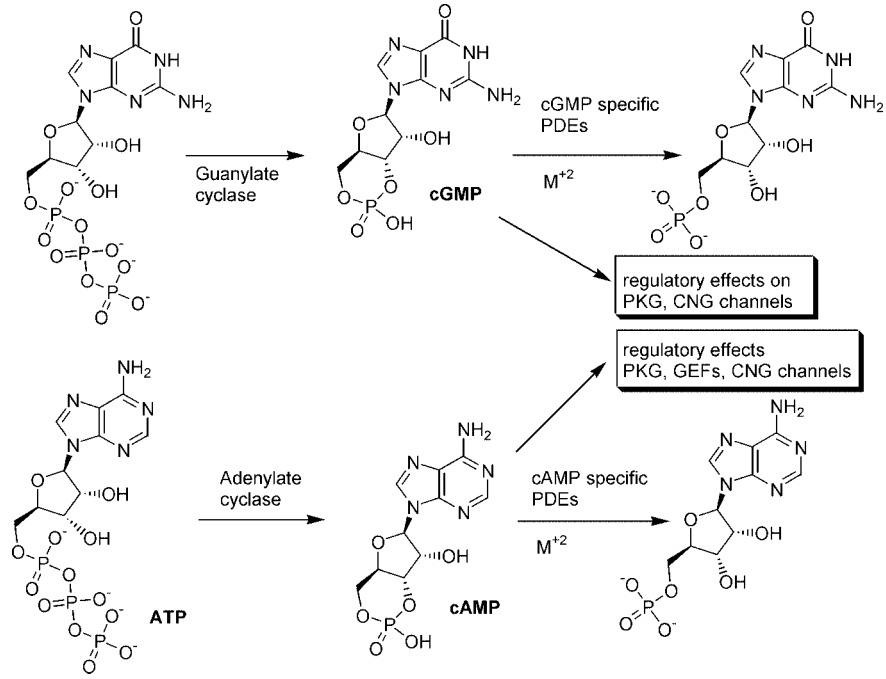
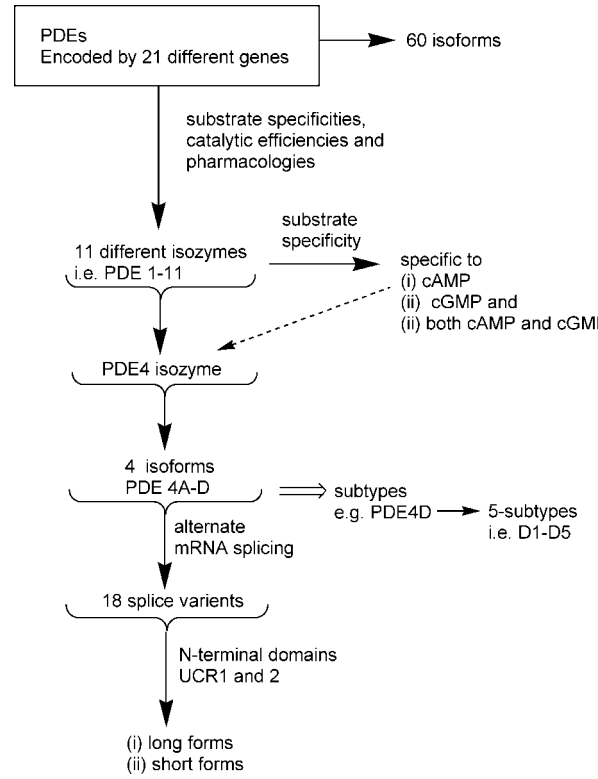


Figure 1. Synthesis of cGMP and cAMP (that regulate several effectors) and their degradation by PDEs.

AMP and 5′-GMP. In humans, PDEs are encoded by 21 different genes with over 60 isoforms expressed as a result of alternative splicing patterns. [3] Although a wide variation can be observed among full-length enzymes, the catalytic or terminal domains share common functionality and similar molecular structures with several conserved amino acid residues (270 amino acids fused to additional N- and/or C-terminal sequences that contain distinct domains unique to the members of a PDE family). Key residues in the catalytic core form a hydrophobic pocket that accommodates the plannar nucleotide in a spatial orientation, thereby facilitating H-bonding to an absolutely conserved glutamine residue. [4] These catalytic domains determine several properties of the PDEs. For example, variations in the amino acid sequence at the catalytic domain and elsewhere give rise to unique substrate specificities, catalytic efficiencies, and pharmacologies. Therefore, PDEs can be subdivided into 11 different groups or isozymes, e.g., PDE1 to PDE11 (Chart 1 and Table 1).

However, on the basis of their substrate specificity (or nucleotide selectivity), all these PDEs can be classified as those that are (i) specific to cAMP and (ii) specific to cGMP and (iii) those that act on both cAMP and cGMP. The cAMP specific PDE4 isozymes[5a] (which require a divalent metal ion, e.g., Zn for catalysis) are encoded by four genes (A−D) that give rise to four isoforms, e.g., from PDE4A to PDE4D (Table 2). They are located on three different chromosomes. Each of the PDE4 isozyme genes can result in multiple splice variants, leading to a number of subtypes (for example, subtypes of PDE4D include D1, D2, D3, D4, and D5). All are specific for cAMP, but they differ in their tissue, cellular, and subcellular distribution. They also differ in their sensitivity to inhibitor (Table 2) such as rolipram. The PDE4A, B, and D isoforms are expressed in many inflammatory cells in human (and are therefore of more relevance to the asthmatic disease state), whereas the PDE4C enzyme is mainly found in testis, skeletal muscle, the central nervous system (CNS), and human fetal lung.[5b] A further complexity arising through alternative mRNA splicing generates a number of distinct PDE4 splice variants with unique N-terminal regions.

Chart 1. Classifications of PDEs



There are at least 18 different splice variants of the four PDE4 isoforms known. These variants are termed as long and short forms depending on the presence or absence of two unique N-terminal domains called upstream conserved regions 1 and 2 (UCR1 and 2), a structural feature unique to the cAMP-specific PDE4 family. UCR1 and UCR2 have been shown to form a module necessary for the activation of PDE4 upon phosphorylation by the cAMP-dependent kinase (PKA). Moreover, UCRs appeared to mediate dimerization[6] of the cAMP-specific PDE4. It is generally accepted that most PDEs exist as dimers or

Table 1. Pharmacological Properties/Characterizations of PDEs[a]

PDE	regulatory mechanism	distribution: lung	distribution: inflammatory cells	distribution: other tissues	inhibited by
PDE1	Ca^{2+}/calmodulin-stimulated	yes		heart	calmidazolium chloride, chlorpromazine·HCl, 8-methoxymethyl-3-isobutyl-1-methylxanthine, vinpocetine
PDE2	c-GMP-stimulated	yes		heart	erythro-9- (2- hydroxy-3- nonyl) adenine hydrochloride (EHNA·HCl)
PDE3	cGMP-inhibited	yes	yes	heart	cilostamide, milrinone, trequinsin·HCl
PDE4	cAMP-specific	yes	yes	kidney	denbufylline, etazolate·HCl, PDE-4 inhibitors, Ro-20-1724, rolipram
PDE5	cGMP-specific	yes		smooth muscle cells	dipyridamole, 4-{[3′,4′-(methylenedioxy)benzyl]amino}-6-methoxyquinazoline, 1-(3-chloroanilino)-4-phenylphthalazine (MY-5445), PDE V inhibitor II, zaprinast, sildenafil
PDE6	photoreceptor cGMP-specific			photoreceptors	zaprinast, dipyridamol, (sildenafil)
PDE7	cAMP-specific, high-affinity, rolipram-insensitive	yes	yes	skeletal muscle cells	3-(*N*,*N*-dimethylsulfonamido)-4-methylnitrobenzene (BRL 50481), IC242, not sensitive to rolipram
PDE8	cAMP-selective, IBMX-insensitive		yes	testes	sensitive to dipyridamole, insensitive to other inhibitors
PDE9	cGMP-selective, IBMX-insensitive	yes		kidney	zaprinast, sildenafil (~750-fold less active than PDE5)
PDE10	cGMP-sensitive			testes, brain	none disclosed
PDE11	cGMP-sensitive, dual-specificity			prostate	none disclosed

[a] 3-Isobutyl-1-methylxanthine (IBMX) and pentoxifylline are nonselective inhibitors of PDEs.

Table 2. Pharmacological Properties/Characterizations of PDE4

subtype (mcM)	isoforms (kDa)	primary tissue distribution	subcellular localization	IC_{50} to rolipram (μM)
PDE4A	PDE4A1 (80)	lung	cytosol	0.29 ± 0.09
	PDE4A4B	inflammatory cells	cytosol	1.25 ± 0.15
			particular fraction	0.15 ± 0.07
	PDE4A10 (120)	inflammatory cells	cytosol	0.06 ± 0.01
		brain, lung	particular fraction	0.05 ± 0.01
PDE4B	PDE4B1 (84)	lung	cytosol	0.08 ± 0.03
			particular fraction	0.05 ± 0.01
	PDE4B2 (64)	lung	cytosol	0.02 ± 0.01
			particular fraction	0.21 ± 0.03
	PDE4B3 (82)	lung	cytosol	0.05 ± 0.01
		particular		0.10 ± 0.03
	PDE4B4 (85)	liver, brain	cytosol only	0.08 ± 0.02
PDE4C	PDE4C-791	only in lung		
	PDE4C-461	lung		
	PDE4C-δ54	only in testis		
	PDE4C-δ109	testis		
PDE4D	PDE4D1 (68)	lung	cytosol only	0.05 ± 0.01
	PDE4D2 (68)	lung	cytosol only	0.05 ± 0.01
	PDE4D3 (95)	lung	cytosol	0.14 ± 0.01
			particular fraction	0.32 ± 0.05
	PDE4D4 (119)	lung	cytosol	0.06 ± 0.01
			particular fraction	0.05 ± 0.02
	PDE4D5 (105)	lung	cytosol	0.08 ± 0.01
			particular fraction	0.59 ± 0.05

oligomers and the dimerization domain is located at the N terminus of the protein. Thus, the overall structural organization of a PDE4 splice variant includes a catalytic domain flanked by regulatory and dimerization domains at the N terminus. UCRs are also thought to bind to signaling molecules such as lipids.

3. Selective Inhibition of PDE4: A Major Pharmaceutical Focus

It is now better understood that the intensity of inflammatory process increases as COPD progresses.[7] Mediators of inflammatory response include immunocompetent cells such as T cells, B cells, monocytes, neutrophils, eosinophils, and macrophages. cAMP plays a critical role in mediating inflammatory, and thereby cytokine, responses.[8] In the inflammatory cells cAMP plays the role of a negative regulator of the primary activating pathways such as cytokine release by T-cells. Levels of cAMP on the other hand are regulated by cAMP-specific PDE isozymes (e.g., PDE4 predominantly expressed in inflammatory and immune cells in addition to brain[9]) in the case of mediators of inflammatory response. Inhibition of the PDE4 in these cells effectively elevates the intracellular cAMP levels, thereby activating specific protein phosphorylation cascades that elicit a variety of functional responses. This in turn inhibits the release of inflammatory mediators such as cytokines [tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interleukin-12 (IL-12), leukotriene B4 (LTB4), interferon-γ (IFN-γ[a])], as well as activation of inflammatory cells and thereby inactivation of the anti-inflammatory response. Since the cellular mediators play a key role in the inflammatory diseases such as asthma and COPD (along with rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, and multiple sclerosis), the development of PDE4 inhibitors as therapeutic agents therefore has been a major pharmaceutical focus (Charts 2 and 3). Additionally, elevation of intracellular cAMP levels via inhibition of PDE4 activity led to smooth muscle relaxation and thereby bronchodilation that is beneficial for the management of respiratory diseases like asthma or COPD.

Elevation of cAMP levels has several other beneficial effects that include inhibition of mast cell mediator release, suppression of neutrophil degranulation, inhibition of basophil degranulation, and inhibition of monocyte and macrophage activation. Moreover, the connection between PDE4 activity and cognition has been speculated ever since the discovery that the cAMP-regulating dunce gene of the fruit fly encodes a PDE4 homologue.[10,11] Another two important roles for PDE4D have recently been identified in cardiovascular tissue; they are (i) a correlation for PDE4 polymorphism with stroke and (ii) an involvement in vascular smooth muscle cell proliferation.[12a] Finally correlation between PDE4D and osteoporosis has also

[a] Abbreviations: NANC, non-noradrenergic, noncholinergic transmitter; CD8+ T cells, cytotoxic T cells with cluster of differentiation 8 (CD8) surface protein; CD11b, cluster of differentiation molecule 11b; IFN-γ, interferon-γ; GST-PDE4A, PDE4A fused to glutathione *S*-transferase; PEG200, polyethylene glycol 200.

Chart 2. Therapeutic Actions of PDE4 Inhibitors (Asthma)

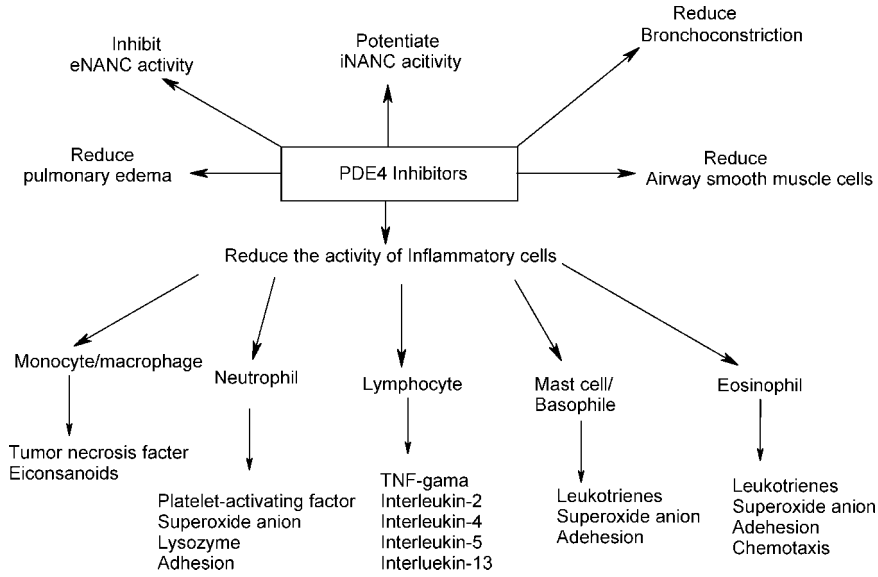
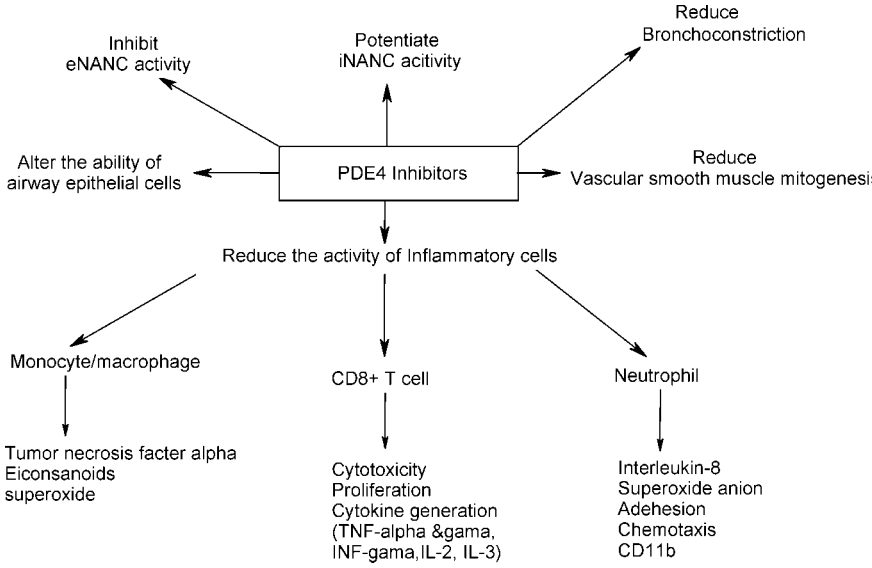


Chart 3. Therapeutic Actions of PDE4 Inhibitors (COPD)



been reported. Specifically single nucleotide polymorphisms mapping to the PDE4D gene are linked to variability in bone mineral density.[12b] However, in view of five subtypes of PDE4D, it was hypothesized that inhibition with the selectivity order of D5 > D3 > B > A > D2 could lead to the decreased side effects associated with most of the first and second generation PDE4 inhibitors (see later for a detailed discussion), and improved therapeutic index.[13a−c] This was supported by the observation that dual PDE4A/B inhibition has a significant correlation with the inhibition of lipopolysaccharide (LPS)-stimulated TNF-α secretion and with T-cell proliferation in peripheral blood monocytes. However no such correlation was found for PDE4D inhibition. Notably, the PDE4C not generally found in proinflammatory cells is highly expressed in the CNS[13d] where most of the PDE4 inhibitors are believed to promote many of their side effects such as emesis. Thus, selectivity for PDE4D at the same time minimizing PDE4C inhibition could be a useful drug design strategy for the development of novel PDE4 inhibitors. It is, however, worth mentioning that gastrointestinal (GI) side effects and vascular toxicity are other major challenges[14a−d] in addition to emesis in the development of PDE4 inhibitors.

Inflammatory leukocytes infiltrate the airways of asthmatic patients in which eosinophils are the major component and are accumulated in the lungs. When activated, they synthesize and release inflammatory cytokines such as interleukin-1 (IL-1), IL-2, and TNF-α[14e,f] and also inflammatory mediators. Overproduction of TNF, a serum glycoprotein, is associated with a number of autoimmune and inflammatory diseases such as asthma, septic shock, AIDS, and rheumatoid arthritis. Since TNF-α production in proinflammatory cells is reduced by an elevation of intracellular cAMP (which in turn is regulated by PDE family of enzymes), the selective inhibition of PDE4, thereby inhibiting the conversion of cAMP to 5′-AMP, reduces the TNF-α production and its release into the blood.

4. PDE4 Inhibitors as Therapeutic Agents

As mentioned earlier, development of PDE4 inhibitors involves several challenges, e.g., emesis, GI side effects, and vascular toxicity. While a wide variety of inhibitors have been

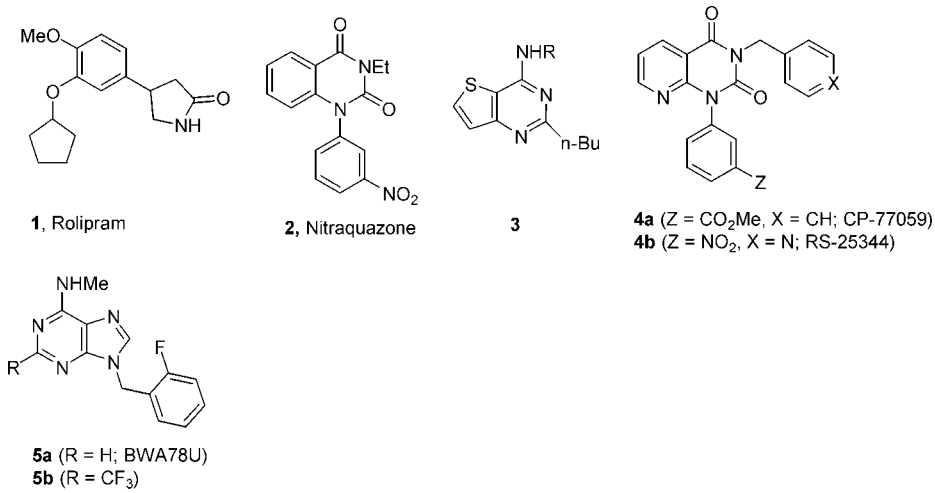


Figure 2. Structures of rolipram (**1**), nitraquazone (**2**), and its analogues.

reported, until recently no PDE4 inhibitor has been launched for use by patients. Initially, on the basis of the structural features, selective PDE4 inhibitors were broadly divided into several classes, e.g., (i) structural analogues of rolipram, (ii) structural analogues of nitraquazone, (iii) structures related to xanthines, (iv) substituted phthalazines, and (v) others. However, such a broad classification was found to be irrelevant at a later stage mainly because of the wide structural diversity of PDE4 inhibitors reported to date. Alternatively, PDE4 inhibitors could be classified on the basis of the strategies followed to design them. The recent approaches that are generally followed to obtain potent and selective inhibitors with an improved therapeutic index include development of new compounds (i) selective toward one subtype, e.g., PDE4A and B, (ii) with original chemical structures, which are completely unrelated to catechol ether derivatives (e.g., rolipram), and (iii) structurally related to the natural substrate cAMP. While first two approaches were found to be effective to obtain PDE4 inhibitors having reduced emetic side effects in ferrets, the third one yielded compounds that produced fewer gastrointestinal side effects in rats. Nevertheless, several interesting recent PDE4 inhibitors are presented in the following sections.

5. Rolipram: The Earliest PDE4 Inhibitor and Related Agents

Rolipram (**1**, Figure 2) or (4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone) from Schering belongs to the diether derivative of catechol class and is one of the earliest and most extensively studied PDE4 inhibitors. A detailed SAR study suggested that the 4-(3,4-dialkoxyphenyl) moiety was important for PDE4 inhibition where the catechol ether oxygens played a crucial role in binding to the enzyme. The substituent at the 4-position of the phenyl ring was restricted to small lipophilic groups, preferably methoxy, indicating that this group might occupy a small lipophilic pocket. In contrast, various alkoxy substituents were well tolerated at the 3-position, suggesting that 3-substituents either fill a large hydrophibic cavity or were located near the enzyme surface. Thus, a 3-cyclopentyloxy substituent yielded a 10- to 100-fold enhancement in potency. Rolipram was clinically tested as an antidepressant for several years before the discovery of its potent and selective PDE4 inhibitory activity.[15] It has an IC_{50} of approximately 1.0 μM (lower under some conditions) and shows at least a 100-fold selectivity for this PDE family. However, its development as a therapeutic has been halted mainly because of undesired side effects such as nausea, vomiting, and increased gastric acid secretion (rolipram causes emesis in ferrets even at doses as low as 0.1 mg/kg po). Notably, these are also common side effects of many other PDE4 inhibitors including 4-(3-butoxy-4-methoxyphenyl)methyl-2-imidazolidone (Ro20-1724), another diether derivative of catechol. It was initially believed that in addition to the PDE4 catalytic site (rolipram $K_i = 1-2$ μM) there exists a high affinity rolipram binding site (rolipram $K_d = 1-2$ nM). Analogues of rolipram[16,17] and other PDE4 inhibitors like nitraquazone (**2**, Figure 2)[18,19] and congeners[20] exhibited rolipram binding site affinity in the nanomolar range. Since the high-affinity rolipram binding site seemed to be responsible for emesis and nausea, hence to obtain more effective agents potentially useful as antiasthmatics, it became important to establish selectivity for PDE4 inhibitory activity over this binding site. This goal was achieved using several rolipram analogues[21,22] and other series of compounds (see later for a detailed discussion). The compound nitraquazone (**2**) is known for its notable anti-inflammatory and analgesic pharmacological profile. Several compounds structurally related to nitraquazone have been developed as PDE4 inhibitors. For example, a series of thieno[3,2-*d*]pyrimidines[23a] (**3**, Figure 2) showed good activity in both PDE4 inhibition and cAMP potentiation and displayed an improved ratio with respect to the [^{3}H]rolipram specific binding site. The analogue **4a** (Figure 2) showed interesting anti-inflammatory properties in the carrageenan-induced rat paw edema,[23b] and **4b** (Figure 2) was identified as a potent PDE4 inhibitor. [23c]

On the basis of the earlier report that 9-benzyladenine derivative **5a** (Figure 2) possesses anticonvulsant effects in addition to anxiolytic and sedative properties, a series of 9-substituted adenines[24] were prepared and tested for PDE inhibiting properties. Accordingly, compound **5b** (NCS 613, Figure 2) was identified as a highly selective inhibitor of PDE4 (IC_{50} values of 40, 380, 0.04, and 5 μM for PDE1, PDE3, PDE4, and PDE5, respectively), and other than the high-affinity rolipram binding sites, another site of action (partially overlapped with the cAMP binding site)[25] in PDE4 was postulated for this adenine derivative.

6. Alternative Pharmacophores for Pyrrolidinone Ring

Among the most potent analogues of rolipram (**1**) in which the pyrrolidinone ring was replaced by an appropriate pharmacophore (Figure 3) are **6** (piclamilast),[26] **7** (roflumilast),[27] and **8**.[28] Indeed, a detailed SAR work[26] on the effect of changes to

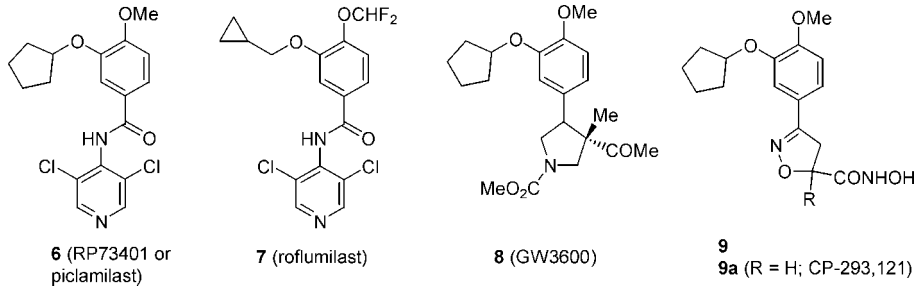
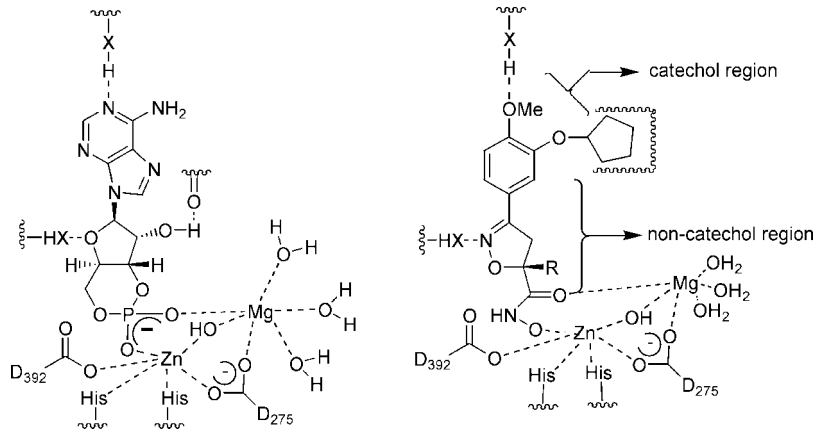


Figure 3. Structures of piclamilast (**6**), roflumilast (**7**), and other compounds.

Scheme 1. cAMP and Compound **9** Bound in the Hypothetical Active Site of PDE4 Represented by Wavy Lines[29b]



alkoxy groups, amide linkages, and *N*-phenyl ring of benzamide moiety on PDE4 inhibition led to the identification of 3,5-dichloropyridyl-4-carboxamide as an effective pharmacophore. However, the clinical development of **6** (Sanofi-aventis) and **8** has been discontinued.

On the basis of the fact that (i) the zinc ion in the active site (because PDE4 requires a divalent metal ion for catalysis) is coordinated by two histidines and two aspartates[29a] and (ii) the hydroxamic acid group is a well-known metal chelator, two novel series of rolipram analogues, 3-aryl-2-isoxazoline-5-hydroxamic acids **9** (Figure 3) and their acyclic counterparts *N*-aroylaminohydroxamic acids, were synthesized and tested for PDE4 inhibitory activity.[29b] The (*R*)-isomer **9a** (Figure 3), the most potent compound with an IC_{50} of 0.024 μM, was identified as a promising inhibitor of PDE4 and TNF-α release in human whole blood (HWB). By overlap of the structures of these compounds with cAMP (based on close structural resemblance of the noncatechol regions of these series to the ribose 3′,5′-phosphate group of cAMP) and by taking into account their ability to bind to a metal in the active site of PDE4, a hypothetical model of interaction between inhibitor and enzyme was proposed (Scheme 1).

Another potent PDE4 inhibitor **10a** (Figure 4) (PDE4 IC_{50} = 4.5 nM and rolipram K_i binding 60 nM),[30] derived from rolipram by modifying the pyrrolidinone moiety, significantly reduced antigen-induced bronchoconstriction in animal models and in asthmatic patients. However, it suffered from extensive metabolism in vitro and thereby a short half-life in vivo. While its major metabolic pathway involved para-hydroxylation on the pendent phenyl group in rat and glucuronidation of the pyridine ring in human hepatocytes, other major sites of metabolism were centered on the catechol portion of the molecule with hydroxylation, dealkylation, and glucuronide and sulfate conjugation. Thus, highly potent [GST-PDE4A IC_{50} = 4.2 nM; HWB (TNFα) IC_{50} = 0.67 μM] and metabolically stable PDE4 inhibitor **10b** (Figure 4) containing a stable bis-difluoromethoxy catechol and a pendent bistrifluoromethyl-carbinol was developed.[31a] Athough it was found to be active in various models of in vivo [e.g., ovalbumin-induced bronchoconstriction model in guinea pig (58% at 1.0 mg/kg ip) and in the ascaris-induced bronchoconstriction models in sheep (85−95% at 2 mg/kg iv) and squirrel monkey (96% at 3 mg/kg po)] and was well tolerated in ferret with an emetic threshold greater than 30 mg/kg po, an excessively long half-life (>48 h

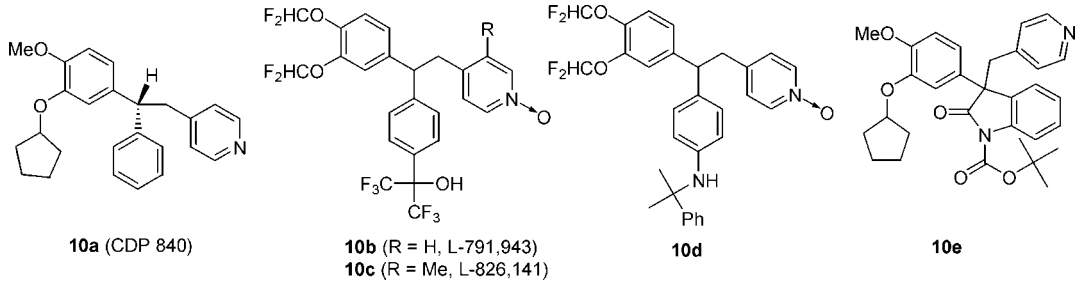


Figure 4. Triarylethanes and related compounds.

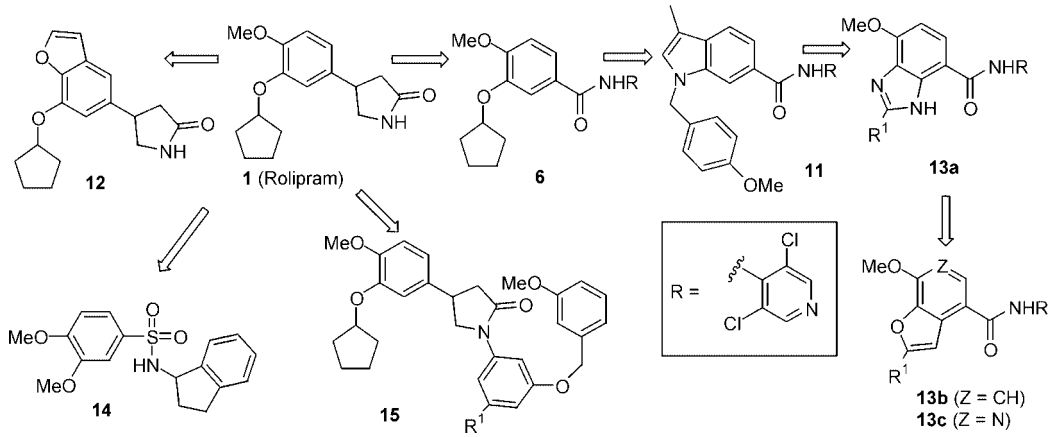


Figure 5. Compounds containing isoster for catechol moiety of rolipram (**1**).

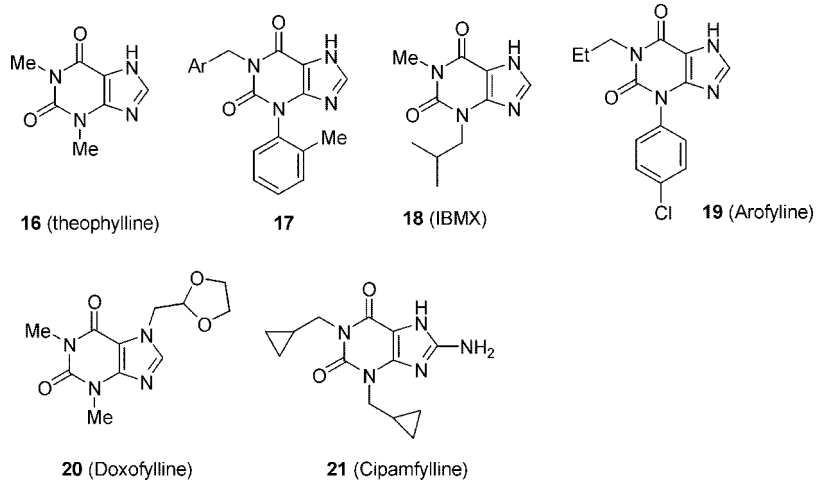


Figure 6. Theophylline (**16**) and related derivatives.

in rat, squirrel monkey, and dog) precluded the development of this compound. But the introduction of a soft metabolic site on the structure of **10b** permitted the identification of **10c** (Figure 4) as a potent PDE4 inhibitor [GST-PDE4A IC_{50} = 1.3 nM; HWB (TNFα) IC_{50} = 0.30 μM] that showed a shorter half-life (10, 36, and 19 h in rat, squirrel monkey, and dog, respectively) than **10b** and was well absorbed in a variety of animal species.[31b] It showed a bioavailability of 60% and a plasma level of 0.4 mM at 6 h when administered po at 3 mg/kg in 60% PEG200 in rat. The efficacy of **10c** was demonstrated in different in vivo models (82% at 1.0 mg/kg ip in guinea pig, 26−38% at 0.5 mg/kg po in squirrel monkey, and 62−90 at 2.0 mg/kg iv in sheep), and it showed no emetic side effects in ferret up to 30 mg/kg po. In order to reduce the half-life further, metabolically resistant phenyl bistrifluoromethylcarbinol of **10b** was replaced by a substituted aminopyridine residue, and a SAR study led to the identification of **10d** (Figure 4).[31c] Compound **10d** exhibited a good PDE4 inhibitor activity [GST-PDE4A IC_{50} = 0.8 nM; HWB (TNFα) IC_{50} = 0.12 μM] and an improved pharmacokinetic profile over **10b** (rat $t_{1/2}$ = 2 h). It was well tolerated in ferret with an emetic threshold of 30 mg/kg (po) and was found to be active in the ovalbumin-induced bronchoconstriction model in guinea pig (54%, 0.1 mg/kg, ip) as well as the ascaris-induced bronchoconstriction model in sheep (64%/97%, early/late, 0.5 mg/kg, iv). In another approach based on the report of impressive selectivity shown by **10a**, novel conformationally constrained quaternary substituted oxindole analogues of **10a** as exemplified by **10e** (Figure 4) were evaluated for PDE4 inhibitory activities.[31d] Owing to their observed PDE4 activity in the submicromolar range (IC_{50} = 0.7 μM; K_i rolipram binding of 2.8 μM) and reasonable selectivity for the catalytic binding site over the rolipram binding site, these compounds had potential for addressing the emetic side effects.

7. Uncovering Isostere for Catechol Moiety

While the presence of 3-methoxy-4-cyclopentoxy motif (catechol region of rolipram) was found to be common in the majority of PDE4 inhibitors, the indole[32] or indazole[33] skeleton was identified as an effective isoster for catechol. Thus, a novel series of potent PDE4 inhibitors was reported by replacing the catechol-like rolipram motif of **6** (Figure 3) with a 3,4-substituted indole derivative (**11**, Figure 5; PDE4 IC_{50} = 12 nM and K_i rolipram binding of 13 nM). Notably, benzofuran was also found to be an isoster for catechol as exemplified by potent PDE4 inhibitors **12** (Figure 5).[34,35] In a further effort to develop PDE4 inhibitors and at the same time to address several issues associated with other inhibitors like susceptibility to oxidative metabolism and low water solubility, a new series **13a** (Figure 5) was developed where the 3,4-dialkoxyphenyl group of **6** was replaced with a 2-substituted-7-methoxybenzimidazole moiety.[36a] These compounds including their *N*-oxide derivative retained the required inhibitory activity against PDE4 (**13**, R^1 = CH_2OMe, PDE4 IC_{50} = 42 nM and K_i rolipram binding of 25 nM) and showed good oral bioavailability (35−87%) in mouse. The equipotency of the *N*-oxide derivative with their parent compound suggested that the enzyme−inhibitor

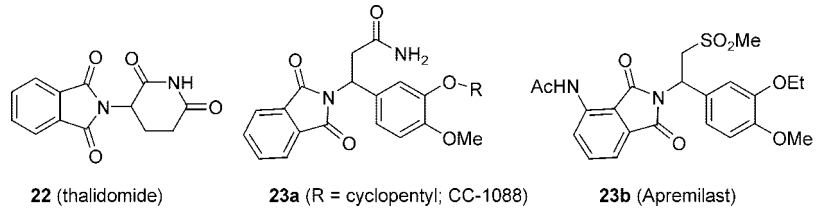


Figure 7. PDE4 inactive thalidomide (**22**) and its PDE4 active derivatives.

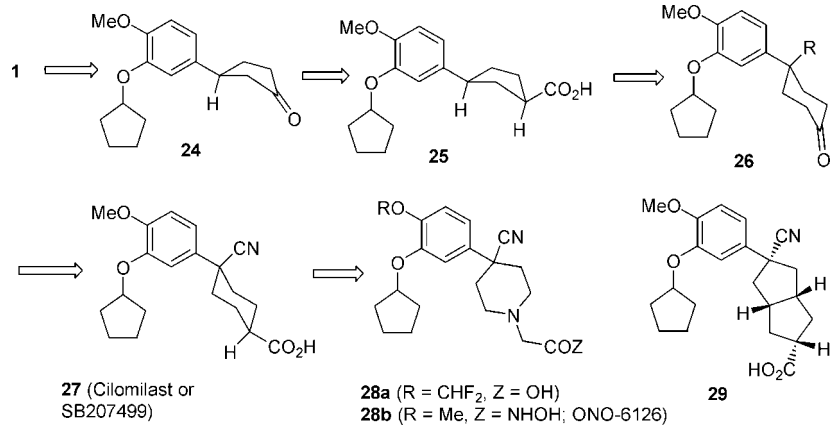


Figure 8. Cilomilast and related inhibitors.

interaction was perhaps mediated by a water bridge instead of a direct H-bond. However, in general the PDE4 activity to rolipram binding ratio of benzimidazoles was not acceptable, and therefore, this series was not investigated further. Following a similar strategy, 2-alkyl-7-methoxybenzofuran derivatives **13b** (Figure 5) as potent inhibitors of PDE4 (PDE4 $IC_{50} = 0.4-2$ nM, TNF-α $ED_{50} = 20-50$ mg/kg po) were reported.[36b,c] However, observed emetic reaction in dogs (when administered iv at 0.3 mg/kg) caused by these compounds precluded their further development. At a later stage replacement of the 7-methoxybenzofuran moiety of **13b** by 7-methoxyfuro[2,3-*c*]pyridine (**13c**) was reported[36d] where the incorporation of the nitrogen atom was intended to improve the pharmacokinetic properties of this series over the benzofurans. 7-Methoxy substituent of **13c**, as indicated by SAR study, was critical for PDE4 inhibition, and replacing it with a difluoromethoxy group enhanced the potency. The most potent compound (R = Et; PDE4 $IC_{50} = 14.0$ nM, rolipram binding affinity $IC_{50} = 84$ nM) showed good oral bioavailability (54% at 3 mg/kg po in guinea pig) and 40% inhibition of lung eosinophila at 10 mg/kg po in guinea pig lung eosinophila model. Interestingly, to identify an orally active PDE4 inhibitor devoid of nausea/emesis in man but maintaining the full spectrum of beneficial biological actions, a novel sulfonamide series **14** (Figure 5, PDE4 $IC_{50} = 6$ μM and 12% rolipram binding affinity at 10 μM when R = indan-1-yl) was identified.[37] These compounds showed good selectivity for the catalytic site of PDE4 over the high affinity rolipram binding site and no emetic side effects up to the highest dose (10 mg/kg ip) tested in ferret. N-Arylation of rolipram[38a] led to the identification of another series **15** (Figure 5) as highly potent and selective PDE4 inhibitors that showed promising activity in an animal model of airway inflammation. Very recently, 1-phenyl-2-pyridynyl alkylene alcohols have been reported as inhibitors of PDE4.[38b]

8. Xanthine-Based Inhibitors

Theophylline (**16**, Figure 6), a dimethylxanthine that has been used to treat asthma for over 60 years, was found to be a weak and nonselective inhibitor of PDE4[39] (39% PDE4 inhibition at 200 μM and 27% rolipram binding affinity at 100 μM). However, its clinical use was limited by adverse reactions on the cardiovascular and central nervous systems as well as its narrow therapeutic index and high interindividual variability in absorption, metabolism, and clearance. [40] Since theophylline has only weak affinity for the rolipram binding affinity, its observed side effects were thought to be associated with activity against other PDE enzymes and adenosine receptors. A further SAR (structure−activity-relationship) study on theophylline analogues resulted in identification of a novel series of xanthines[41] **17** (Figure 6) with acceptable PDE4 activity and improved selectivity (PDE4 $IC_{50} = 4.2$ μM and K_i rolipram binding of 1.02 μM when Ar = 2-thienyl). No emesis or CNS related side effects were observed when dosed orally to ferrets at 10 mg/kg. Other members that belong to the family of xanthine derivatives include 3-isobutyl-1-methylxanthine (IBMX, **18**), arofylline (**19**) from Almirall presently in phase 3 clinical trial, doxofylline (**20**) from Instituto Biologico Chemioterapico already launched, and cipamfylline (**21**) from GSK. Although some of the xanthine derivatives are either under clinical trials or launched, in general these inhibitors are nonselective and relatively weak inhibitors of PDE4.

9. Thalidomide and Its Derivatives

Owing to its effective immunomodulatory properties, thalidomide **22** (Figure 7), known to be a selective inhibitor of TNF-α production (TNF-α $IC_{50} \approx 200$ μM) in activated monocytes, has never totally disappeared from pharmaceutical use in spite of its teratogenic properties. By use of PDE4 inactive thalidomide as a lead structure, its analogues **23** (Figure 7) derived from 3-amino-3-arylpropionic acids were developed that were found to be potent inhibitors of TNF-α as well as PDE4.[42] Although thalidomide was inactive against PDE4, the TNF-α inhibitory properties of its analogues **22** were proposed to be due to their PDE4 inhibition. Two of these compounds, **23a** (PDE4 $IC_{50} = 1.1$ μM, TNF-α $IC_{50} = 2.5$ μM) of Celgene and **23b** (Apremilast), are presently undergoing phase 2 clinical trial.

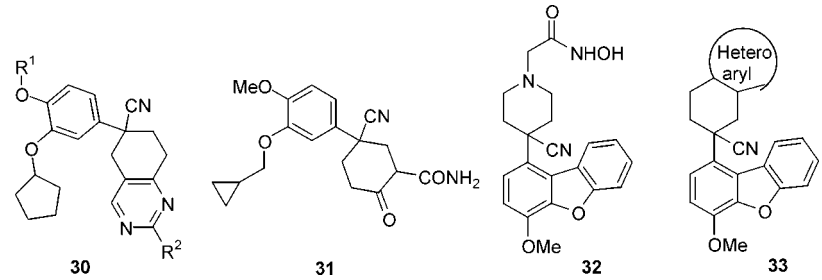


Figure 9. Cyclohexane-based inhibitors.

10. Benzylic Nitrile as an Effective Pharmacophore: Cilomilast and Related Agents

To identify novel, selective (for PDE4 versus other PDE isozymes), orally active second-generation PDE4 inhibitors as anti-inflammatory and antiasthmatic agents with decreased potential for the side effects, *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid[43] was designed and explored. On the basis of the earlier information available on the steric and electronic structural requirements for selective PDE4 inhibition, the 3-(cyclopentyloxy)-4-methoxyphenyl group and an appropriately positioned hydrogen bond acceptor moiety were identified as the minimum pharmacophoric requirements, consistent with results subsequently reported by other researchers.[44a] Moreover, a comparison of cyclopentanone derivative **24** (Figure 8) with (*R*)- and (*S*)-isomer of **1** (Figure 2) revealed that the NH function of the rolipram pyrrolidinone ring was not essential for PDE4 inhibition. The carboxyl function of cyclopentanecarboxylate **25** (Figure 8) served as a slightly poorer hydrogen bond acceptor, as it was less potent than **24** in terms of PDE4 inhibition. To avoid the conformational flexibility caused by the five-membered ring of **24** or **25** (as the aryl group could adopt either a pseudoaxial or pseudoequatorial orientation with respect to the carbonyl or carboxyl moieties), cyclohexanone **26** (Figure 8) was designed and evaluated. Since the cyano ketone (**26**, R = CN) showed improved PDE4 activity and selectivity over others (**26**, R = H, acetylene, amide, aldehyde, or fluoromethyl), further SAR work was carried out maintaining the benzylic nitrile as a third pharmacophore element. Accordingly, compound **27** (Figure 8) was identified as the best compound and has completed phase 3 clinical trial. While **27** showed 10-fold selectivity for PDE4D versus other PDE4 isozymes, in clinical trials, it still induced emesis at the first and/or second doses, albeit this effect apparently disappeared with continued treatment.[44b]

Nevertheless, the markedly improved therapeutic index of **27** over **1** was due to the selective inhibition of PDE4 compared to its low activity toward the [^{3}H]rolipram-binding site and its selective inhibition of PDE4D subtype in comparison to **1**, a known competitive inhibitor of PDE4A.[45] However, the limited penetration of **27** into the central nervous system (CNS) due to the negative charge at the physiological pH was also thought to be responsible for decreased occurrence of side effects. On the basis of this hypothesis that the dose-limiting side effects of PDE4 inhibitors such as nausea and emesis could be caused by an effect on the CNS[46] (and to address the issue of configurational isomerism of **27** without losing the inhibitory activity), the design and synthesis of piperidine derivatives as hydrophilic inhibitors, which were predicted to show limited penetration of the CNS, were carried out.[46] The lead compound **28a** (Figure 8) showed better potency (PDE4 IC_{50} = 65 nM, TNF-α HWB IC_{50} = 0.84 μM) than **27** and was expected to show an improved side effect profile as indicated by study on

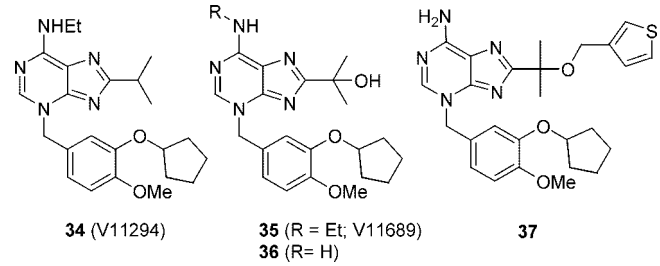


Figure 10. Purine-based inhibitors.

inhibition of gastric emptying in rats (ID_{50} = 4.7 mg/kg po) and emesis in ferrets (no emesis up to 10 mg/kg po). A hydroxylamine derivative **28b** (Figure 8) was advanced to phase 2 clinical trial.

In a separate study, it was hypothesized that more rigid spatial fixing of the three pharmacophores (the carboxylic acid, nitrile, and aromatic moieties) of **27** within their optimized stereochemistry using a bicyclo[3.3.0]octane template instead of a cyclohexane template could lead to the discovery of a new inhibitor. Accordingly, design, synthesis, and evaluation of bicyclo[3.3.0]octane derivatives were carried out[47] and one of the lead molecules **29** (Figure 8) showed improved therapeutic potential (PDE4 IC_{50} = 9.0 nM, TNF-α HWB ID_{50} = 21 μM) with fewer side effects (compound **29** caused more than 50% inhibition of gastric emptying at 10 mg/kg po in rats).

Recently, cyclohexane derivatives as PDE4 inhibitors maintaining the benzylic nitrile as one of the pharmacophore have been reported (PDE4 IC_{50} = 0.003−1.0 μM).[48] The cyclohexane ring was essentially fused with a six- or five-membered heterocyclic ring as exemplified by compound **30** (Figure 9). Among the other cyclohexanone/piperidine derivatives reported are compounds **31**,[49] **32**,[50] and **33**.[51]

11. Focus on PDE4 Subtype Selectivity: Purine Derivatives

In the search of a purine-based nonemetogenic PDE4 inhibitors, a detailed SAR work around the purine ring skeleton particularly at N-3, C-8, and the amino group was carried out. This effort resulted in identification of **34**[52] (possessing "rolipram-like" disubstituted catechol moiety at N-3) (Figure 10) that in addition to its activity in a number of in vitro and in vivo models of inflammation[53] selectively inhibited human lung hPDE4 (K_i = 436 nM) and was found to be nonemetogenic. Interestingly, the 8-benzyloxy analogue of **36** derived from **35**, the 8-hydroxy human metabolite[54] of **34**, was found to be more potent than its parent compound. Subsequent SAR[55] study led to the identification of highly potent and selective PDE4 inhibitors (with potencies in the range 10−300 nM) as exemplified by compound **37**, which showed 6−10 nM potency at PDE4B, D3, and D5 and a 20- to 200-fold selectivity over A and D2.

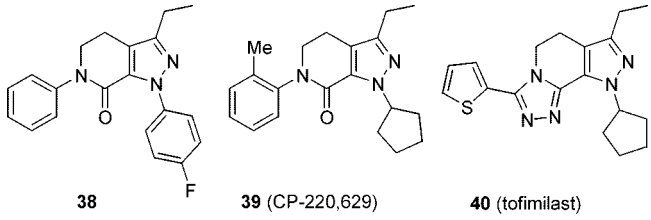


Figure 11. Tofimilast (**40**) and related compounds.

12. Tofimilast for Inhaled Delivery

Eosinophils are believed to be a critical proinflammatory target cell for asthma.[56] Because PDE4A, B, and D are expressed in eosinophils,[57] inhibition of PDE4 in these cells would block the release of various mediators, thereby inactivating the inflammatory response. To identify a novel and selective PDE4 inhibitor (and its evaluation against human eosinophil), PDE random file screening was conducted that led to the discovery of the pyrazolopyridine, **38** (Figure 11). Despite its structural dissimilarity with rolipram, SAR optimization[58] of **38** indicated that the ethyl and 4-fluorophenyl moieties of **38** could mimic the methoxy and cyclopentoxy moieties of rolipram. Moreover, this SAR work involving the optimization of substituents at the 1-, 3-, and 6-position of the pyrazolopyridine bicycle of **38** yielded analogues that were 50-fold more potent ($IC_{50} \approx 0.03-1.6\ \mu M$) than **38**. For example, compound **39** ($IC_{50} = 0.44\ \mu M$) (Figure 11) showed efficacy in the guinea pig aerosolized antigen induced airway obstruction assay ($ED_{50} = 2.0$ mg/kg, po) and demonstrated a significant reduction in eosinophil (55%), neutrophil (65%), and IL-1β (82%) responses to antigen challenge in atopic monkeys (10 mg/kg, po) (atopy means the genetic tendency to develop the classic allergic disease, e.g., atopic dermatitis, allergic rhinitis, and asthma). This compound also showed a reduced emetic liability (when dosed at 1.0 mg/kg, sc, in ferrets) compared to **1**.

The selective inhibition of specific PDE4 isoforms (A−D) without affecting the other isoforms (thought to be responsible for undesired side effects such as nausea and emesis observed with most of the PDE4 inhibitors) is the commonly used strategy to develop a safer drug. Another method for potentially minimizing the emetic liability and at the same time maximizing the efficacy of a PDE4 inhibitor is to administer the compound directly into the lung, thereby minimizing the systemic exposure. Since inhaled delivery of drug could serve this purpose, development of an inhaled (nonisoform selective) PDE4 inhibitor was undertaken. [59] Thus, incorporation of the lactam moiety of compound **39** into a triazolo ring followed by SAR around the 3-position of the resulting series led to the identification of a novel and potent PDE4 inhibitor **40** (tofimilast, Figure 11) with low oral bioavailability, low emetic liability (no emesis-associated behaviors in ferrets at plasma concentrations up to 152 ng/mL), and physical properties conducive to formulation development in an inhaler device. Low exposure (1.2% and 3% oral bioavailability in rats and dogs) was thought to be the decreased emetic liability of compound **40** that is presently in clinical development.

13. Dual PDE3/4 Inhibitors

Because PDE4 is involved in the regulation of airway smooth muscle tone together with PDE3 (a major PDE present in airway smooth muscle), it was observed that either a combination of PDE3 and PDE4 inhibitors or dual PDE3/4 inhibitors produced a much larger bronchorelaxant effect than individual isoenzyme-selective agents alone.[60,61] Thus, by combinination of the structural elements of a dual inhibitor zardaverine **41** (Figure 12) [pIC_{50} (PDE3) = 6.24, human platelet; pIC_{50} (PDE4) = 6.80, canine trachea], a PDE4 selective pyridopyridazinone **42** (Figure 12) [pIC_{50} (PDE4) = 8.30, human lymphocyte], and a catechol ether moiety, novel PDE4 selective inhibitors were designed.[62] The *cis*-4-aryl phthalazinone **43** (Figure 12) was identified as a potent PDE4 inhibitor [pIC_{50} (PDE4) = 7.9, human neutrophils] of this class. The cis-fused cyclohexene ring played an important role both in enzyme inhibition (as indicated by the molecular modeling studies) and in in vivo anti-inflammatory activities in a mouse ear edema assay.[63] Moreover, the 4-(3,4-dialkoxyphenyl) pattern was essential for potent PDE4 inhibition as indicated by the detailed SAR study.[64] Since PDE3 and PDE4 isoenzymes apparently act in a synergistic manner in human airway smooth muscle, it was hypothesized that combining the bronchodilatory and anti-inflammatory activities in a dual PDE3/PDE4 inhibitor would be more effective in the treatment of bronchial asthma. Accordingly, novel potent dual PDE3/PDE4 inhibitors were designed and one of the lead molecules, **44** (Figure 12), showed PDE3/PDE4 inhibition in vitro [pIC_{50} (PDE3) = 7.50, human platelet; pIC_{50} (PDE4) = 7.80, human neutrophils] and anti-inflammatory activities in vivo after oral dose (47% at 30 μmol/kg po in AA-induced mouse ear edema).[65] However, advantages of combining both PDE3 and PDE4 inhibitory activity in a single molecule remained inconclusive because of the observed lack of correlation between the anti-inflammatory and the PDE3 and/or PDE4 inhibitory activities.

While **41** was a dual inhibitor of PDE3/PDE4, the 6-aryl-4,5-heterocyclic-fused pyridazinones[66] **45** (Figure 13), however, showed a good selectivity profile toward the PDE4 family and greatly attenuated affinity for the rolipram high-affinity binding site. Their low affinity toward A1 and A2 adenosine receptors identified these derivatives as potential antiasthmatic and anti-inflammatory agents, devoid of CNS and cardiovascular side effects. Thus, **46**[67a] and **47**[67b] (Figure 13), related to the potent PDE4 inhibitor **4b**, have been described as PDE4 inhibitors with exceptional biological activity in arachidonic acid-induced ear mouse edema. The 3,5-dihydro-1,3,5,6-tetraza-cyclopenta[*a*]naphthalene-4-one based PDE4 inhibitor[68] **48** (Figure 13) was related to the bronchodilator but weak PDE4 inhibitors **49**[69] and **50**[70] (Figure 13), which exhibited extremely high activity in the

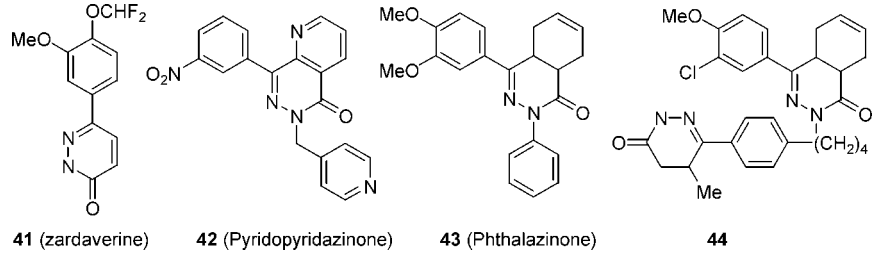


Figure 12. Zardaverine (**41**) and related compounds.

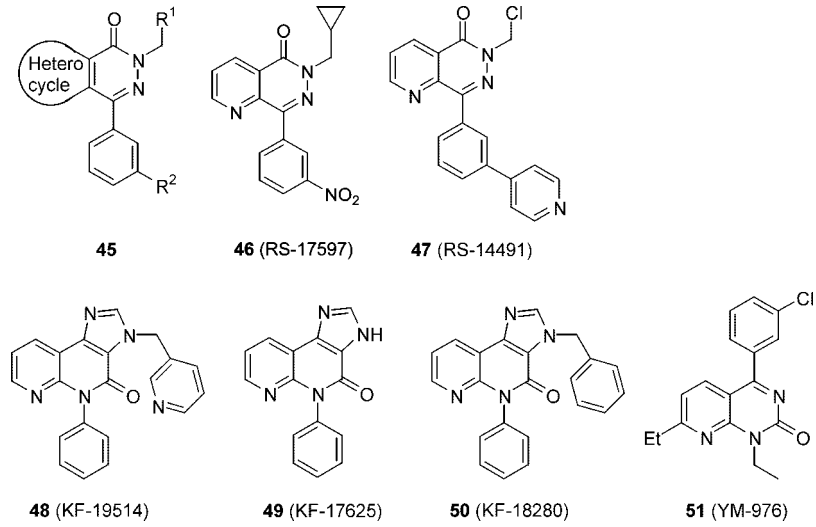


Figure 13. Other inhibitors derived from zardaverine (**41**) and naphthyridinone.

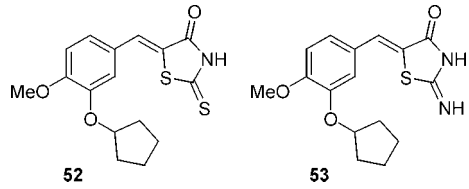


Figure 14. Rhodanine based inhibitors **52** and **53**.

carrageenan-induced rat paw edema, zymosan-induced rat paw edema, and the reverse passive Arthus reaction-induced paw edema in the rat, have an anti-inflammatory profile like glucocorticoid. Notably, a closely related derivative **51** (Figure 13) has been discontinued recently.

14. Rhodanine-Based Inhibitors

Recently, as a result of assaying a lead generation library against PDE4 via high throughout screening, a novel series of rhodanine based PDE4 inhibitors was identified.[71] The lead compound **52** (Figure 14) showed good selectivity for PDE4B inhibition ($IC_{50} = 0.89\ \mu M$) over PDE3A ($IC_{50} = 10.83\ \mu M$). On the basis of the published crystal structures of PDE4 in complex with rolipram and other catechol ether inhibitors, it was hypothesized that during the interaction of benzylidenerhodanines with PDE4, the alkoxy groups were bound within two hydrophobic pockets while the catechol oxygens participated in hydrogen bonding with a suitable donor residue.[71] The replacement of rhodanine moiety with structurally related heterocycles led to the identification of pseudothiohydantoin **53** (PDE4B $IC_{50} = 0.31\ \mu M$) that was 3-fold more potent than **52** and inactive against PDE3A.

15. PDE4D-Selective Inhibitors

Encouraged by the progress of clinical trials on moderately PDE4D selective inhibitor **27**, 1,7-naphthyridines were developed as a novel class of potent and PDE4D selective inhibitors.[72a] The purpose was to evaluate if these compounds retain the broad anti-inflammatory properties of "universal", non-PDE4 subtype selective inhibitors and at the same time display better safety profiles. Accordingly, compound **54a** (human PDE4D $IC_{50} = 1.0$ nM) (Figure 15) was found to be 55, 175, and 1000 times more potent in inhibiting PDE4D over PDE4B, PDE4A, and PDE4C and reduced more than 50% the influx of eosinophils, T-cells, and neutrophils into bronchoalveolar lavage fluid (BALF) samples obtained from antigen-challenged animals when dosed 1 mg/kg po in a Brown−Norway rat model of allergic asthma. Thus, PDE4D-selective inhibitors of the 1,7-naphthyridine class have the potential as an oral therapy for treating asthma. Notably, compound **54a** was nonselective in terms of its ability to inhibit PDE4D catalytic activity versus ability to compete for the high-affinity [^{3}H]rolipram binding site, a profile very similar to **27**, which was also found to be about twice as potent in inhibiting [^{3}H]rolipram binding versus inhibiting PDE4D catalytic activity. Nevertheless, compound **54a** contained a sterically nonhindered aromatic nitro functionality known to be responsible for potential mutagenic and carcinogenic properties, and consequently, an appropriate replacement of the nitro group was required. Thus, a new series, i.e., 6-benzo[1,2,5]oxadiazole substituted 1,7-naphthyridines, was evaluated and a 6,8-disubstituted 1,7-naphthyridine **54b** (Figure 15) was characterized as a potent and selective inhibitor of PDE4D ($IC_{50} = 1.5$ nM).[72b] The compound inhibited TNFα-release from human peripheral blood mononuclear cells and was orally active in a model of adjuvant-induced arthritis in rats.

By combination of known pharmacophores that displayed low incidence of emesis with highly potent ones, 8-biarylquinolines were identified as a novel class of PDE4 inhibitors and the lead molecule **54c** (Figure 15) [PDE4 $IC_{50} = 3.0$ nM, HWB (TNF-α) $IC_{50} = 0.16\ \mu M$] was selected as a development candidate.[73,74] However, the possibility of potential drug−drug interaction [compound **54c** was found to be a competitive inhibitor of the cytochrome P450 2C9 ($IC_{50} = 0.2\ \mu M$)] and isomerization related issues (the olefin moiety of **54c** was found to isomerize in human by the addition and elimination of glutathione) triggered a backup effort. Accordingly, two compounds **54d** and **54e** (Figure 15) were identified[75] that addressed the liabilities associated with **54c** and found to be less emetic in squirrel monkeys. The acid **54e** exhibited superior efficacy in the guinea pig model of bronchoconstriction. In another effort to replace the olefin moiety of **54c** by a suitable linker, the group bearing amide moiety was found to be most promising and compound **54f** (Figure 15), identified as a potent inhibitor of PDE4, showed activity in an ovalbumin-induced bronchoconstriction assay in conscious guinea pig.[76]

In the process of identifying an emetic probe, it was observed that a large substituent at the 3′-position of an 8-arylquinoline derivative led to compounds with an improved emetic threshold in the ferret while maintaining similar potency. For example,

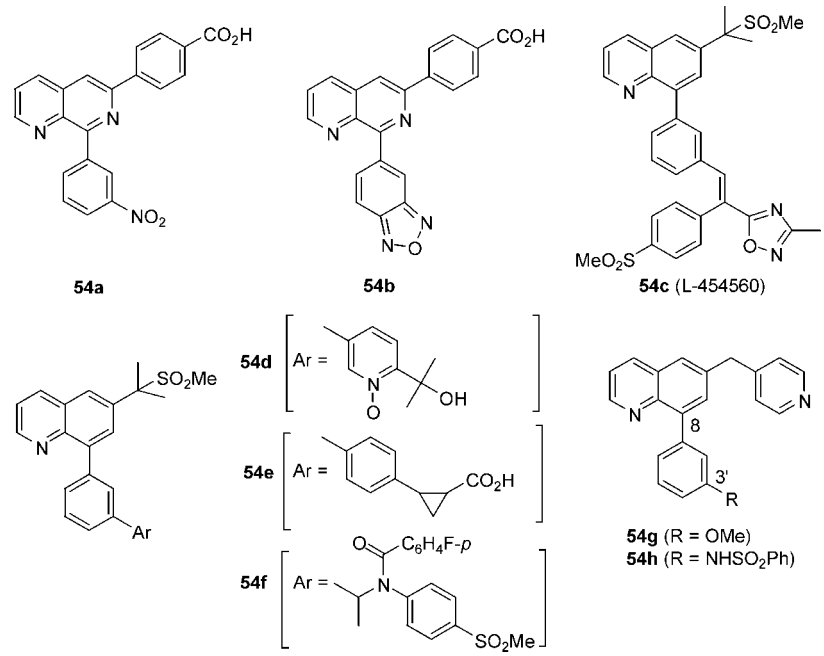


Figure 15. 1,7-Naphthyridines and 8-biarylquinolines as PDE4 inhibitors.

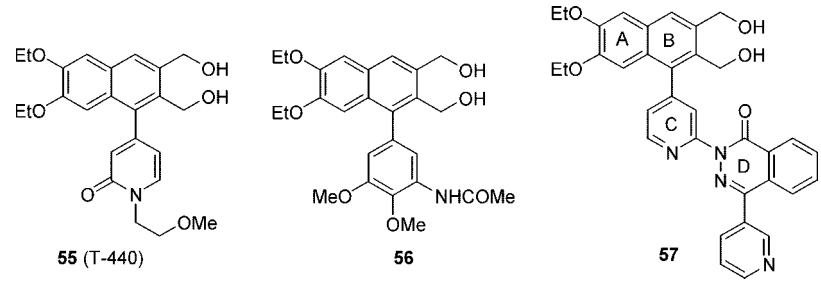


Figure 16. Structure of few naphthalene derivatives.

54g[77] and **54h**[78] (Figure 15) showed similar HWBA IC_{50} (8.3 and 7.8 μM, respectively). The emetic threshold for **54g** in the ferret was 1.0 mpk po (C_{max} = 0.2 μM), while **54h** was nonemetic up to 10 mpk po (C_{max} = 2.0 μM). Thus, incorporating the structural features from **10a**, which were different from those of rolipram, into the 3′-position of the 8-arylquinoline afforded potent PDE4 inhibitors, and subsequent SAR work around this class led to the identification of **54c** (Figure 15).

16. 1-Arylnaphthalene or 1-Heteroarylnaphthalene Derivatives

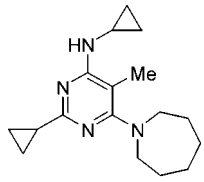
1-Arylnaphthalene lignans were initially identified as a new structural class of selective PDE4 inhibitors, and compounds **55** and **56** (Figure 16) were identified as lead molecules in this series.[79] To enhance the potency and selectivity for PDE4 inhibition, 1-pyridylnaphthalene derivatives were designed as a hybrid compound of **55** and compound **56**. In this approach, (i) the naphthalene part (A,B-ring) was fixed, (ii) the heterocyclic compound having a carbonyl group (D-ring) was introduced, and (iii) the pyridyl group (C-ring) was selected on the basis of the scope of chemical modification. Accordingly, compound **57** (T-2585·HCl, Figure 16) (PDE4 inhibition IC_{50} = 0.13 nM, PDE3/4 ratio of 14 000) showed potent antispasmogenic activities (ED_{50} = 0.063 mg/kg for reduction of antigen-induced bronchoconstriction, iv; ED_{50} = 0.033 mg/kg for reduction of histamine-induced bronchoconstriction, id) without significant changes in heart rate.[80] Also, compound **57** induced significantly weaker emetic effects than piclamilast on both orally administered ferrets and intravenously administered dogs and therefore was selected for further evaluation as an antiasthmatic agent.

17. Combination of Muscarinic M_3 Antagonism with PDE4 Inhibition

Since selective muscarinic M_3 antagonists (anticholinergic bronchodilators), currently the preferred choice for the symptomatic management of COPD, are more effective and have fewer side effects than β_2-adrenoceptor agonists, it was hypothesized that combination of selective muscarinic M_3 antagonism with selective PDE4 inhibition, thereby combining both bronchodilating and anti-inflammatory properties, might lead to a new class of drugs. Accordingly, 4,6-diaminopyrimidines were identified as potent dual M_3 antagonists and PDE4 inhibitors, and **58** (Figure 17) (PDE4 IC_{50} = 0.63 μM; M_3 K_i = 3.2 nM), which belongs to this class, was characterized as having the most interesting profile on both targets. [81]

18. Tetrahydrodiazepinoindoles and Pyrazolopyridines

During evaluation of aminobenzodiazepine-based library of compounds (originally prepared as cholecystokinin receptor antagonists) against PDE4 enzymes, novel tetrahydrodiazepinoindoles were identified as PDE4 inhibitors. The SAR studies led to the identification of a candidate for development,[82] **59a** (Figure 18) (PDE4 IC_{50} = 1.1 μM). Further work on this series led to the discovery of 9-aminotetrahydrodiazepinoindoles, and



58 (ucb-101333-3)

Figure 17. 4,6-Diaminopyrimidine as dual M_3 antagonist and PDE4 inhibitor.

the lead compound[83] **59b** (Figure 18) (PDE4 $IC_{50} = 0.27$ μM) showed improved in vitro PDE4 activity and selectivity versus other PDEs. While this compound displayed promising in vivo activity in models of antigen-induced eosinophil recruitment ($ED_{50} = 3.2$ mg/kg po in Brown−Norway rats) and production of LPS-induced TNFα in rats ($ED_{50} = 2.8$ mg/kg po) and no emetic liability in ferrets, its further development was halted because of the toxic side effects. The aniline structural fragment contained in its structure was considered as a toxicophore, as oxidation of the aromatic ring either ortho or para to the nitrogen or of the aniline nitrogen could lead to reactive metabolites, thereby causing toxic side effects. Thus, replacing the aniline moiety by substituted azaindoline led to the triazabenzo[*cd*]azulen-9-one system,[84] and the lead compound **59c** (Figure 18) showed promising in vitro activities (PDE4 $IC_{50} = 4.3$ μM).

On the basis of the known phosphodiesterases inhibiting properties of 1*H*-pyrazolo[3,4-*b*]pyridines, pyrazolopyridines[85] were evaluated via the high throughput screening (HTS) technique.[85a] Accordingly, 1,3-dimethyl-4-anilino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide **60** (Figure 19) (PDE4 $IC_{50} = 0.04$ μM, inhibition of TNFα = 48% at 10 mg/kg po in rats) was identified as an initial lead and structural optimization of the aniline moiety led to the identification of orally active PDE4 inhibitor **61** (Figure 19) (PDE4 $IC_{50} = 0.01$ μM; inhibition of TNFα production $ID_{50} = 1.0$ mg/kg po in rats). Compound **61** showed 50% inhibition of SRS-A-induced bronchoconstriction at $ID_{50} = 10$ mg/kg and improved oral bioavailability (33%) in rats and did not cause emesis at oral doses up to 10 mg/kg.

19. 8-Methoxyquinolines and Phthalazines

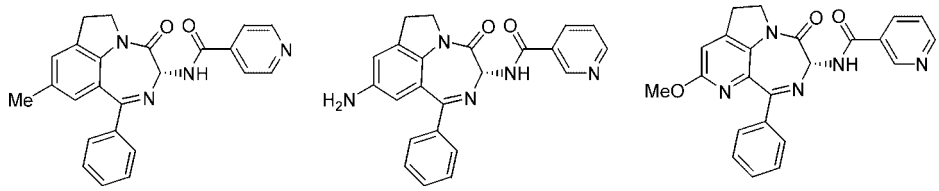
Encouraged by the improved pharmacokinetic profiles of benzimidazoles, 8-methoxyquinoline-4-carboxamides were evaluated, which led to the identification of 8-methoxyquinoline-4-carboxylic acid (3,5-dichloropyridyl-4-yl)amide **62** (Figure 20) as a potent and selective PDE4 inhibitor[86] (PDE4 $IC_{50} = 0.17$ μM; RBA $IC_{50} = 0.53$ μM). This compound showed good pharmacokinetic profile (62% oral bioavailability in guinea pig) and showed reasonable levels of oral activity in a functional model of inflammation (30 mg/kg po in guinea pig) along with reduced liability for emetic and CNS side effects. To improve its pharmacological profile further, novel 2-substituted 8-methoxyquinolines were evaluated and the 2-trifluoromethyl derivative **63** (Figure 20) was found to be a potent PDE4 inhibitor (PDE4 $IC_{50} = 0.051$ μM; RBA $IC_{50} = 0.077$ μM).[87] Compared with **62**, compound **63** showed an improved plasma half-life in vivo when dosed orally to guinea pigs (oral bioavailability 78%) and significantly improved activity in a guinea pig lung eosinophilia model at 10 and 3 mg/kg po. However, rat PK study on **63** showed the presence of a major metabolite whose levels were higher than the parent compound at time points greater than 3 h and was identified as the pyridine *N*-oxide **64** (Figure 20).[88] A further investigation identified **64** as a potent and selective inhibitor of PDE4 (PDE4 $IC_{50} = 0.06$ μM; RBA $IC_{50} = 0.15$ μM) with an excellent PK profile and significant activity in an in vivo model at doses that showed no emetic side effects. However, compound **64** was (a) found to cause vasculopathy in monkeys and (b) metabolized to the more potent **63** with a varied conversion rate in different species. These two major issues prompted the search for a superior surrogate of the dichloropyridine *N*-oxide moiety of **64**. As a result, substituted quinolyloxazoles were discovered as a novel and highly potent series of PDE4 inhibitors. [89] Published crystal structures of PDE4 and modeling studies on **64** and related compounds suggested that the quinoline moiety binds to the adenosine recognition site, while the amide portion serves as a linker to anchor a group containing a polar atom which provides favorable interactions with the metal ion binding site of PDE4. SAR studies revealed that the oxazole core, with 4-carboxamide and 5-aminomethyl groups, was a novel pharmacophore for PDE4 inhibition. Moreover, modeling studies indicated that the oxazole ring served as a linker for (a) positioning the primary amino group to simultaneously coordinate with both the Zn^{2+} and His-234 and (b) anchoring the amide oxygen to coordinate with the Mg^{2+} in the catalytic site. The lead molecule **65** (Figure 20) showed high selectivity toward PDE4B (PDE4B $IC_{50} = 19$ nM, PDE10 $IC_{50} = 430$ nM, PDE11 $IC_{50} = 2000$ nM), exceptionally high plasma level in rats ($C_{max} = 9.5$ μM at 10 mg/kg po), efficacy in the rat LPS (lipopolysaccharide)-induced pulmonary inflammation model (64% inhibition at 3 mg/kg po), and no emetic effect in Cynomolgus monkeys at 30 mg/kg po.

In another approach phthalazines were identified as potent inhibitors of PDE4 via modification of the amide linker attached to the dichloropyridine moiety of **6** (Figure 3) and the lead compound **66** (Figure 20) (PDE4 $IC_{50} = 53$ nM; RBA $K_i = 149$ nM; TNFα $IC_{50} = 254$ nM) showed good therapeutic potential.[90] On the basis of its reduced ability to increase acid secretion ($IC_{50} = 7.0$ μM) in isolated whole rat stomach and to induce emesis in dog ($ED_{50} > 10$ μM/kg iv), compound **66** was selected for further studies. However, its decreased potency in comparison to piclamilast (thought to be due to the unfavorable interaction between the cyclopentyloxy group and the peri-hydrogen at the C-4 position of the phthalazine nucleus) prompted further SAR work on this class of compounds. Accordingly, **67** (PDE4 $IC_{50} = 37$ nM; RBA $K_i = 271$ nM; TNFα $IC_{50} = 46$ nM) and **68** (PDE4 $IC_{50} = 241$ nM; RBA $K_i = 383$ nM; TNFα $IC_{50} = 72$ nM) (Figure 20) were identified as potent inhibitors of PDE4.[91] Both the compounds showed good in vitro metabolic stability and oral bioavailability in the rat (27%), inhibition of eosinophil infiltration in the guinea pig (34−37% at 30 umol/kg ip), reduced ability to increase acid secretion in isolated whole rat stomach, and no sign of emesis in the dog (>30 μM/kg iv). A further SAR study on this series led to the identification of another phthalazine series represented by **69** (Figure 20).[92]

20. Pyrazole-Based Inhibitors

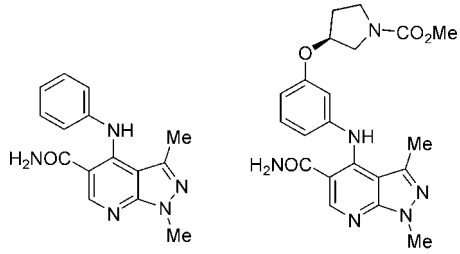
In view of their promising pharmacological properties pyrazolo[1,5-*a*]-1,3,5-triazines were explored as potent and selective PDE4 inhibitors.[93] Compounds **70** and **71** (Figure 21) were identified as the most potent PDE4 inhibitors with IC_{50} values of 13 and 11 nM, respectively, which was 100-fold more potent than **1** (1.2 μM) and 2.5-fold when compared to **27** (30 nM). Moreover, these compounds showed strong inhibition of cytokine production in LPS-induced human blood and were selected for further biological evaluation.

On the basis of the fact that compound **72** (Figure 21) inhibited several tissue PDEs with moderate potency, its PDE4



59a (CI-1018) **59b** (CI-1044) **59c**

Figure 18. Tetrahydrodiazepinoindoles as PDE4 inhibitors.



60 **61**

Figure 19. Pyrazolopyridine-based inhibitors.

inhibitory properties were investigated recently.[94] Compound **72** potently inhibited purified human PDE4A, B, C, and D with IC_{50} values of 54, 65, 239, and 166 nM, respectively, and effectively blocked LPS-induced TNF (TNF-α, $IC_{50} = 6.2\ \mu M$) and *N*-formyl-Met-Leu-Phe (fMLP)-induced leukotriene $(LT)_4$ B_4 biosynthesis ($IC_{50} = 2.5\ \mu M$) in human whole blood, which were 3- and 6-fold more potent than cilomilast. Notably, ibudilast was about 3-fold less potent against the PDE4D isozyme (which was implicated in mediating the emetic response) and was well tolerated in clinic as either an ophthalmic solution or an oral formulation.

21. Conclusion: Toward a Better Understanding of Emetic Side Effects and Uncovering Alternative Options

It is evident that as a result of intense effort devoted toward the development of PDE4 inhibitors, numerous candidates have been placed into clinical trials for asthma and/or COPD (Table 3). While a few of these drugs appeared to be promising (e.g., cilomilast and roflumilast), many were discontinued from development because of a narrow window between efficacy and the undesired side effects of nausea and emesis. These adverse side effects were thought to be due to the inhibition of PDE4 in the gut and brain. It was hypothesized that these side effects could be due to either binding at a high-affinity allosteric binding site of PDE4 (called the rolipram binding site), affecting gastric acid secreting cells in the gut, or activation of emetic centers within the CNS. Thus, to improve the therapeutic window, efforts were devoted initially for the identification of compounds that are more potent for inhibiting the enzyme activity and have less affinity toward rolipram binding site. However, it was shown later that the high-affinity rolipram-binding site of the enzyme is simply the cofactor (e.g., Mg^{2+}) bound active site on the holoenzyme (active form of the enzyme) and the conformational difference between the PDE4 apoenzyme and the holoenzyme is responsible for the differential binding of inhibitors. Subsequently, efforts have been directed toward the identification of isozyme-selective compounds and a number of PDE4 inhibitors were developed that showed selective inhibition of PDE4D. Incidentally, at a later stage evidence suggested that emesis resulting from administration of PDE4 inhibitors was due to the selective inhibition of PDE4D (and that emesis was related to the ability of PDE4 inhibitors to penetrate the CNS rather than their affinity for PDE4). Also, it was not clear if PDE4A and/or PDE4C could regulate emesis, and inhibition of PDE4D was essential for anti-inflammatory and/or immunomodulatory activities. While answers to these questions are beginning to emerge, evidence to date suggests that selective inhibitors of PDE4A and/or PDE4B should have a much improved therapeutic ratio compared with currently used PDE4 inhibitors and could have promising therapeutic utility in asthma and COPD. Also, administering the drug directly into the lung via inhaled delivery, another strategy for maximizing the efficacy of a PDE4 inhibitor while minimizing the emetic liability, could have enormous potential. Finally, development of dual inhibitors could be another way forward. For example, PDE7, a cAMP specific inactivator of cAMP, is involved in T cell activation, and therefore, a dual PDE4−PDE7 inhibitor could be more effective in asthma and COPD. Similarly, a dual PDE3−PDE4 compound might provide enhanced bronchodilator and bronchoprotective effect in addition to the beneficial PDE4 effects. Recently, development of dual therapy by combining PDE4 inhibition with Ca^{2+} channel antagonism has been described as a potential option for the management of severe COPD. Overall, owing to their unique anti-inflammatory and immunomodulatory properties coupled with their potential for disease modification, PDE4 inhibitors would continue to be preferred and the potential option for the treatment of severe asthma and COPD, and clinical approval of one of the PDE4 inhibitors would provide a better understanding of the long-term efficacy and the potential for disease modification.

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Arumugam Kodimuthali received his Master degree in Organic Chemistry (1995−1997) from Bharathidasan University, Trichy, India. He then joined Indian Institute of Technology, Chennai, as a Project Associate. In 2000 he moved to Shasun Chemicals and then to Sanmar Speciality Chemicals, Chennai, in 2003. In 2004 he moved to Hyderabad, India, where he joined the New Drug Discovery Department of Matrix Laboratories Limited. His research interest includes synthesis of new chemical entities in the area of metabolic disorder and inflammation.

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Manojit Pal received his Ph.D. degree from Jadavpur University, Kolkata, India, in 1995. He then moved to Vadodara, Gujarat, where he worked as a Research Officer—R&D (1995−1997) in Alembic Chemical Works Co Ltd. and as an Executive—Organic Synthesis (1997−1998) in Sun Pharma Advanced Research Center. In 1998 he joined Dr. Reddy's Laboratories Limited in Hyderabad as a

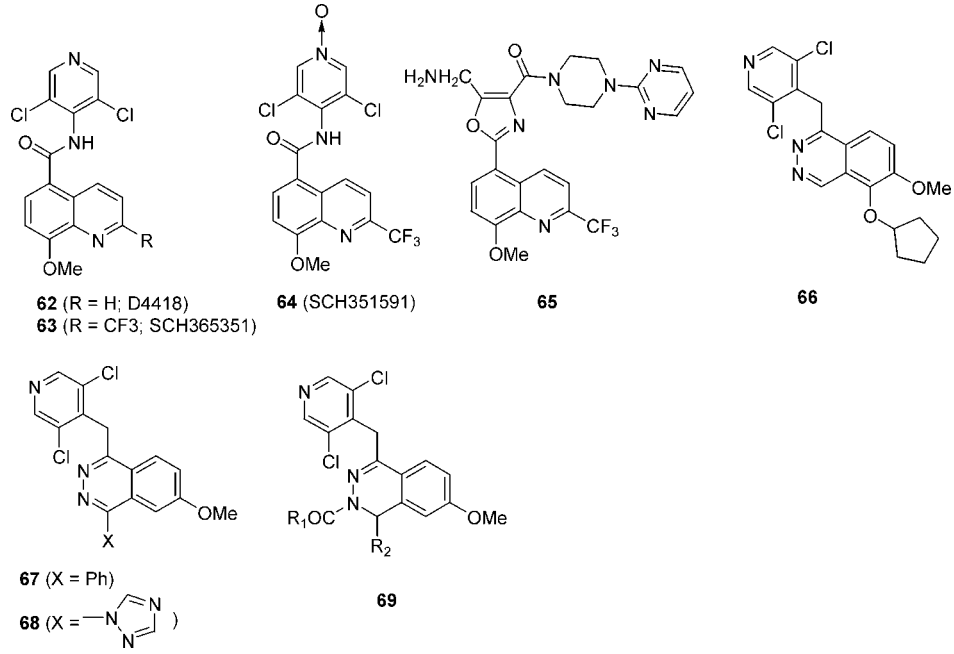

Figure 20. 8-Methoxyquinoline and phthalazine-based inhibitors.

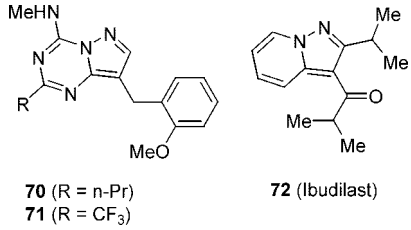

Figure 21. Ibudilast (**72**) and related compounds.

Table 3. Status Summary of Selected PDE4 Inhibitors

compd	route	company	status
roflumilast	oral	Altana	phase 3
tetomilast	oral	Otsuka	phase 3
arofylline	oral	Almirall Prodesfarma	phase 3
oglemilast	oral	Glenmark	phase 2
apremilast	oral	Celgene	phase 2
4-acetylenic substituted cyclohexy amine (GSK256066)[95]	inhaled	GSK	phase 2
cilomilast	oral	GSK	discontinued
C3193[95]	oral	Merck	discontinued
23a	oral	Celgene	discontinued
28b	oral	Ono	discontinued
N-(3,5-dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxyindole-3-yl]-glyoxylic acid amide (AWD-12-281/GSK842470)[95,96]	inhaled	Elbion-GSK	phase 2
tofimilast	inhaled	Pfizer	phase 2

Senior Scientist and became Senior Director—Discovery Chemistry in 2006. In 2007 he joined the New Drug Discovery Department of Matrix Laboratories Limited, Hyderabad, and presently leads the department. He is a recipient of CSIR Certificate of Merit 1989 and Bioorganic and Medicinal Chemistry Letters Most Cited Paper 2003−2006 Award and has been listed in the Marquis Who's Who in Medicine and Healthcare, 5th edition (2004−2005). His research interests include development of new chemical entities under the new drug discovery program in various therapeutic areas. He has authored or coauthored more than 50 research publications and a number of patents.

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