Dual PDE3/4 and PDE4 Inhibitors: Novel Treatments For COPD and Other Inflammatory Airway Diseases

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Abstract: Selective phosphodiesterase (PDE) 4 and dual PDE3/4 inhibitors have attracted considerable interest as potential therapeutic agents for the treatment of respiratory diseases, largely by virtue of their anti-inflammatory (PDE4) and bifunctional bronchodilator/anti-inflammatory (PDE3/4) effects. Many of these agents have, however, failed in early development for various reasons, including dose-limiting side effects when administered orally and lack of sufficient activity when inhaled. Indeed, only one selective PDE4 inhibitor, the orally active roflumilast-n-oxide, has to date received marketing authorization. The majority of the compounds that have failed were, however, orally administered and non-selective for either PDE3 (A,B) or PDE4 (A,B,C,D) subtypes. Developing an inhaled dual PDE3/4 inhibitor that is rapidly cleared from the systemic circulation, potentially with subtype specificity, may represent one strategy to improve the therapeutic index and also exhibit enhanced efficacy *versus* inhibition of either PDE3 or PDE4 alone, given the potential positive interactions with regard to anti-inflammatory and bronchodilator effects that have been observed pre-clinically with dual inhibition of PDE3 and PDE4 compared with inhibition of either isozyme alone. This MiniReview will summarize recent clinical data obtained with PDE inhibitors and the potential for these drugs to treat COPD and other inflammatory airways diseases such as asthma and cystic fibrosis.

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality and is increasing in prevalence. The WHO estimates that by 2020, COPD will be the third leading cause of death and the fifth leading cause of disability worldwide [1]. The disease is characterized by progressive airflow obstruction resulting from mucosal inflammation and oedema, bronchoconstriction, mucus hypersecretion and loss of elastic recoil.

Short-acting β_2 agonists and muscarinic receptor antagonists are the first line of treatment, and as the disease progresses, long-acting β_2 agonists (LABAs) and muscarinic antagonists (LAMAs), with the combination of a LABA and a LAMA, often becoming necessary as a maintenance therapy. In addition, LABAs are typically used in combination with inhaled corticosteroids (ICS) [2]. Despite the beneficial effects of these therapies and their various combinations, modest beneficial effects on health-related quality of life and FEV_1 have been observed [3], and corticosteroids are relatively ineffective at suppressing the airway wall thickening and luminal occlusion in patients with COPD [4] and the consequent disease progression. Indeed, most patients with COPD remain symptomatic and experience exacerbations of their disease, characterized by increased airway obstruction and worsening lung function, associated with enhanced airways inflammation. Patients with severe COPD and frequent exacerbations experience poorer

health and faster disease progression, including decline in lung function and a higher risk of death [5]. There is also an increasing concern that the regular use of corticosteroids to treat patients with COPD makes such patients more susceptible to infections such as pneumonia [6].

Chronic obstructive pulmonary disease is considered to be among the costliest inpatient conditions, with costs in the UK estimated to be £800 million, over half of which relates to hospital-based care. COPD is also estimated to be responsible for 24 million lost working days/year. Thus, there is a high unmet medical need that requires novel effective therapies [2]. Inflammation is a characteristic feature of COPD that contributes to the airway obstruction and lung tissue destruction. Whilst CD68^{+} macrophages and CD8^{+} T-lymphocytes are the predominant cells in the bronchial mucosa of patients with COPD, the presence of other inflammatory cells such as B-lymphocytes, neutrophils and dendritic cells, together with increased levels of pro-inflammatory mediators (e.g. IL-6, IL-1β, TNF-α, Gro-α, MCP-1, IL-8 and IL-32) in the lung [7–9], and increased levels of IL-1β, IL-6 and TNF-α in the blood, has also been observed. This inflammatory response persists even in the absence of overt infection [10].

Exacerbations of COPD reflect a worsening of the underlying chronic inflammation in the airways and are characterized by marked elevations in neutrophils and their products (e.g. neutrophil elastase) in the airways [11,12], together with a significant increase in cytokines such as IL-8 and TNF-α which are known to act as chemoattractants for neutrophils

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[11]. Interestingly, there is a significant correlation between the percentage of neutrophils in bronchoalveolar lavage (BAL) fluid and severity of airways obstruction assessed by FEV_1/ FVC ratio [11]. Increases in eosinophils have also been reported in patients undergoing exacerbations [13].

Mucus hypersecretion and reduced mucociliary clearance are also distinctive features of COPD that are thought to contribute to airway obstruction and the progressive decline in FEV_1. Very importantly, ICS do not appear to have any effect on the accelerated decline in FEV_1 [14], suggesting the need for novel classes of anti-inflammatory drugs to reduce the progression of this debilitating disease. It is interesting to note that cystic fibrosis transmembrane conductance regulator (CFTR), a chloride ion channel which plays an integral role in controlling the electrolyte/fluid balance and regulating mucociliary clearance [15], is reduced in COPD patients, with expression inversely correlating with disease severity [16].

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) regulate a variety of cellular processes including airway smooth muscle relaxation and inflammatory mediator release [17]. The phosphodiesterase (PDE) enzyme family hydrolyses, cAMP and cGMP, to inactive 5′AMP and 5′GMP, respectively, and thus, inhibition of PDEs represents a potential mechanism by which cell functions such as airway smooth muscle relaxation and inflammatory mediator release can be modulated [18]. 11 PDE gene families have been identified, denoted PDE1-11, which differ in primary structures, affinities for cAMP and cGMP, responses to specific effectors, sensitivities to specific inhibitors and mechanisms of regulation [19]. Each family contains at least one member, and in some cases, the members are products of more than one gene.

PDE4 is a low km (~1–10 μM) cAMP-specific PDE that only has a weak affinity for cGMP ($k_m > 50$ μM). The PDE4 family is comprised of four genes (PDE4A, B, C, D), with each gene having multiple splice variants. PDE4 gene products have a broad tissue distribution; including the brain, gastrointestinal tract, spleen, lung, heart, testis and kidney [20]. In addition, PDE4 is expressed in almost all inflammatory cell types except blood platelets [21–39].

PDE3 hydrolyses both cAMP and cGMP with relatively high affinities (k_m cAMP < 0.4 μM; k_m cGMP < 0.3 μM), although the V_{max} for cAMP hydrolysis is nearly 10 times higher than for cGMP. Two genes have been identified for PDE3, known as PDE3A and PDE3B, which have >80% amino acid identity for the catalytic region. Splice variants have only conclusively been demonstrated for the 3A isoform. PDE3A is expressed in platelets, vascular smooth muscle, cardiac myocytes, oocytes [40] and B-lymphocytes [26], whereas PDE3B is relatively highly expressed in adipocytes, hepatocytes and spermatocytes and has also been detected in vascular smooth muscle cells, pancreas, T-lymphocytes and macrophages [40]. No inhibitors have been described that clearly distinguish between PDE3A and PDE3B, although there are two reports of compounds showing some selectivity [41,42].

Whilst PDE4 inhibitors are very efficacious at inhibiting the activation and release of inflammatory mediators from certain cells (e.g. neutrophils, eosinophils) [43–46], there is little evidence for selective PDE3 inhibitors inhibiting inflammatory cell function. However, there is growing evidence to suggest that dual inhibition of PDE3 and PDE4 can be additive or even synergistic at suppressing inflammatory mediator release from other cell types which also express PDE3 that are thought to play a role in COPD (e.g. macrophages, dendritic cells, epithelial cells, lymphocytes, airway smooth muscle cells and endothelial cells) [27,39,47–50].

PDE3 inhibitors cause bronchodilation in man [51], and there is evidence from pre-clinical studies to suggest that combined inhibition of PDE3 and PDE4 suppresses spasmogen-induced contraction of human and bovine airway smooth muscle, in a synergistic fashion [52,53]. PDE3 and PDE4 inhibitors have also been shown to activate CFTR-mediated Cl^- secretion, suggesting they may be able to stimulate mucociliary clearance [15]. This diverse spectrum of biological effects has thus implicated PDE4 and PDE3/4 inhibitors as potential therapeutic agents for a range of disease indications involving inflammation and altered mucus production such as COPD, asthma and cystic fibrosis. Indeed, both the FDA and EMEA approved roflumilast-n-oxide, an orally administered selective PDE4 inhibitor in 2010 for the maintenance treatment of severe COPD associated with bronchitis and a history of frequent exacerbations, as an add-on to standard treatment. Orally administered PDE4 inhibitors, do, however, have a low therapeutic ratio, with particular concerns for the gastrointestinal side effects which to date have stopped their development or limited their wider use. It is conceivable therefore that administration of PDE inhibitors, particularly a dual PDE3/4 inhibitor by the inhaled route, may offer increased efficacy with a reduced side effect potential *versus* an orally administered PDE4 inhibitor.

Pre-clinical Data with PDE3 and PDE4 Inhibitors

Bronchodilation.

PDE3 together with PDE4B and D is expressed in human airway smooth muscle cells [23,30,54]. Some studies have demonstrated that PDE4 inhibitors can relax inherent tone in isolated human bronchial muscle [52,55], and in a study using siRNA targeted to PDE4D5, this PDE4 splice variant was shown to be the key physiological regulator of β_2-adrenoceptor-induced cAMP turnover within human airway smooth muscle [23,30]. Further evidence to support a key role for the D isoform in the contractile response of airway smooth muscle can be derived from a study with $PDE4D^{-/-}$ mice, in which a significant disruption in airway smooth muscle contractility was observed in isolated tracheas from $PDE4D^{-/-}$ mice, highlighted by a marked reduction in maximal tension and reduced sensitivity to muscarinic cholinergic agonists [56]. PDE3 has also been shown to play an important role in airway smooth muscle contraction, as the PDE3 inhibitor, olprinone dose-dependently antagonized methacholine-induced bronchoconstriction in the dog [57]. Furthermore, it has recently been demonstrated that PDE3 is up-regulated in airway smooth muscle obtained from patients with asthma [58].

Anti-inflammatory effects.

In vitro. A number of PDE4 isoenzymes (PDE4A, B and D) are expressed in human neutrophils [21,36,38]. Some evidence suggests that PDE4B2 is the predominant PDE4 isoform [38] and PDE4A is exclusively located within a subset of myeloperoxidase (MPO)-containing neutrophil granules [36]. PDE4 inhibitors inhibit the release of a range of pro-inflammatory mediators from human neutrophils including LTB_4 and reactive oxygen species [44,45], and MMP-9 and neutrophil degranulation products such as neutrophil elastase and MPO [46]. PDE4 inhibitors also inhibit PAF-induced CD11b expression and L-selectin shedding [59] and TNF-α and fMLP-mediated neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) [46,60].

PDE4 isoenzyme subtypes (A, B and D) are also present in human eosinophils, with evidence to suggest that PDE4A is exclusively located in all eosinophil granules [36]. PDE4 inhibitors inhibit the release of reactive oxygen species from human eosinophils [44], as well as C5a and PAF-stimulated LTC_4 synthesis [61]. PDE4 inhibitors can also inhibit PAF-induced CD11b expression and L-selectin shedding [59], as well as C5a and PAF-stimulated eosinophil chemotaxis [61].

PDE4 isoenzyme subtypes (A, B, C, D) are also present in human lung macrophages and peripheral blood monocytes [21,62], and PDE3B has been detected in macrophages [40]. PDE4A4 is up-regulated in lung macrophages from smokers with COPD compared with control smokers [21], and PDE4A4 and PDE4B2 are increased in peripheral blood monocytes from smokers versus non-smokers [21]. PDE4 inhibitors can inhibit LPS-stimulated TNF-α release from peripheral blood monocytes [63,64], and interestingly, in PDE4B$^{-/-}$ mice (but not PDE4D$^{-/-}$ mice), there is a marked reduction in the ability of LPS to stimulate TNF-α release from peripheral blood leucocytes [65], suggesting a key role for PDE4B in this response. Further support for a key role of PDE4B in LPS-induced TNF-α release from monocytes comes from a separate study demonstrating that mean IC_{50} values for inhibition of LPS-stimulated TNF-α release significantly correlated with compound potency against the catalytic activity of recombinant human PDE4B (and PDE4A), but not the catalytic activity of recombinant human PDE4D [63].

PDE3 and PDE4 (A, B, D) are both present in monocyte-derived dendritic cells [48], and PDE4A appears to be the predominant PDE4 isoform [66]. The PDE4 inhibitor rolipram partially inhibited LPS-stimulated TNF-α release from dendritic cells [48].

PDE4A, B and D and PDE3 have been detected in human T- and B-lymphocytes [21,22,27,29,48] with PDE3A and PDE3B detected in B-lymphocytes [48] and T-lymphocytes [40], respectively. Knockdown of PDE4B or PDE4D (but not PDE4A), using siRNA, inhibited IL-2 release, whereas knockdown of PDE4D showed the most predominant inhibitory effect on IFN-γ and IL-5 release [35]. In one study, PDE4 inhibitors were described to partially inhibit IL-4 and IL-5 gene expression in T_H2 cells [67], together with IL-4

and IL-5 release from human CD4$^+$ T cells [61], whereas a separate study demonstrated that specific inhibition of PDE4 had no significant effect on T_H2 cell-mediated IL-4 or IL-13 generation, but preferentially inhibited T_H1 cell cytokine generation (IFN-γ) [68]. PDE4 inhibitors also partially inhibit phytohaemagglutinin (PHA) and anti-CD3-/anti-CD28-stimulated proliferation of CD4$^+$ and CD8$^+$ T cells [27,44]. A separate study demonstrated that dual PDE4A/B and PDE4D inhibitors inhibited antigen-stimulated human T-cell proliferation, with mean IC_{50} values significantly correlating with compound potency against the catalytic activity of recombinant PDE4A or B, but not with the catalytic activity of recombinant PDE4D [63]. In contrast, a PDE4D siRNA (but not PDE4A or B siRNAs) also significantly inhibited anti-CD3-/-CD28-stimulated CD4$^+$ proliferation [35]. The reason for this apparent difference in PDE4 subtype involvement in this proliferative response is unclear, but could be related to the fact that different T-cell populations were used, or the fact that different stimuli were used to elicit proliferation.

PDE4 (A, C and D) and PDE3 are expressed in human airway epithelial cells [25,39]. The PDE4 inhibitor, rolipram, partially inhibited IL-1β-stimulated GMCSF release from human airway epithelial cells and A549 cells [39], and whilst a separate study demonstrated that rolipram inhibited LPS-stimulated IL-6 release from human airway epithelial cells, relatively high concentrations were required to see an inhibitory effect, with an IC_{50} of 24 μM, suggesting the effect could have been mediated through other PDE enzymes such as PDE3 [69]. In a more recent study, roflumilast-n-oxide inhibited RSV infection of human bronchial epithelial cells, prevented the loss of ciliated cells and markers, reduced the increase in CLCA1 and inhibited the increase in IL-13, IL-6, IL-8, TNFα and ICAM-1. Additionally, roflumilast-n-oxide reversed the reduction in Nrf2, HO-1 and GPx mRNA levels and consequently restored the total antioxidant capacity and reduced H_2O_2 formation [70].

Endothelial cells express PDE4 (A, B and D) [24,32,33] and PDE3 [71]. PDE4 inhibition in combination with appropriate activation of adenylate cyclase inhibited TNF-α-induced E selectin expression on human lung microvascular endothelial cells [49]. Rolipram has also been shown to potently block H_2O_2-induced endothelial permeability when combined with PGE_1 [71], suggesting that this inhibition of enhanced vascular permeability may be an additional beneficial effect of PDE4 inhibition.

In vivo. The orally administered PDE4 inhibitors, cilomilast and roflumilast-n-oxide, are effective at suppressing neutrophil recruitment to the lung in mice that have been acutely exposed to cigarette smoke [72,73]. In more chronic cigarette smoke exposure studies, roflumilast-n-oxide (oral treatment for 7 months) fully prevented emphysema in mice [73], and the PDE4 inhibitor GPD-1116 (oral treatment for 8 weeks) also markedly attenuated the development of cigarette smoke-induced emphysema in the senescence-accelerated mouse P1 strain [74]. PDE4 inhibitors have also been shown to reduce

MMP-9 and TGF-β release during LPS-induced lung injury in mice [75].

Local administration of PDE4 and dual PDE3/4 inhibitors directly to the lung also inhibits LPS-induced neutrophil recruitment to the lung in a range of species: rats [76,77], ferrets [76] and pigs [76]. In addition, local administration of an inhaled PDE3/4 inhibitor SDZ ISQ 844 in dogs decreased airways responsiveness to inhaled methacholine at a dose which did not affect base-line respiratory resistance [78].

Studies with PDE4 subtype-deficient mice suggest that PDE4B and PDE4D (but not PDE4A) are important in mediating LPS-induced neutrophil recruitment to the lung, as neutrophil migration was inhibited by 31% and 48% in PDE4B$^{-/-}$ and PDE4D$^{-/-}$ mice, respectively. These effects were associated with a reduction in the expression of CD18, but not CD11 [79], suggesting that PDE4B and PDE4D inhibition of adhesion molecule expression may contribute to the reduced neutrophil recruitment. PDE4 inhibitors have also more recently been shown to promote PKA-dependent resolution of established neutrophilic inflammation in an *in vivo* setting (LPS-induced neutrophil recruitment to the pleural cavity in mice) by inducing apoptosis of neutrophils mediated by activation of caspase-3 which correlated with inhibition of the PI3K/AktMcl-1 pathway in the mouse [80].

Mucus secretion and mucociliary clearance.
CFTR is the primary cAMP-activated chloride channel on the apical membrane of airway epithelia, and a number of groups have shown that acquired CFTR dysfunction may cause delayed mucociliary clearance, even in the absence of the congenital CFTR dysfunction seen in cystic fibrosis patients [15]. Cigarette smoke causes CFTR dysfunction *in vitro* and *in vivo*, an effect which has been suggested to contribute to COPD pathogenesis by causing a pre-disposition to epithelial dysfunction, mucus retention and chronic bronchitis. Inhibition of PDE4 (in particular the D isoform) and PDE3 activated CFTR-mediated chloride secretion in an epithelial cell line [15,81,82], suggesting that PDE3/4 inhibitors may have additional benefit in COPD by being able to enhance mucociliary clearance.

Interestingly, roflumilast-n-oxide was recently shown to partially restore CFTR activity in cigarette smoke exposed human bronchial epithelial cells, an effect which was additive with ivacaftor (the recently approved CFTR potentiator compound) [83]. In addition, roflumilast-n-oxide improved depleted airway surface liquid depth of human bronchial epithelial monolayers exposed to smoke**.** CFTR activation by roflumilast-n-oxide also induced CFTR-dependent fluid secretion in murine intestine, increasing the wet/dry ratio and diameter of ligated murine segments [83], an effect which has been suggested to likely be responsible for the diarrhoea that has been seen clinically in some patients administered roflumilast-n-oxide. Roflumilast-n-oxide has also recently been shown to inhibit RSV-induced increase in MUC5AC [70]. PDE3 and PDE4 inhibitors have also been shown to accelerate ciliary beat frequency in upper and lower airway tissue *in vitro* [84].

Airway remodelling.
Fibroblasts and airway smooth muscle cells are thought to contribute to the airway remodelling that has been observed in COPD and other obstructive lung diseases, through the release of growth factors, cytokines and extracellular matrix proteins [85]. The PDE4 inhibitor, piclamilast, inhibits the differentiation of lung fibroblasts to myofibroblasts (induced by TGFβ) [86]. In addition, PDE4 inhibitors can inhibit TNF-α-stimulated pro-MMP1 and pro-MMP2 release from human lung fibroblasts [87], as well as the chemotaxis of foetal lung fibroblasts towards fibronectin [88]. Roflumilast-n-oxide has also been shown to inhibit TGF-β-induced fibronectin deposition in human airway smooth muscle cells and also TGF-β-induced CTGF, collagen I and fibronectin expression in human bronchial tissue rings [85].

Combined Inhibition of PDE3 and PDE4 can have Additive or Synergistic Effects

Anti-inflammatory.
Whilst PDE4 inhibitors can completely suppress LPS-stimulated TNF-α release from monocytes, PDE4 inhibitors only have a partial inhibitory effect on LPS-stimulated TNF-α release from human alveolar macrophages [47] and dendritic cells [48] which appears to be due to the presence of PDE3B [40], as combined inhibition of PDE3 and PDE4 can completely suppress this effect [47,48]. In a separate study, in human alveolar macrophages, whilst a PDE3 or PDE4 inhibitor alone only caused about 20% inhibition of LPS-induced cytokine release in the presence of PGE$_2$, combined inhibition of PDE3/PDE4 effectively inhibited the LPS-induced cytokine secretion by about 90%. Interestingly, this inhibitory effect was sustained in the presence of oxidative stress. This was in contrast to the inhibitory effect of the corticosteroids, budesonide and dexamethasone, which was reduced from 90 to 30% under conditions of oxidative stress [50].

There is also evidence that combined inhibition of PDE3 and PDE4 can have synergistic anti-inflammatory effects in CD4$^+$ and CD8$^+$ human T-lymphocytes, as whilst the PDE3 inhibitor, SK&F 95654, had no effect alone on mitogen-stimulated IL-2 release, it potentiated the inhibitory effect of the PDE4 inhibitor rolipram [27,44]. In addition, with regard to epithelial cells, rolipram only partially inhibited IL-1β-stimulated GMCSF release from human airway epithelial cells and A549 cells, whereas the dual PDE3/4 inhibitor (ORG-9935) completely suppressed this effect [39].

Combined inhibition of PDE3 and PDE4 has also been shown to have a synergistic inhibitory effect on VCAM-1 expression and eosinophil adhesion to activated human lung microvascular endothelial cells [49].

Bronchodilator effects.
Whilst some studies have demonstrated that PDE4 inhibitors can relax inherent tone in isolated human bronchial muscle [52,55], other studies have found that PDE3 or PDE4 inhibitors alone are ineffective, but in combination effectively relax

inherent tone [54]. In addition, PDE3 or PDE4 inhibition alone had no effect on allergen or LTC_4-induced contraction of human airway smooth muscle, but in combination, they acted synergistically to inhibit contraction [52]. Furthermore, we have recently shown that the PDE3/4 inhibitor RPL554 is a very effective drug at relaxing human bronchial smooth muscle pre-contracted by a range of contractile agents. Furthermore, RPL554 was able to interact synergistically with muscarinic receptor antagonists and to a lesser extent with β_2 agonists in relaxing human bronchial smooth muscle [89].

Clinical Data

PDE4 inhibitors.

Roflumilast-n-oxide. Roflumilast-n-oxide was first identified in 1993 and shown to have subnanomolar potency for PDE4, with high selectivity against other PDE enzymes. Pre-clinical studies demonstrated beneficial effects on tobacco smoke-induced inflammation, lung fibrosis, remodelling and mucociliary malfunction [90].

Two pivotal 1-year, phase 3 clinical trials in patients with COPD have shown that roflumilast-n-oxide reduces exacerbations and produces a modest improvement in lung function [91,92]. In addition, roflumilast-n-oxide has demonstrated anti-inflammatory effects in the clinic, specifically, in a cross-over study in which 38 patients with COPD received 500 μg roflumilast-n-oxide or placebo once daily for 4 weeks. In this study, roflumilast-n-oxide significantly reduced the absolute number of neutrophils and eosinophils/g sputum compared with placebo, as well as levels of soluble interleukin-8, neutrophil elastase, eosinophil cationic protein and alpha(2)-macroglobulin in sputum and the release of TNF-alpha from blood cells compared with placebo. Furthermore, this study also showed that post-bronchodilator FEV_1 improved significantly during treatment with roflumilast-n-oxide compared with placebo, with a mean difference between treatments of 68.7 ml [93]. Roflumilast-n-oxide, whilst having fewer adverse effects than other PDE4 inhibitors that did not reach the market, still exhibits a significant number of GI effects that are dose limiting.

Roflumilast-n-oxide was licensed in the European Union in 2010 for the maintenance treatment of severe COPD (GOLD stages 3 and 4; i.e. post-bronchodilator $FEV_1 < 50\%$) associated with chronic bronchitis and a history of frequent exacerbations as an add-on to standard therapy. Two 6-month, phase 3 clinical trials have demonstrated beneficial effects of roflumilast-n-oxide in patients already receiving treatment with the long-acting β_2 agonist, salmeterol or the anticholinergic, tiotropium bromide [90].

Other PDE4 inhibitors. As many other orally active PDE4 inhibitors have been stopped in the development because of unacceptable adverse effects [94], this has led to alternative strategies to find PDE4 inhibitors with improved therapeutic ratios. Thus, a number of highly potent PDE4 inhibitors have been developed, including RP73401 [55], AWD 12-281 [76], UK-500,001 [45], CP-325366 [95], GSK 256066 [96] and

more recently a novel class of benzoic acid derivatives [97]. Unfortunately, a number of clinical trials conducted to date with some of these inhaled PDE4 inhibitors have not demonstrated any major therapeutic effect, either in patients with asthma [98,99] or COPD [100,101]. Why these drugs have failed clinically is not known at this time, but it is hoped that newer compounds [97] may show improved effectiveness.

Dual PDE3/4 inhibitors.

Compounds in pre-clinical development. Researchers at the Leiden/Amsterdam Center for Drug Research have reported the synthesis and SAR of a series of potent dual PDE3/4 inhibitors which are able to inhibit arachidonic-acid-induced ear oedema in the mouse, giving 44% inhibition at an oral dose of 16 mg/kg [102].

Altana Pharmaceuticals (now part of Takeda) have filed several patent applications for benzonaphthyridine derivatives as dual PDE3/4 inhibitors some of which reached the clinic (see below), but there are limited data available on these compounds beyond the investigation of pumafentrine [103].
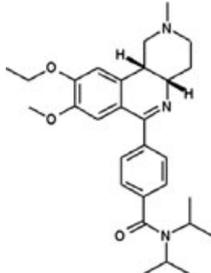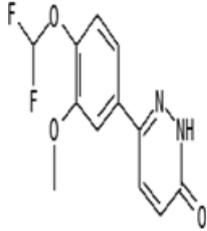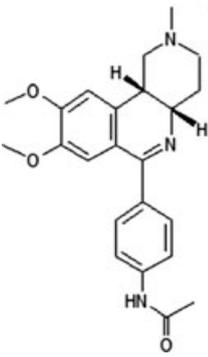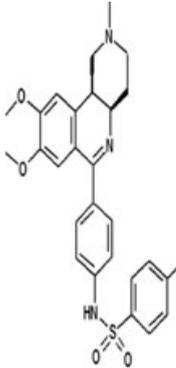
Kyorin have also recently reported a series of potent hybrid PDE3/4 dual inhibitors which potently suppress histamine-induced bronchoconstriction following i.v. administration and also exhibit anti-inflammatory activity in pre-clinical animal models when administered by the inhaled route [104]. However, most of these agents have not been developed beyond the pre-clinical stage because they have been demonstrated to have unwanted effects in the gastrointestinal system.

Compounds reaching clinical trials. More importantly, to date, there have been at least five dual PDE3/4 inhibitors which have reached clinical development (zardaverine, benzafentrine, pumafentrine, tolafentrine and RPL554). Four of these appear to have been discontinued (zardaverine, benzafentrine, pumafentrine, tolafentrine) (see table 1 for summary).

Zardaverine [105] has bronchodilator [106,107] and anti-inflammatory [108] effects in animal models. To our knowledge, two clinical trials have been reported with this compound. In the first study [109], zardaverine was reported to have a modest and short-lasting bronchodilating activity when given by inhalation to twelve patients with reversible bronchial obstruction. In this study, four puffs of either zardaverine (total dose 6 mg) or placebo were inhaled at 15-min. intervals. Compared with placebo, specific airway conductance (sGaw) and FEV_1 increased significantly during the first hour of repeated inhalations. In seven patients, FEV_1 increased by >15%, but the duration of action varied considerably between patients. Three patients, however, had side effects (headache, drowsiness, vertigo, nausea), and one patient dropped out of the study due to vomiting. In 1995, a second study was published in which zardaverine was administered by metered dose inhaler at single doses of 1.5 mg, 3.0 mg or 6.0 mg, and compared with salbutamol (0.3 mg) and placebo (administered on separate days) to 10 patients with partially reversible airway obstruction. In this trial, zardaverine did not improve airway function, whilst salbutamol did [110]. No further clinical studies have been published with

Table 1.

Discontinued PDE3/4 inhibitors that reached clinical trials

	Pumafentrine	Zardaverine	Ben(z)afentrine	Tolafentrine
IC_{50} PDE3	28 nM	110 nM	1.74 μM (platelet)	
IC_{50} PDE4	7 nM	210 nM	1.76 μM	
	• Ph2 for asthma • Safety and tolerability expectations met in phase 1 study • Anti-inflammatory potency demonstrated by TNF-α reduction (whole blood *ex vivo*)	• Ph2 for asthma • Modest & short-lived bronchodilation (inhalation) in patients with airflow obstruction • No effect in separate clinical study in patients with reversible airflow obstruction • Nausea and vomiting	• Dose-dependent bronchodilation (inhaled and i.v.) against Mch challenge in HV at doses that had no effect on blood pressure or heart rate	• Ph1 for pulmonary hypertension/asthma
Reason for discontinuation	Limited duration of action (discontinued Nov 2002)	Unknown Duration of action? GI side effects?	Unknown Duration of action?	Unknown

zardaverine, and to the best of our knowledge, this drug appears to have been discontinued.

Ben(z)afentrine [111] has been administered to healthy volunteers by the oral, i.v. and inhaled route. When given at doses up to 90 mg orally, benzafentrine failed to protect against methacholine-induced bronchoconstriction. However, when given by inhalation, ben(z)afentrine produced a dose-dependent bronchoprotection to methacholine challenge, with an ED_{50} of approximately 9.2 mg. Interestingly, when the drug was given by i.v. infusion (at 20 or 40 mg), a short-lived bronchodilatator response was also observed without affecting blood pressure or heart rate [112]. Unfortunately, a detailed analysis of pharmacokinetic data is missing from this report. However, this pilot study does imply that an inhaled approach delivering a PDE3/4 inhibitor directly to the lung may provide a good therapeutic window and demonstrates that furthermore, even after i.v. administration, drugs having PDE3/4 activity may be able to provide improvements in lung function without causing significant effects on the cardiovascular system. However, despite these somewhat encouraging early clinical results, ben(z)afentrine appears to have been discontinued from development.

Pumafentrine from Altana (now Takeda) [113], also known as BY343, was in phase 2 clinical trials for the treatment of asthma, but was discontinued in 2002 reportedly due to a short duration of action. It has been speculated that the focus shifted to an active metabolite of pumafentrine – hydroxy-pumafentrine, although there have been no published clinical data on this compound [114].

Tolafentrine is cited as a dual PDE3/4 inhibitor, although there is a distinct lack of published information regarding the ability of the drug to inhibit PDE3 and PDE4 enzymes, respectively. The compound is effective by inhaled delivery in rodent models of pulmonary hypertension [115,116], and in 2002, it was reported to be in phase 1 clinical trials for the treatment of primary pulmonary hypertension [117] (having previously been described as synergistically prolonging the vasodilating properties of prostanoids in secondary pulmonary hypertension) [118].

More recently, another dual PDE3 and PDE4 inhibitor RPL554 has been described that is derived from a series of analogues of trequinsin which exhibits both bronchodilator and anti-inflammatory activities in pre-clinical *in vitro* and *in vivo* model systems [119]. RPL554 also produces a rapid, significant and sustained bronchodilator effect in patients with mild–moderate COPD and also in patients with asthma, when administered by the inhaled route [120] and is also bronchoprotective against methacholine challenge. Importantly, RPL554 appears to be at least as effective as the β_2 agonist, salbutamol as a bronchodilator in the same patients and achieves these beneficial effects on lung function without significant effects on the cardiovascular system. At the same

dose that elicits bronchodilation, RPL554 also exhibits highly significant anti-inflammatory effects in man, as it is able to reduce the ability of LPS to induce recruitment of inflammatory cells into the airways, particularly the absolute numbers of neutrophils, eosinophils, lymphocytes and macrophages [120], again without significant effects on the cardiovascular system. RPL554 has limited systemic bioavailability with rapid plasma clearance and no evidence of drug accumulation. RPL554 has been administered to more than 100 people to date (healthy volunteers, asthma patients and patients with COPD) in clinical trials, with adverse events being generally mild and of equal frequency to placebo-treated groups. RPL554 is an effective and well-tolerated bronchodilator, bronchoprotective and anti-inflammatory drug that shows promise for the treatment for COPD, asthma and potentially other obstructive airway diseases such as CF.

Adverse Effects of PDE3 and PDE4 Inhibitors

The therapeutic window of orally administered selective PDE4 inhibitors is currently limited by GI side effects such as nausea, vomiting, diarrhoea, abdominal pain and dyspepsia, although some of these GI effects do appear to resolve over time. Despite these observations, roflumilast-n-oxide was still recently approved for the treatment of severe COPD in a number of countries, including the USA.

Additionally, regulatory agencies have been particularly concerned by the observation that mesenteric vasculitis develops in some laboratory animal species with some PDE4 inhibitors, although this has also been seen with theophylline, a drug that has been widely used clinically for almost a century [121,122] questioning the relevance of the pre-clinical toxicology findings. To date, there have been no reports of mesenteric vasculitis in patients treated with roflumilast-n-oxide or with another orally active PDE4 inhibitor cilomilast that was widely used in patients with asthma and COPD in phase 3 clinical trials. It is plausible therefore that rats and dogs have an increased susceptibility to drug-induced vascular lesions as such arteriopathies commonly occur in these species that are regularly used for pre-clinical toxicology studies [123,124], and species differences have been shown to exist in terms of both PDE4 expression and also the functional effects of PDE4 inhibitors. For example, a recent study demonstrated that levels of PDE4 enzyme activity are much higher in rats than human beings in multiple tissues, which could explain why rats are more susceptible to PDE4 inhibitor-induced toxicities [125]. In addition, the PDE4 inhibitor IC542 significantly enhanced LPS-induced IL-6 release from rat whole blood [126], whilst having no potentiating effect on LPS-induced IL-6 release from human or non-human primate blood. The potential for developing vasculitis with systemically active PDE inhibitors does, however, require careful monitoring in man, and there have been attempts to identify potential predictive biomarkers for this unwanted effect. Indeed, the tissue inhibitor of metalloproteinase 1 appears to be an early and sensitive predictive biomarker of the inflammatory and tissue remodelling components of PDE4 inhibitor-induced vascular injury in rats [127].

Some insight into the potential isoform subtypes responsible for mediating side effects of PDE4 inhibition can be gleaned from studies with subtype knockout mice, although potential species differences need to be considered. PDE4A$^{-/-}$ and B$^{-/-}$ mice have normal neonatal growth survival and fertility, whereas this is impaired in PDE4D$^{-/-}$ mice [128]. Furthermore, PDE4D$^{-/-}$ mice have been shown to suffer from various cardiovascular complications [128]. However, PDE4D$^{-/-}$, but not PDE4B$^{-/-}$ mice have shortened α_2 adrenoceptor-mediated anaesthesia, which is thought to be a behavioural correlate of emesis [129], and thus, it has been suggested that the D isoform is responsible for the emesis which has been observed with orally administered PDE4 inhibitors.

Even some inhaled PDE inhibitors have been shown to elicit gastrointestinal side effects in patients (e.g. Zardaverine), suggesting that the inhaled route is not sufficient alone to reduce this troublesome side effect profile (presumably via the swallowed portion of the inhaled dose). Rather, there is a need to ensure that only non-emetic pharmacophores are developed such as RPL 554.

PDE3 inhibitors were developed in the 1980s as 'safer' alternatives to cardiac glycosides for the treatment of dilated cardiomyopathy, and in the short-term, beneficial effects on the force of myocardial contraction and vascular smooth muscle tone were reported. However, chronic treatment (more than 28 days systemic treatment) resulted in a significant increase in mortality [130] which has somewhat sensitized the field to avoid developing drugs with significant PDE3 inhibitory activity. However, the PDE3 inhibitor, milrinone, that was implicated in these early clinical trials, is currently still in regular clinical use for the short-term therapy of severe congestive heart failure, and four other PDE3 inhibitors (amrinone, cilostazol, pimobendane and enoximone) are also used clinically for a number of indications, including prevention of stroke and for their anti-platelet activity. Importantly, however, in the discussion about the potential side effect liability of PDE3 inhibitors, it needs to be appreciated that some of these drugs are not just PDE3 inhibitors, but exhibit a range of other pharmacological actions which may have contributed to the adverse effects seen with these compounds clinically, particularly when they were administered chronically by the systemic route. For example, milrinone is an adenosine 1 receptor antagonist [131]. The relative contribution of inhibition of PDE3A and B to these adverse effects is unclear, not least, as these inhibitors can inhibit both PDE3 isoforms. However, some insight into the potential roles of PDE3A and 3B can be derived from knockout mouse studies as PDE3A$^{-/-}$ and PDE3B$^{-/-}$ mice have normal neonatal growth and survival, but PDE3A$^{-/-}$ mice have an increased heart rate and are infertile [132]. However, PDE3B$^{-/-}$ mice do not share these characteristics. Metabolic dysregulation, including systemic insulin resistance, has, however, been observed in PDE3B$^{-/-}$ mice [133].

Given that additive and synergistic effects of dual PDE3/4 inhibition have been observed in terms of efficacy end-points, a clear concern is that additive or synergistic effects could also be seen with respect to potential adverse events. In this regard, the phenotype of dual PDE3$^{-/-}$ and PDE4$^{-/-}$ mice would

clearly be of interest to investigate, but we are not aware of such mice being available. It is of interest, however, that whilst selective inhibition of PDE3 or PDE4 in wild-type cardiomyocytes causes elevated calcium transients, sarcoplasmic reticulum Ca^{2+} content and phospholamban phosphorylation [134], combined PDE3 and PDE4 inhibition caused no further increases in sarcoplasmic reticulum function. The reason for this perhaps unexpected finding is unclear, but could be related to compartmentalization of pools of cAMP. Nevertheless, no pre-clinical findings were identified in toxicology studies which prevented 5 inhaled dual PDE3/4 inhibitors progressing to early clinical trials, one of which, RPL554, is currently in phase 2 clinical trials, and to date, this drug has not been associated with any clinically significant cardiovascular side effects [120].

Conclusion

Combined inhibition of PDE3 and PDE4 would appear to be an attractive strategy to treat COPD, and other inflammatory airway diseases given the broad anti-inflammatory and bronchodilator effects of these agents, together with their potential to stimulate mucociliary clearance. It is clear that dual inhibition of PDE3 and PDE4 is required to optimally inhibit the activity of certain key inflammatory cell types (e.g. macrophages, lymphocytes, epithelial cells and airway smooth muscle cells) involved in the pathogenesis of COPD and the inhaled route of administration would be a sensible strategy to take with PDE3/4 inhibitors to maximize the therapeutic ratio and reduce the adverse effects that have been previously seen with older systemically administered PDE3 and PDE4 inhibitors which has limited their therapeutic utility. Designing an agent with subtype selectivity could also potentially offer an advantage based on data generated with knockout mice, together with the expression profile of PDE3B, suggests that a compound which could selectively inhibit PDE3B, rather than PDE3A could be beneficial to potentially mitigate cardiovascular risk, although selective inhibitors of PDE3A and PDE3B would clearly be required to confirm this. It would seem that PDE4 A, B and D play important roles in mediating the anti-inflammatory effects of PDE4 inhibitors and that 4D may contribute to effects on airway smooth muscle, although no PDE4 inhibitor to date has exhibited any acute bronchodilator activity. The availability of an inhaled dual PDE3/4 inhibitor, RPL 554, that has both bronchodilator and anti-inflammatory activity and that is rapidly cleared from the systemic circulation may provide a novel approach to treating COPD and other inflammatory airway diseases going forward.

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