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Novel Roflumilast analogs as soft PDE4 inhibitors

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ABSTRACT

PDE4 inhibitors are of high interest for treatment of a wide range of inflammatory or autoimmune diseases. Their potential however has not yet been realized due to target-associated side effects, resulting in a low therapeutic window. We herein report the design, synthesis and evaluation of novel PDE4 inhibitors containing a γ-lactone structure. Such molecules are designed to undergo metabolic inactivation when entering circulation, thereby limiting systemic exposure and reducing the risk for side effects. The resulting inhibitors were highly active on both PDE4B1 and PDE4D2 and underwent rapid degradation in human plasma by paraoxonase 1. In contrast, their metabolites displayed markedly reduced permeability and/or on-target activity.

Cyclic nucleotides, including cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP), play a key role in mediating cellular responses to various hormones and neurotransmitters. The levels of cAMP and cGMP are tightly regulated in their synthesis and degradation.[1] Cyclic nucleotide phosphodiesterases (PDE) represent a superfamily of intracellular enzymes that degrade cyclic nucleotides.[2] In humans, 21 PDE genes are known and can be classified into 11 families, which differ in their intracellular localization and affinity for cAMP and cGMP. The cAMP-specific phosphodiesterase 4 (PDE4) family, encoded by four genes (PDE4A-D), contains over 20 identified isoforms and is the largest of the 11 PDE families.[1] PDE4 isoforms are widely distributed and highly present among pro-inflammatory, inflammatory and immune cells, where they represent the main cAMP-metabolizing enzymes.[1,3] As increased cAMP levels inhibit inflammatory cell function, PDE4 inhibitors are of potential interest for the treatment of a wide range of inflammatory or autoimmune diseases,[1] including chronic obstructive pulmonary disease (COPD),[4] asthma,[3,4] dry eye disease,[5] psoriasis[6] or inflammatory bowel disease (IBD).[7]

Safety-related issues represent a significant challenge in the development of PDE4 inhibitors. Reported side effects resulting from systemic exposure to PDE4 inhibitors include emesis, nausea, dyspepsia, diarrhea, abdominal pain and headache. Arteritis, which is characterized by blood vessel inflammation, hemorrhage, and necrosis, has been reported in animal models.[8,9] Such side effects can at least in part be associated with on-target activity. For instance, a correlation has been found between the emetic effect of PDE4 inhibitors and their occupancy of the high affinity Rolipram (Fig. 1) binding site of PDE4 in brain.[10] In spite of such issues, the PDE4 inhibitor Roflumilast (Fig. 1) has been approved in the EU (as Daxas®) and in the US (as Daliresp®) for treatment of severe COPD associated with chronic bronchitis and a history of exacerbations.[11] Apremilast (Otezla®) has been recently approved for the treatment of psoriatic arthritis.[12] Many development programs of PDE4 inhibitors have however been discontinued due to side effects,[8,9] and the full potential of PDE4 inhibitors is yet to be exploited.

Several attempts have been made to mitigate the side effects resulting from PDE4 inhibition. It has been suggested that occupancy of the high affinity binding site for Rolipram was responsible of emetic effects and that PDE4 inhibitors targeting the low affinity binding site of Rolipram would display an improved therapeutic window.[10] However, Cilomilast, which displays selectivity towards the low affinity binding site still causes side effects.[13] Isoform

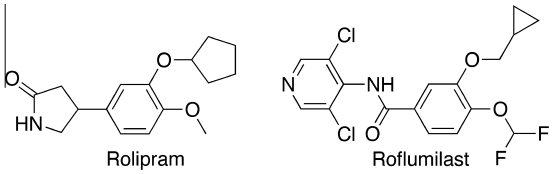

Figure 1. Structure of the PDE4 inhibitors Rolipram and Roflumilast.

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selectivity has also been investigated, as PDE4B was proposed as the main PDE4 isoform mediating TNF-α release,[14] while knockout experiments suggested that inhibition of PDE4D would be associated with emesis.[15] However, the improved therapeutic window observed with several PDE4 inhibitors during preclinical studies was not always confirmed in clinical trials.[8]

Another possibility is to optimize PDE4 inhibitors towards topical delivery.[8,16,17] In this context a soft drug approach represents an attractive way towards novel PDE4 inhibitors with improved therapeutic window. Soft drugs, sometimes known as antedrugs, are biologically active compounds that are designed to undergo metabolic inactivation by controlled conversion of the parent molecule into a predictable, nontoxic metabolite.[18,19] This strategy has recently been applied to a series of boron-containing PDE4 inhibitors, in view of dermatologic applications.[20] We have recently reported the design and evaluation of soft inhibitors of ROCK, a kinase target of relevance for the treatment of respiratory diseases, including COPD.[21] Herein, we discuss the design and initial evaluation of soft PDE4 inhibitors which are analogs of Roflumilast.

Crystal structures of PDE4 in complex with several inhibitors, including Roflumilast, have been reported and provide a structural basis for their activity.[22] Those structures revealed multiple hydrophobic interactions with highly conserved residues sandwiching the inhibitor in the active site, as well as H-bonding interactions between the (halo)alkoxy group found in several inhibitors and an invariant glutamine. The lipophilic nature of the Roflumilast binding site was further emphasized in a later publication wherein a linker of sufficient length had to be included between the main inhibitor scaffold and ionic solubilizing groups.[5] Interestingly, such findings suggest an inactivation mechanism for the design of soft PDE4 inhibitors, since generation of a charged species by drug metabolizing enzymes could result in a metabolite with decreased potency. The associated change in physical–chemical properties can also result in reduced membrane permeability, which represents another way of modulating the functional activity of compounds inhibiting an intracellular target.

For the design of soft PDE4 inhibitors, we chose to favor a γ-lactone structure, which was previously used in soft corticosteroids[23] and more recently in the design of soft ROCK inhibitors.[24] Hydrolysis of this lactone will result in a negatively charged species, with increased polar surface area and decreased hydrophobicity. Candidate soft PDE4 inhibitors were prepared in 5 or 6 steps, starting from the commercially available 3,4-dihydroxybenzaldehyde **1** (Fig. 2). Sequential alkylation of **1** first involved selective difluoromethylation on position 4, using sodium chlorodifluoroacetate as a source of difluorocarbene. Alkylation of the remaining hydroxyl function of **2** was performed with alkyl dibromides, yielding intermediates **3a** and **3b**. In the next step, the aldehyde function was oxidized to the corresponding benzoic acids **4a** and **4b,** using sodium chlorite (Lindgren oxidation) in combination with sulfamic acid as chlorine dioxide scavenger. Coupling with 3,5-dichloropyridin-4-amine, after in situ conversion of **4a** and **4b** into acid chlorides, resulted in intermediates **5a** and **5b**. Further nucleophilic substitution with either 3-amino- or 3-sulfanyldihydrofuranone yielded final parent compounds **6**–**9** in a single step. The corresponding metabolites **6M**–**9M** could be easily obtained by hydrolysis of the lactone structure with LiOH. As N-oxide analogs of Roflumilast and related PDE4 inhibitors were reported to possess significant inhibitory activity against PDE4,[11,25] compound **10**, representing the N-oxide analog of **6** was synthesized using the same procedure as described above, by replacing 4-amino-3,5-dichloropyridine with 4-amino-3,5-dichloropyridine-N-oxide in the corresponding reaction step. Finally, compound **11**, an acyclic analog of **6** was synthesized by alkylating **5a** with the methyl ester of glycine. As with **6**–**9**, the corresponding metabolite **11M** was synthesized via hydrolysis of the parent compound with LiOH.

Evaluation of the candidate PDE4 inhibitors revealed strong on-target activity. Comparative data with Roflumilast and Rolipram are given in Table 1. Parent compounds **6**, **7**, **8** and **9** displayed similar IC_{50} values in the low nanomolar range. Replacement of the pyridine moiety by a pyridine N-oxide resulted in a significant drop in potency, as illustrated by **10**. This finding was surprising, as the N-oxide analogs of Roflumilast are known to possess significant inhibitory activity against PDE4.[11,25] Compound **11**, representing an acyclic analog of **6**, displayed slightly reduced (2–4 fold) potency. Significant differences were found between the expected metabolites **6M**, **7M**, **8M**, **9M** and **11M**. Indeed, **6M** and **8M**, which resulted from hydrolysis of a homoserine lactone structure, were markedly less active than their parent compounds, while their sulfanyl analogs **7M** and **9M** essentially retained their on-target activity. This conserved activity was not the result of re-lactonization of the metabolites under assay conditions or storage in DMSO stock solutions, as both hypotheses were excluded by HPLC–MS analysis. A major drop of potency was also found for **11M**.

An important part of the observed structure–activity relationships can be explained by the charge state of the different species, and by the distance between charged groups and the main inhibitor scaffold. Indeed, it is known that highly polar moieties are poorly tolerated in the essentially hydrophobic binding site of PDE4, unless a spacer of sufficient length is placed between the ionizable group and the main inhibitor scaffold.[5] All parent compounds remain neutral at physiological pH and under assay conditions, and consequently display strong on-target activity. The fact that metabolites from sulfanyl analogues **7M** and **9M** retain strong on-target activity suggests that the carboxylic group resulting from

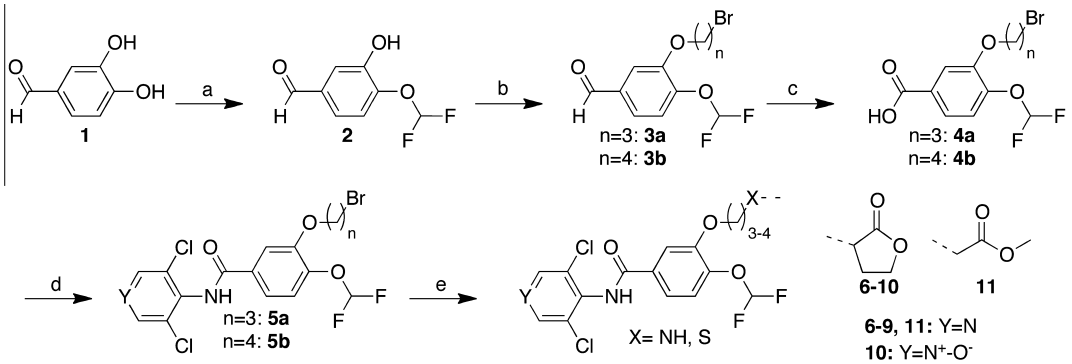

Figure 2. Overview of compound synthesis: (a) $ClF_2CCOONa$, NaOH, DMF, H_2O, 120 °C, 2 h; (b) $Br(CH_2)_nBr$, K_2CO_3, ACN, reflux, 4 h; (c) H_2NSO_3H, $NaClO_2$, AcOH, H_2O, 10 °C, 30 min; (d) (1) $SOCl_2$, toluene, 90 °C, 1.5 h; (2) 3,5-dichloro-4-aminopyridine or 3,5-dichloro-4-aminopyridine N-oxide, 60% NaH, THF, 10 °C, 1 h; (e) 2-oxo-3-amino-oxolane, 2-oxo-3-mercapto-oxolane or glycine methyl ester; K_2CO_3, ACN, reflux, 1.5 h.