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Novel Class of Benzoic Acid Ester Derivatives as Potent PDE4 Inhibitors for Inhaled Administration in the Treatment of Respiratory Diseases

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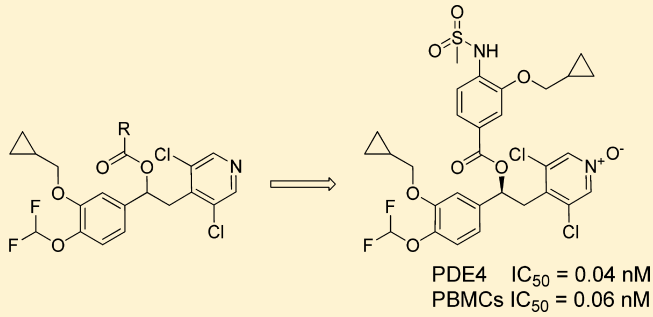
Supporting Information

ABSTRACT: The first steps in the selection process of a new anti-inflammatory drug for the inhaled treatment of asthma and chronic obstructive pulmonary disease are herein described. A series of novel ester derivatives of 1-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl) ethanol have been synthesized and evaluated for inhibitory activity toward cAMP-specific phosphodiesterase-4 (PDE4). In particular, esters of variously substituted benzoic acids were extensively explored, and structural modification of the alcoholic and benzoic moieties were performed to maximize the inhibitory potency. Several compounds with high activity in cell-free and cell-based assays were obtained. Through the evaluation of opportune in vitro ADME properties, a potential candidate suitable for inhaled administration in respiratory diseases was identified and tested in an in vivo model of pulmonary inflammation, proving its efficacy.



INTRODUCTION

The phosphodiesterase 4 (PDE4) enzyme is highly expressed in inflammatory and immune cell types relevant for the pathogenesis of airway inflammatory disorders.[1] Indeed, PDE4 inhibition, resulting in an increase of intracellular cAMP concentration, exerts a broad range of anti-inflammatory effects, including the inhibition of cellular trafficking and microvascular leakage, cytokine and chemokine release, reactive oxygen species production, and cell adhesion-molecule expression.[2] PDE4 is also expressed in lung structural cells: fibroblasts, airway epithelial and endothelial cells, and airway and vascular smooth muscle cells. Therefore, PDE4 inhibitors have the potential to alleviate not only pulmonary inflammation but also fibrotic airway and vascular remodeling and mucociliary dysfunction (mucus hypersecretion and decreased mucus transport), which are pathophysiological features common to various pulmonary diseases.[3] These beneficial effects of PDE4 inhibitors have been demonstrated in a broad array of in vitro and in vivo experimental models of airway inflammation.[4] Consequently, there has been great interest in developing PDE4 inhibitors for the treatment of respiratory diseases.

The development of first-generation PDE4 inhibitors, such as rolipram (**1**), has been hampered by the occurrence of mechanism-associated side effects, nausea and emesis, at effective anti-inflammatory doses.[5] Second-generation inhibitors, roflumilast[6] (**2**) and cilomilast[7] (**3**), were generally better-tolerated, but this was likely after administration of doses that did not achieve sustained PDE4 inhibition[8,9] (Figure 1). The recent registration of roflumilast in Europe and the U.S. has confirmed

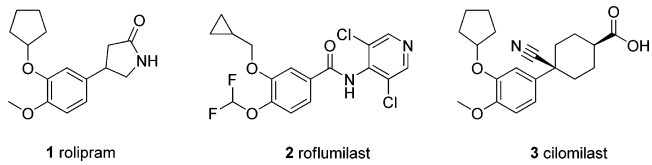


Figure 1. Structures of rolipram (**1**) (first-generation PDE4 inhibitor) and roflumilast (**2**) and cilomilast (**3**) (second-generation PDE4 inhibitors for oral administration).

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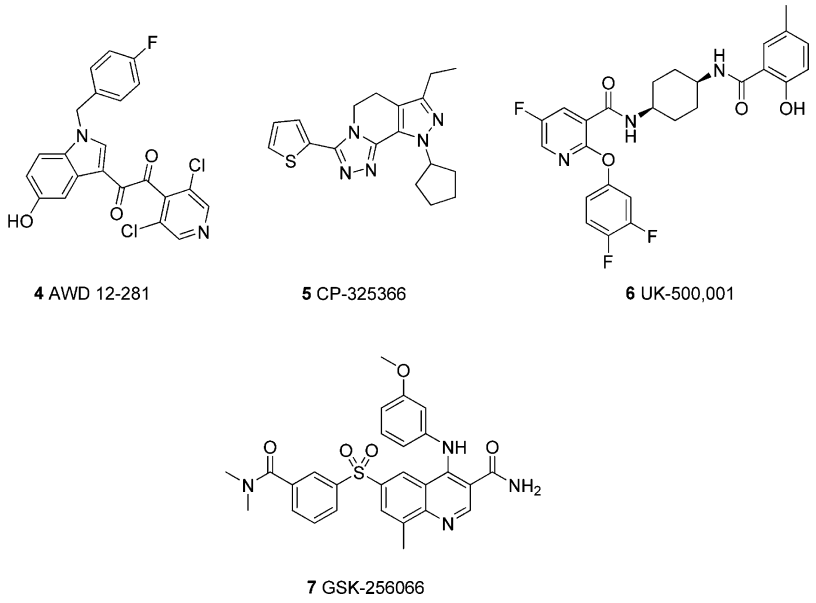

Figure 2. Structures of the most significant PDE4 inhibitors for inhaled administration.

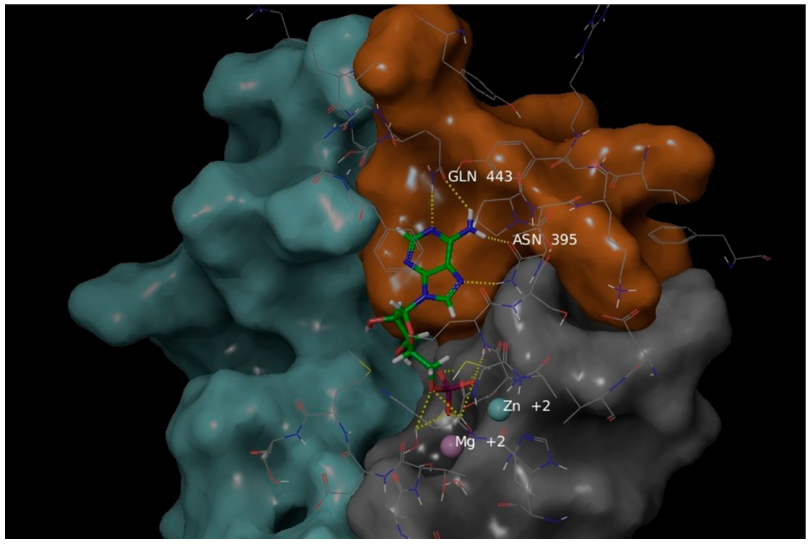

Figure 3. AMP bound to PDE4B (orange, hydrophobic region; gray, metal region; and turquoise, solvent exposed region) (PDB ID: 1TB5).

the therapeutic utility of targeting PDE4 in severe chronic obstructive pulmonary disease (COPD) patients and has renewed interest in this target, prompting the search for third-generation inhibitors endowed with a greater therapeutic window.[10]

A number of strategies are being followed to achieve this objective. The synthesis of compounds selective for the PDE4B isoform[11,12] or that are devoid of affinity for the high-affinity rolipram binding site[13] has been pursued. However, because of the lack of very selective compounds, it remains elusive whether these approaches may be a viable option for improving the therapeutic window of PDE4 inhibitors. It has been hypothesized that compounds characterized by a limited blood-brain barrier penetration could be endowed with a reduced emetic profile. Nevertheless, this approach has also been questioned because compounds that preferentially distribute to the brain are relatively free from emesis.[14] Moreover, the chemoreceptor trigger zone in the brain stem is accessible to the free drug within the circulation,[15] and PDE4 inhibitors may interfere with local regulation of acid secretion in the gastrointestinal tract.[16]

A different strategy to achieve a larger therapeutic window between efficacy and side effects involves drug administration via inhalation directly to lung tissue. Topically administered compounds require much lower doses to block local PDE4 activity than oral drugs and should lack the adverse effects associated with systemic exposure. This idea prompted the clinical development of a number of inhaled inhibitors such as **4** (AWD-12-281),[17] **5** (CP-325366),[18] **6** (UK-500,001),[19] and **7** (GSK-256066)[20] (Figure 2).

Unfortunately, a lack of efficacy in phase II trials in asthma and COPD led to the discontinuation of the development of **4** (AWD-12-281), **5** (CP-325366), and **6** (UK-500,001).[21] Given the suboptimal anti-inflammatory activity displayed by these compounds in cell-based assays, it can be hypothesized that their clinical efficacy has been compromised by the administration of doses lower than the ones required to achieve adequate and sustained inhibition of PDE4 in the lung tissue. **7** (GSK-256066),

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which is at least 3 orders of magnitude more potent than the other inhaled compounds tested in the clinic, showed promising efficacy in an allergen-challenge study in mild asthmatics and in a 4 week study in COPD patients at a dose of 87.5 μg/day. In the latter study, a significant inhibition of markers of PDE4 activity was demonstrated in sputum cells, suggesting that the administration of extremely potent compounds may be necessary to affect the activity of PDE4 in the lung tissue significantly.[22]

We report here the discovery of a novel series of potent PDE4 inhibitors, which are characterized by an in vitro profile that is promising for inhaled administration. Such PDE4 inhibitors specifically suitable for inhaled administration at a low dose could offer a solution to the issue of emesis and nausea for long-term treatment of chronic airway diseases and could also improve the clinical efficacy. Our medicinal chemistry strategy was initially based on the analysis of the PDE4 catalytic binding pocket. This includes three subdomains: a hydrophobic region, which contains residues Gln443 and Asn395 that are important for nucleotide recognition, a metal region where two cations (Zn^{2+} and Mg^{2+}) are linked to polar residues and to six coordinated water molecules, and a solvent-exposed region, which is generally filled by water molecules. In Figure 3, the crystal structure of PDE4B in complex with the hydrolytic product AMP (PDB ID: 1TB5) reveals that the main interactions of the endogenous ligand are formed exclusively in the hydrophobic pocket and in the metal region of the catalytic binding pocket.[23] The adenine fragment of AMP forms four hydrogen bonds with residues Gln443 and Asn395 in the hydrophobic region, the ribose ring is shared by the hydrophobic and metal regions, and the phosphate group interacts near the metals. The left part of Figure 3 shows the solvent-exposed region in turquoise; additional binding energy could be achieved through interacting with the residues of the solvent-exposed region.

Because an inhaled administration could reduce the side effects of PDE4 inhibitors, particular attention should be given to the physicochemical properties that can affect the level of drug in plasma. High lipophilicity is an important physicochemical property for high plasma protein binding,[24] which can contribute to the reduction of unwanted systemic side effects. A comparison of the predicted $LogD_{7.4}$ values[25] for the PDE4 inhibitors, included in Figures 1 and 2 and Table 1, confirmed that most

Table 1. Calculated $logD_{7.4}$ Values for Oral and Inhaled PDE4 Drug Candidates

compd	administration	calculated $LogD_{7.4}$[a]
rolipram	oral	2.00
roflumilast	oral	2.30
cilomilast	oral	−0.78
AWD-12-281	inhalation	4.92
CP-325366	inhalation	4.22
UK-500,001	inhalation	5.05
GSK-256066	inhalation	2.18

[a]ACD/Labs logD v.11.

inhaled clinical candidates are more lipophilic than the oral ones. Even if, in principle, a high lipophilicity could reduce the free fraction available for activity in the lung, there are also cases that suggest that a high lipophilicity is compatible with a good efficacy in the lung (i.e., the clinical efficacy of Fluticasone Furoate[26] with a predicted $LogD_{7.4}$ 4.10). Therefore, the aim of our project was the identification of a novel class of PDE4 inhibitors endowed with high plasma protein binding and able to undertake extended interaction with all three regions of the catalytic binding pocket (Figure 3), resulting in potentially improved inhibitory potency. Starting from the structure of roflumilast, we replaced the amide linker connecting the two aromatic systems with a hydroxyethylene one, which offered an anchoring point for the introduction of an additional portion potentially oriented to the solvent-exposed region. Ester derivatives of 1-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl) ethanol were synthesized, and the benzoic acid ester class was extensively explored. In such compounds, the dialkyloxyphenyl and the dichloropyridinyl groups could ensure the interaction with the hydrophobic region and the metal region of the PDE4 protein, similar to the binding mode of roflumilast,[23] whereas the ester portion could potentially interact with the solvent-exposed region. The importance of the stereochemistry of the alcoholic carbon and the effect of N-oxidation of the pyridine ring were investigated, and several patterns of substitution were considered on the benzoic acid portion. Calculated $LogD_{7.4}$ and selected in vitro ADME properties were investigated for a subset of potent compounds to identify a candidate suitable for inhaled administration.

Chemistry. The synthetic pathway employed for the synthesis of ketone and enolic ester derivatives (**14** and **15**) is shown in Scheme 1. The preparation of acyl chloride **11** was accomplished starting from commercially available 3,4-dihydroxybenzaldehyde **8**. Selective alkylation of the 4-hydroxy group using sodium chlorodifluoroacetate and NaOH led to the formation of 4-difluoromethoxy intermediate **8a**, alternatively purchased from a commercial supplier. Further alkylation at position 3 with (bromomethyl)cyclopropane gave aldehyde **9**, which was oxidized to acid **10** by sodium chlorite and sulfamic acid in acetic acid. Intermediate **10** was finally converted into corresponding acyl chloride **11** by treatment with thionyl chloride. 3,5-Dichloro-4-methylpyridine **13** was obtained by methylation of commercially available 3,5-dichloropyridine **12** with methyl iodide in the presence of freshly prepared LDA. The lithium salt of **13** (obtained by means of LDA in THF) was coupled with 0.3 equiv of acyl chloride **11** at −70 °C to afford ketone **14**. When an excess of **11** was employed, ester **15** was obtained.

Esters of (±)-**16** were prepared following the synthetic protocol in Scheme 2. Alcohol (±)-**16** was obtained from aldehyde **9** and intermediate **13** in the presence of potassium *tert*-butoxide at −30 °C. Esters (±)-**18** and (±)-**19a−e** were obtained by coupling intermediate (±)-**16** with suitable carboxylic acids using EDC and DMAP.

Enantiomeric separation of (±)-**18** was obtained by semipreparative HPLC (Chiralcel OD column, hexane/*i*PrOH 95:5) to afford single enantiomers (+)-**18** (first elution) and (−)-**18** (second elution) (Scheme 3). Oxidation of racemic (±)-**18** with *m*CPBA gave the corresponding N-oxide as a racemic mixture, which was resolved by semipreparative chiral HPLC (Chiralcel OD, hexane/*i*PrOH 50:50) to give single enantiomers (+)-**20** (first eluting compound) and (−)-**20** (second eluting compound) (Scheme 3).

The absolute configuration of these compounds was then assigned. Racemic alcohol (±)-**21**, obtained by oxidation of (±)-**16** with *m*CPBA, was condensed with (*R*)-naproxen to afford a mixture of two diastereoisomers that were separated by preparative HPLC (Scheme 4). By means of single-crystal X-ray diffraction (Supporting Information) it was possible to determine the structure of compound (*R**,*S***)-**22** (Figure 4), revealing that the absolute configuration at the alcoholic

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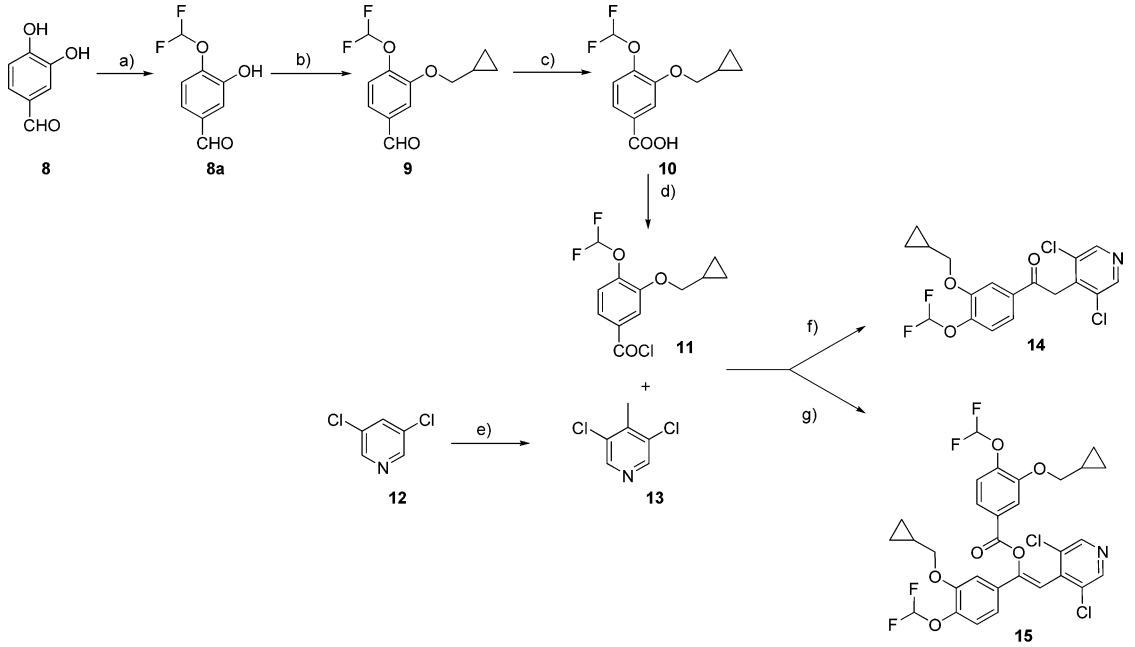
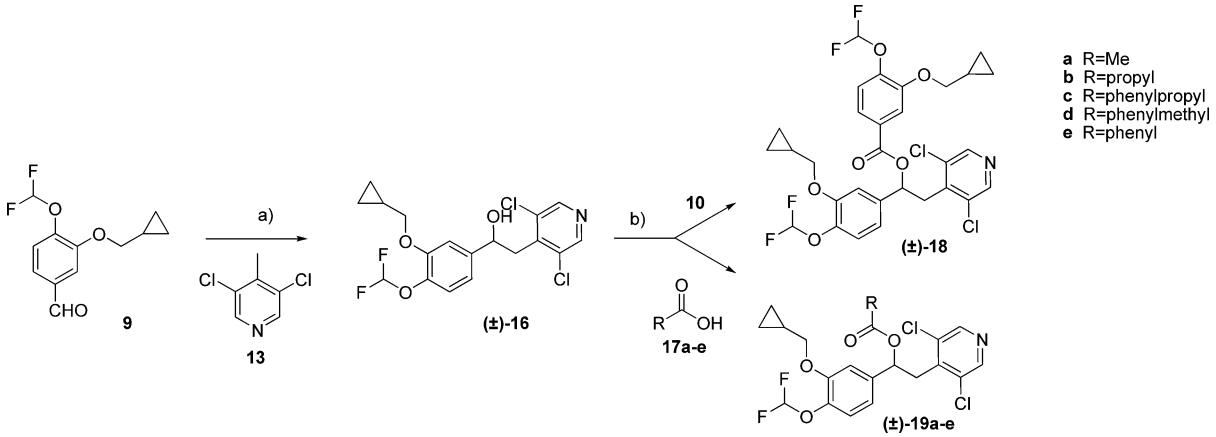
Scheme 1. Preparation of Derivatives 14 and 15[a]



[a]Reagents and conditions: (a) $CF_2ClCOO^-Na^+$, NaOH, DMF, H_2O, 120 °C, 2 h, 44%; (b) (bromomethyl)cyclopropane, K_2CO_3, THF, reflux, 14 h, 97%; (c) H_2NSO_3H, $NaClO_2$, CH_3COOH, H_2O, rt, 1 h, 97%; (d) $SOCl_2$, toluene, reflux, 2 h, 100%; (e) iPr_2NH, BuLi, −10 °C, 30 min, **12**, −20 °C, 30 min, then MeI, −70 °C to rt, 68%; (f) **13**, LDA (1.8 M in THF), −70 °C, 30 min; **11** (0.3 equiv), −70 °C, 15 min, 20%; (g) **13**, LDA (1.8 M in THF), −70 °C, 30 min, then **11** (2 equiv), −70 °C to rt, 30 min, 30%.

Scheme 2. Preparation of Alcohol (±)-16 and Esters (±)-18 and (±)-19a−e[a]



[a]Reagents and conditions: (a) *t*BuOK, THF, −30 °C, 1 h, 75%; (b) EDC, DMAP, DCM or DMF, rt.

stereogenic center was (S). The chemical correlations summarized in Scheme 5, by means of reactions not involving the chiral center (i.e., reduction of N-oxide, cleavage of naproxen ester, esterification with acid **10**, and oxidation with *m*CPBA), allowed the assignment of absolute configuration to compounds (*R*)-(+)-**18**, (*S*)-(−)-**18**, (*R*)-(+)-**20**, and (*S*)-(−)-**20** as well as to the related alcohols. Retention of configuration was further confirmed by esterification of (*S*)-(+)-**16** with (*R*)-naproxen, giving (*R***,*S**)-**23** (Scheme 5, step c).

A synthetic pathway for the large-scale preparation of enantiomerically pure (*S*)-**21** was optimized as shown in Scheme 6. Racemic alcohol (±)-**21** was coupled with (*S*)-(+)-acetylmandelic acid to afford a couple of diastereoisomers, which were separated by fractional crystallization from Et_2O. The less soluble (*S**,*S***)-**24** isomer was obtained in a diastereomeric ratio >95% after several crystallizations, whereas its epimer, (*S**,*R***)-**24**, was recovered from mother liquors and crystallized from *i*PrOH. Solvolysis in a mixture of methanol and aqueous $NaHCO_3$ afforded enantiomerically pure (*S*)-**21** and (*R*)-**21**.

The preparation of esters (*S*)-**26a−s** was achieved by coupling alcohol (*S*)-**21** with suitable carboxylic acids in the presence of EDC and DMAP as condensing agents (Scheme 7). Benzoic acids **25a−s** were either commercially available or were prepared as detailed in the Supporting Information. Aniline derivative (*S*)-**26t** was obtained by reduction of nitro analogue (*S*)-**26q** with $SnCl_2$ hydrate, whereas hydroxyl derivative (*S*)-**26u** was prepared by catalytic hydrogenation of benzyl derivative (*S*)-**26r** with palladium on barium sulfate as catalyst. Reduction of aldehyde (*S*)-**26s** with sodium borohydride in THF gave hydroxymethyl derivative (*S*)-**26v**, whereas oxidation of (*S*)-

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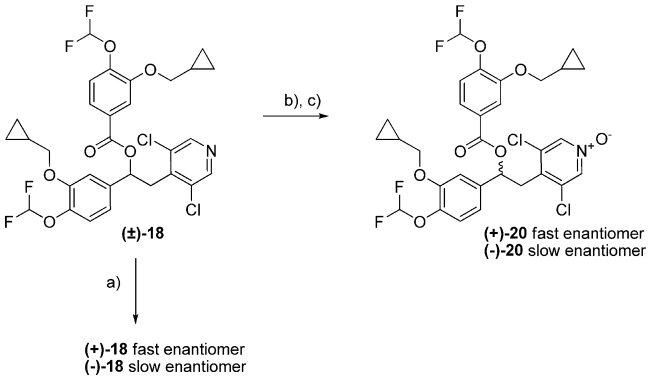
Scheme 3. Preparation of Enantiopure Compounds (+)-18, (−)-18 and (+)-20, (−)-20[a]



[a]Reagents and conditions: (a) chiral HPLC, column: Chiralcel OD, eluant: hexane/*i*PrOH 95:5, flow: 18 mL/min; (b) *m*CPBA, DCM, rt, 18 h, 45%; (c) chiral HPLC, column: Chiralcel OD, eluant: hexane/*i*PrOH 1:1, flow: 18 mL/min.

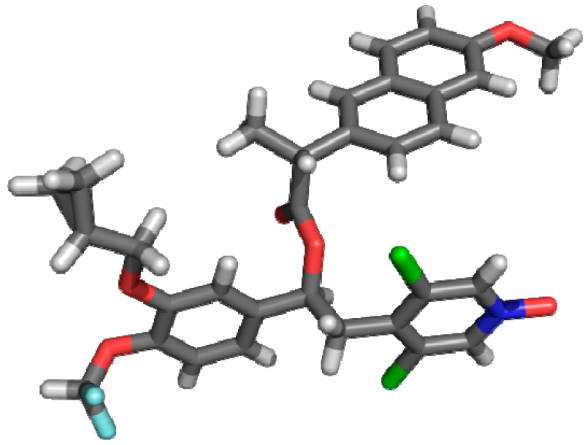


Figure 4. X-ray structure of (*R**,*S***)-**22**.

26s with sodium chlorite and sulfamic acid in acetic acid gave carboxylic acid (*S*)-**26w**.

Sulfonamides (*S*)-**32a−l** were prepared as described in Scheme 8. Intermediates **27a−l** were either commercially available or were prepared as detailed in the Supporting Information. Sulfonilation of anilines **27a−l** with sulfonyl chlorides in pyridine gave sulfonamides **28a−j**. N-Boc protection followed by hydrolysis of methyl esters **29a−j** under basic conditions gave acids **30a−j**, which were coupled with (*S*)-**21** to obtain intermediates (*S*)-**31a−j**. The removal of Boc protection under acidic conditions led to final compounds (*S*)-**32a−j**. Compounds (*S*)-**32k−l** were alternatively prepared by direct hydrolysis of intermediates **28k−l** followed by coupling with alcohol (*S*)-**21**, which avoided the protecting and deprotecting steps.

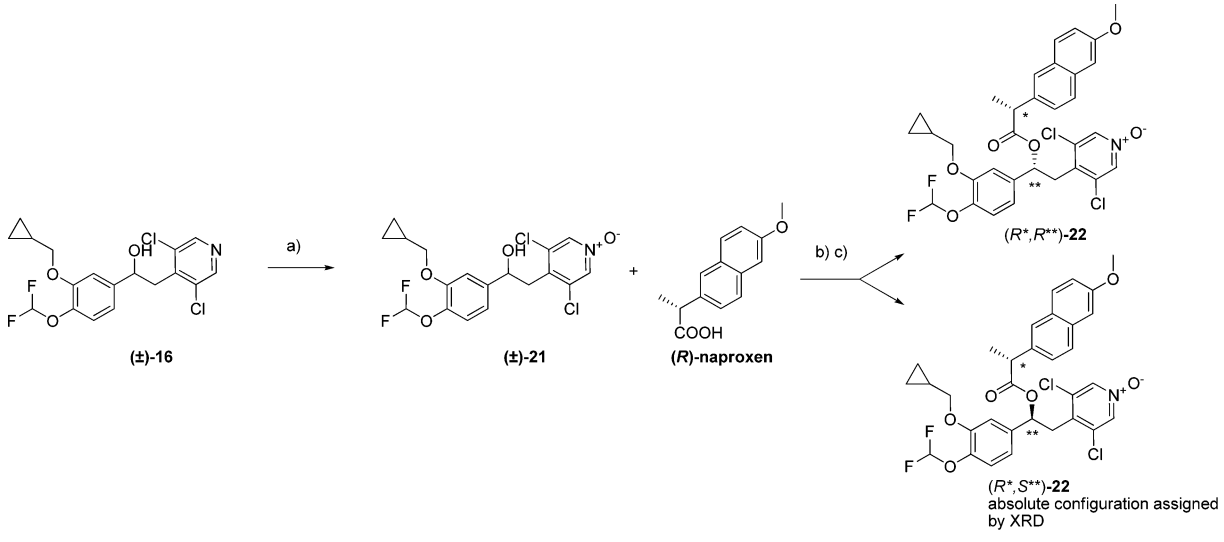
A partially modified synthetic route was followed for compounds (*S*)-**36a−c** (Scheme 9): acids **34a−c** were prepared by hydrogenolysis of corresponding benzylester intermediates **33a−c** (synthesis described in the Supporting Information). The subsequent coupling with (*S*)-**21** followed by cleavage with HCl afforded sulfonamides (*S*)-**36a−c**.

Sulfonamides (*S*)-**38a−e** were obtained by sulfonylation of corresponding anilines (*S*)-**37a−e** according to Scheme 10. Anilines (*S*)-**37a−e** could be prepared either by reduction of the nitro analogues or by deprotection of the N-Boc-protected derivatives obtained by coupling alcohol (*S*)-**21** with suitable intermediates. For the detailed synthesis of intermediates (*S*)-**37a−e**, see the Supporting Information.

Phenol (*S*)-**44** was obtained as summarized in Scheme 11. Aldehyde **40**, obtained by condensation of isovanillin (**39**) with N-Boc-(*S*)-proline, was converted into acid **41** by oxidation with sodium chlorite and sulfamic acid in acetic acid. After coupling with (*S*)-**21** followed by acidic removal of the Boc protecting group, affording diester (*S*)-**43**, a mild basic hydrolysis of the proline ester gave phenolic derivative (*S*)-**44**.

Biology and ADME. The U937 monocytic cell line was used as source of PDE4 enzyme for the in vitro evaluation of compound inhibitory activity in a cell-free assay. Inhibitory activity was determined by assaying cAMP disappearance from the incubation mixture, and it was expressed as an IC_{50} value.

Scheme 4. Synthesis of (*R*)-Naproxen Derivatives (*R,*R***)-22 and (*R**,*S**)-22**[a]



[a]Reagents and conditions: (a) *m*CPBA, DCM, rt, 2 h, 76%; (b) EDC, DMAP, DMF, rt, o/n, 88%; (c) preparative HPLC.

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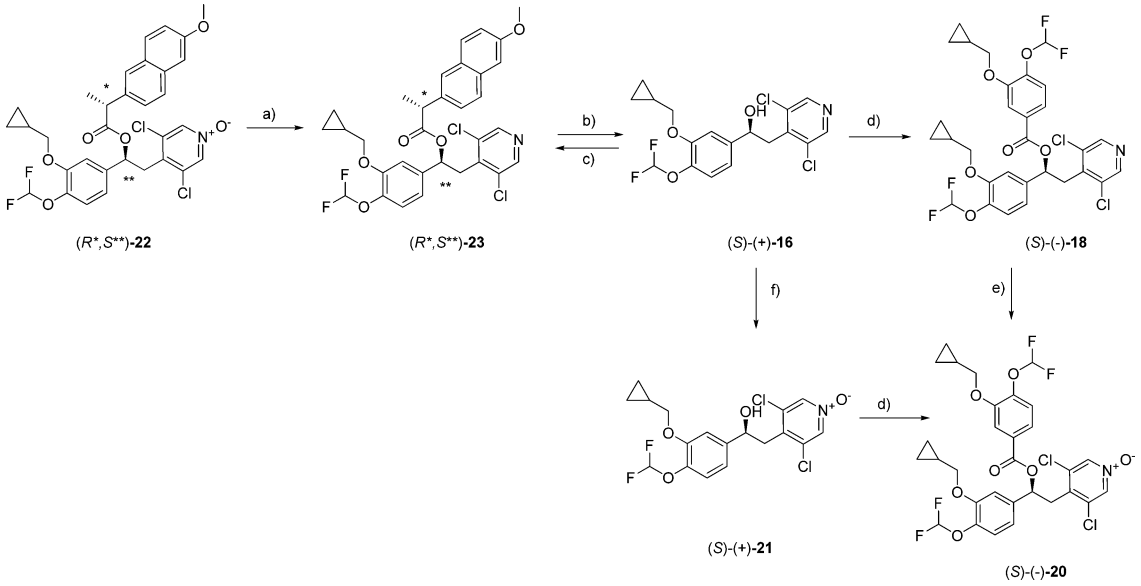
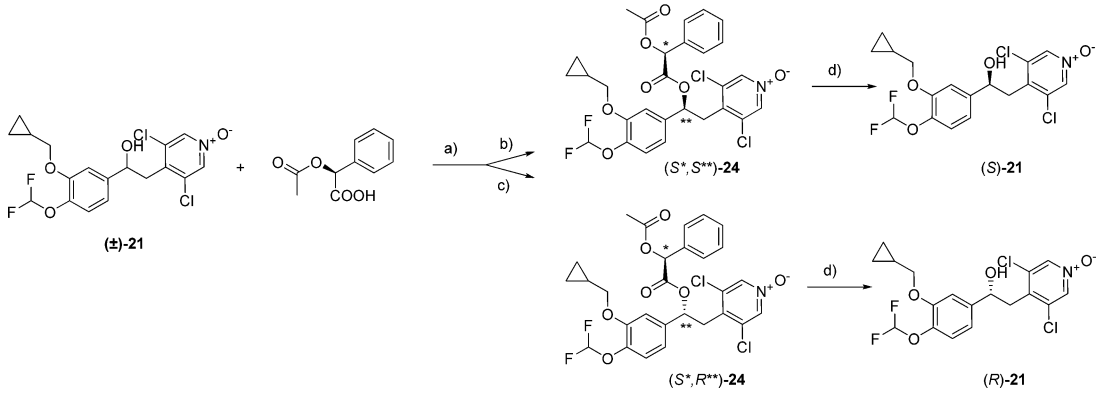
Scheme 5. Chemical Correlations for the Assignment of Absolute Configuration to Alcohols and to the Corresponding Final Esters[a]



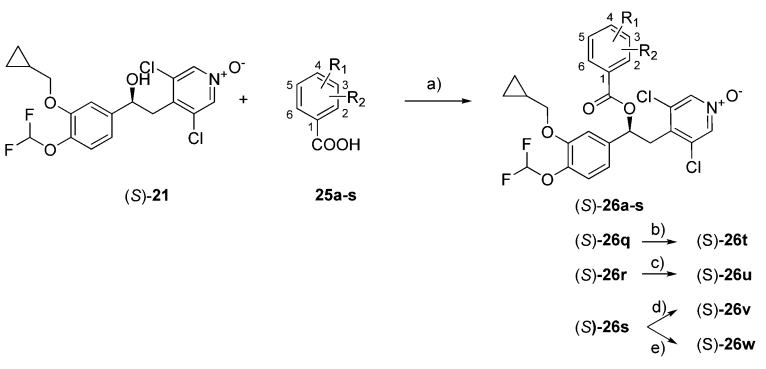
[a]Reagents and conditions: (a) Zn, NH_4Cl, THF, H_2O, rt, 5 min, quantitative; (b) *t*BuOK, MeOH, THF, rt, 20 h, 76%; (c) (*R*)-naproxen, EDC, DMAP, DMF, rt, 20 h, 78%; (d) **10**, EDC, DMAP, DMF or DCM, rt; (e) mCPBA, DCM, rt, 20 h, 68%; (f) *m*CPBA, DCM, rt 68%.

Scheme 6. Large-Scale Preparation of Enantiomerically Pure Alcohol (*S*)-21[a]



[a]Reagents and conditions: (a) EDC, DMAP, DCM, rt; (b) trituration with Et_2O followed by crystallization from *i*PrOH, 34% yield; (c) mother liquor evaporation followed by crystallization from *i*PrOH, 23% yield; (d) aqueous sat. $NaHCO_3$, MeOH, rt, 24 h, 86%.

Scheme 7. Preparation of Compounds (*S*)-26a–w[a]



a	R_1=H	R_2=H
b	R_1=4-Cl	R_2=H
c	R_1=4-Me	R_2=H
d	R_1=4-CF_3	R_2=H
e	R_1=4-CN	R_2=H
f	R_1=4-OMe	R_2=H
g	R_1=4-OMe	R_2=3-OMe
h	R_1=4-OMe	R_2=3-OCyclopropylmethyl
i	R_1=4-OMe	R_2=3-OPh
j	R_1=4-OMe	R_2=3-OCOMe
k	R_1=4-OMe	R_2=3-NHCOMe
l	R_1=4-OMe	R_2=3-SO_2NHMe
m	R_1=4-OMe	R_2=3-SO_2NMe_2
n	R_1=4-NMe_2	R_2=3-OCyclopropylmethyl
o	R_1=4-NHCOMe	R_2=3-OCyclopropylmethyl
p	R_1=3-$NHSO_2$Me	R_2=6-Ocyclopropylmethyl
q	R_1=4-NO_2	R_2=3-OCyclopropylmethyl
r	R_1=4-OBn	R_2=3-OCyclopropylmethyl
s	R_1=4-CHO	R_2=3-OCyclopropylmethyl
t	R_1=4-NH_2	R_2=3-OCyclopropylmethyl
u	R_1=4-OH	R_2=3-OCyclopropylmethyl
v	R_1=4-CH_2OH	R_2=3-OCyclopropylmethyl
w	R_1=4-COOH	R_2=3-OCyclopropylmethyl

[a]Reagents and conditions: (a) EDC, DMAP, DCM or DMF, rt or heating; (b) $SnCl_2{\cdot}2H_2O$, THF, 40 °C, 7 h, 53%; (c) H_2, 5% Pd on $BaSO_4$, EtOAc, 30 psi, 1 h, rt, 78%; (d) $NaBH_4$, THF, rt, 24 h, 28%; (e) NH_2SO_3H, $NaClO_2$, AcOH, H_2O, 3 h, rt, 50%.

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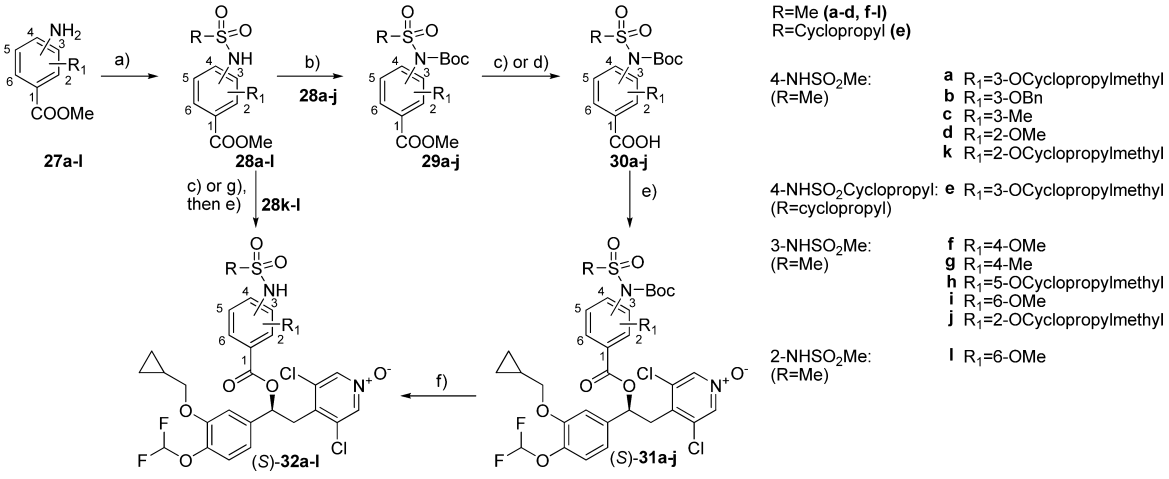
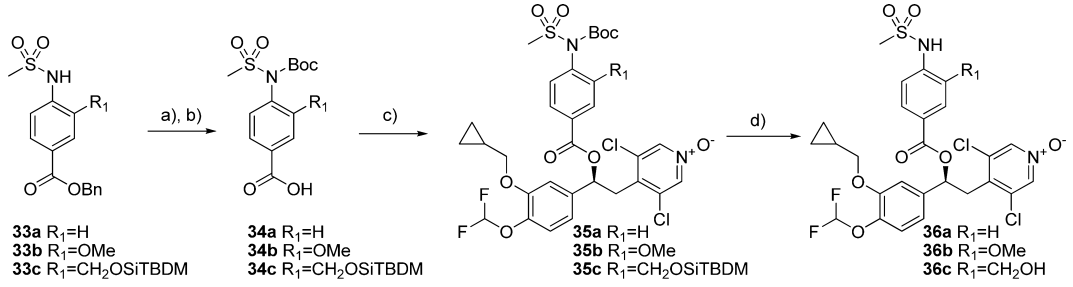
Scheme 8. Synthesis of Sulfonamides (*S*)-32a–l[a]



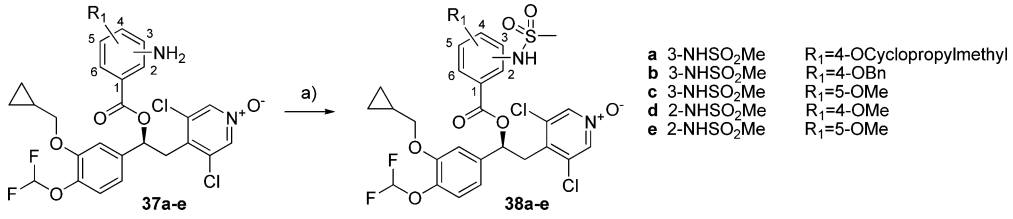
[a]Reagents and conditions: (a) RSO_2Cl, pyridine or DCM/pyridine, rt; (b) Boc_2O, DMAP, DCM, rt; (c) 1 N NaOH, MeOH, rt to 50 °C; (d) 1 N LiOH, THF, rt; (e) (*S*)-**21**, EDC, DMAP, DCM or DMF rt; (f) 4 M HCl in dioxane and DCM or HCl 4 M in EtOAc, rt; (g) conditions for hydrolysis of compound **28l**: KOH, abs. EtOH, reflux, 16 h.

Scheme 9. Preparation of Sulfonamides (*S*)-36a–c[a]



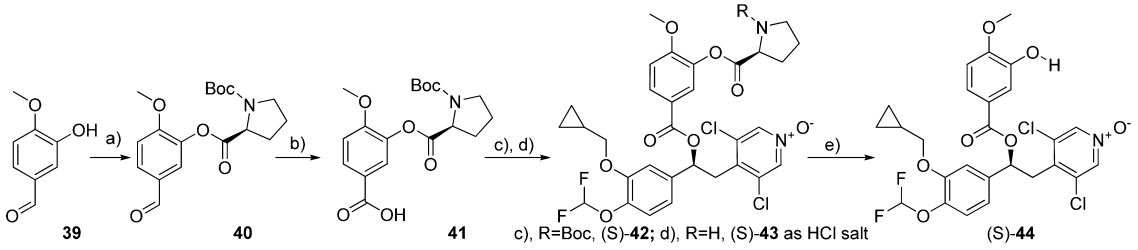
[a]Reagents and conditions: (a) Boc_2O, DMAP, DCM, rt; (b) H_2, 10% Pd/C, MeOH, 30 psi, rt; (c) (*S*)-**21**, EDC, DMAP, DCM or DMF, rt; (d) 4 N HCl in dioxane, DCM or 4 M HCl in EtOAc, rt.

Scheme 10. Preparation of Sulfonamides (*S*)-38a–e[a]



[a]Reagents and conditions: (a) $MeSO_2Cl$, pyridine, DCM or $CHCl_3$, rt.

Scheme 11. Preparation of Compound (*S*)-44[a]



[a]Reagents and conditions: (a) Boc-L-proline, EDC, DMAP, DMF, rt, 95%; (b) $NaClO_2$, H_2NSO_3H, AcOH, H_2O, rt, 76%; (c) (*S*)-**21**, EDC, DMAP, DMF, rt, 1 h, 95%; (d) HCl 4 M in EtOAc, rt, 6 h, 75%; (e) aqueous sat. $NaHCO_3$, MeOH, rt, 3 h, 95%.

The assay for the in vitro evaluation of compound activity in peripheral blood mononuclear cells (PBMCs) was based on the known inhibitory activity exerted by PDE4 inhibitors on lipopolysaccarides (LPS)-induced tumor necrosis factor-alpha

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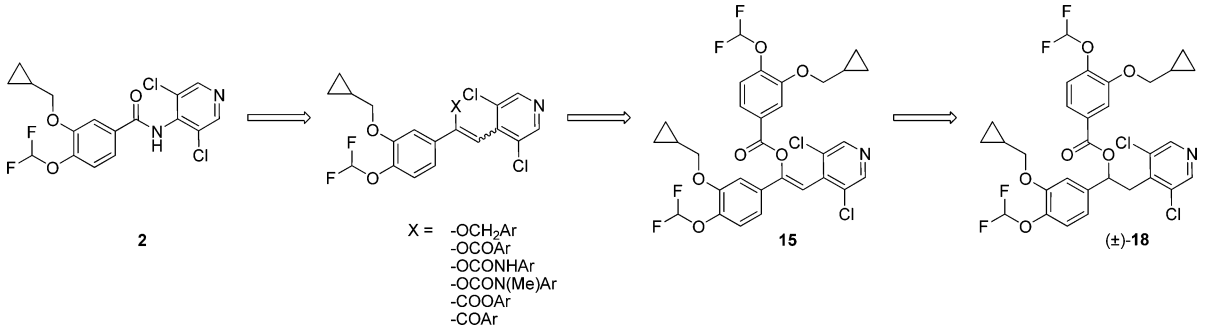


Figure 5. Identification of hit compound (±)-**18**.

(TNF-α) release. The inhibitory activity was determined by measuring LPS-induced TNF-α production after incubation of human PBMCs with different concentrations of the test compounds, and it was expressed as IC_{50} values.

In vitro ADME developability parameters of selected compounds were explored by evaluating the stability in S9 rat and human lung, human and rat plasma protein binding (PPB), and permeability in Caco-2 cells.

Selectivity versus other PDE isoforms, binding kinetics to the PDE4 isoform, stability in human and rat lung slices and in human and rat plasma, Calu-3 permeability, and efficacy in OVA-induced lung eosinophilia in guinea pigs were evaluated for one selected compound.

All methods are described in the Experimental Section.

RESULTS AND DISCUSSION

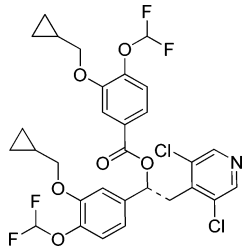
SAR Analysis. According to the earlier-described medicinal chemistry strategy, we started evaluating unsaturated structures that maintain the same geometry as roflumilast and contain a third aryl substituent, potentially able to establish additional interactions, such as enolethers, enolesters, enolcarbamates, α,β-unsaturated ketones, and α,β-unsaturated esters (Figure 5). It was found that benzoic enolester derivatives were the only compounds displaying appreciable activity in a cell-free assay (data not shown).

A more detailed investigation of the enolester class[27] allowed the identification of symmetrically substituted compound **15** as the most interesting compound (cell-free IC_{50} = 1.18 nM). Despite this encouraging result, the enolester class presented some issues because of the difficulty in controlling E/Z isomerism during the synthesis and in avoiding partial isomerization during storage. For these reasons and to investigate if the double bond was required for PDE4 inhibition, the replacement of the enolester scaffold with simple ester derivatives was evaluated.

The substitution pattern shown by enolester **15** was applied to ester (±)-**18**, causing a slight decrease in cell-free assay activity ((±)-**18** IC_{50} = 3.45 nM, Table 2). Selectivity versus other PDE isoforms was evaluated by testing (±)-**18** at the concentration of 10 μM against the enzymatic activity of human PDE5, PDE3, and PDE2, with resulting inhibitions of 58, 18, and 0%, respectively. Given its potency and isoform selectivity, compound (±)-**18** was considered a good starting point for further structure–activity relationship (SAR) investigations.

To confirm our computational hypothesis regarding the relevance of additional interactions with the solvent-exposed region in the enzyme catalytic site, different esters from commercially available carboxylic acids featuring aliphatic chains and/or aromatic groups were prepared and tested in the cell-free

Table 2. Inhibitory Activity of Compounds 15 and (±)-18 in PDE4 Cell Free Assay

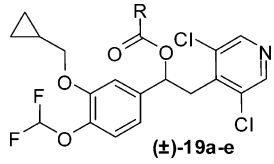


Compd		Cell free IC_{50} (nM)[a]
15	═	1.18± 0.22
(±)-18	—	3.45 ± 0.37

[a]Data are the mean ± SD of three to four experiments performed in duplicate.

assay. As reported in Table 3, all of the derivatives with longer aliphatic chains and/or featuring aromatic residues (**19b–e**)

Table 3. Structure and Inhibitory Activity of Compounds (±)-19a–e in PDE4 Cell Free Assay



compd	R	cell-free IC_{50} (nM)[a]
(±)-**19a**	methyl	4.09 ± 0.83
(±)-**19b**	propyl	1.74 ± 0.55
(±)-**19c**	phenylpropyl	1.34 ± 0.49
(±)-**19d**	benzyl	1.06 ± 0.28
(±)-**19e**	phenyl	1.14 ± 0.06

[a]Data are the mean ± SD of three to four experiments performed in duplicate.

proved to be more active than acetic ester **19a**. Esterification with benzoic acid derivatives was then adopted for the investigation reported in this article because of its high synthetic versatility to allow an extensive exploration of the acidic portion.

To verify the importance of stereochemistry for this class of compounds, our exploration then focused on chiral resolution of (±)-**18**. The two enantiomers were separated, and it was found that the (−) enantiomer, having an (S) configuration, was significantly more active than the corresponding (+)-(R)

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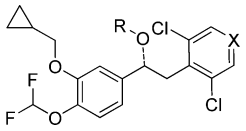
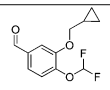
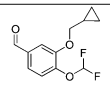
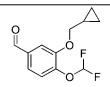
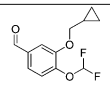
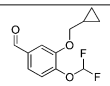
enantiomer (Table 4) (for the assignment of absolute configuration, see the previous section).

Table 4. Inhibitory Activity in Cell-Free and Cell-Based Assays of Compounds (*R*)-18, (*S*)-18, (*R*)-20, (*S*)-20, and (*S*)-21



Compd		R	X	Cell free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(±)-**18**			N	3.45 ± 0.37	-
(*R*)-(+)-**18**			N	44.00 ± 2.45	-
(*S*)-(-)-**18**			N	1.90 ± 0.12	-
(*R*)-(+)-**20**			N=O	2.43 ± 1.18	-
(*S*)-(-)-**20**			N=O	0.22 ± 0.15	1.10 (0.67-1.91)
(*S*)-(+)-**21**		H	N=O	9.44 ± 3.75	479.00 (288.11-798.73)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

The two enantiomers were docked in the catalytic site of the PDE4B structure (PDB ID: 1XMU) in an attempt to explain the difference in their potency. The 1XMU crystal structure was selected because its cocrystallized ligand, roflumilast, shares the same disubstituted catechol scaffold as our benzoic acid ester derivatives. The (S) enantiomer showed a good superposition of its alcoholic fragment on the roflumilast structure in 1XMU, whereas the (R) enantiomer was not able to obtain a good overlay (Figure 6).

It is known that in humans roflumilast is metabolized to the pharmacologically active compound roflumilast N-oxide,[28] which is almost as potent as roflumilast itself.[29] Compounds (*R*)-**20** and (*S*)-**20**, N-oxide analogues of derivatives (*R*)-**18** and (*S*)-**18**, were therefore prepared. As reported in Table 4, the introduction of an N-oxide group in the dichloropyridine moiety unexpectedly and strongly enhanced the inhibitory activity in comparison with roflumilast. Moreover, it was observed that the stereochemistry−activity relationship observed for nonoxidized compounds was maintained in N-oxide analogues, with the (S) enantiomer showing the best activity result. To determine the inhibitory activity exerted by the synthesized compounds in a relevant cellular system that takes into account their cellular penetration capacity, the inhibition of LPS-induced TNF-α production from PBMCs was evaluated. Compound (*S*)-**20** (PBMC IC_{50} = 1.10 nM, Table 4) was about 5-fold less active in the cell-based assay than in the cell-free assay, indicating a moderate difficulty in penetrating the cell. It is important to note that N-oxide alcohol (*S*)-**21** was about 50-fold less active in the cell-free assay and about 450-fold less active in the cell-based assay than corresponding ester (*S*)-**20** (Table 4), proving that ester **20** is stable under the assay conditions, as was also confirmed by HPLC−MS analysis of the tested samples (data not shown). Hence forth, the functional effects of compounds as PDE4 inhibitors in the in vitro model of PBMCs was evaluated to select the best compounds; however, the cell-free assays were also performed as a preliminary evaluation of the enzymatic activity.

Insertion of substituents on the benzoic acid portion was examined, and mono substitution at the para position was considered (Table 5). The best para-substituted compounds thus obtained, in terms of PBMCs activity, were *p*-methoxyderivative (*S*)-**26f** (PBMC IC_{50} = 0.47 nM) and *p*-sulfonamido derivative (*S*)-**36a** (PBMC IC_{50} = 0.13 nM). The disubstituted benzoic acid derivatives of (*S*)-**26f** and (*S*)-**36a** were investigated, as shown in Tables 6 and 7, respectively. In Table 6, the introduction of different substituents at position 3 of the benzoic acid portion already bearing a para-methoxy group was examined. Dialkoxy-

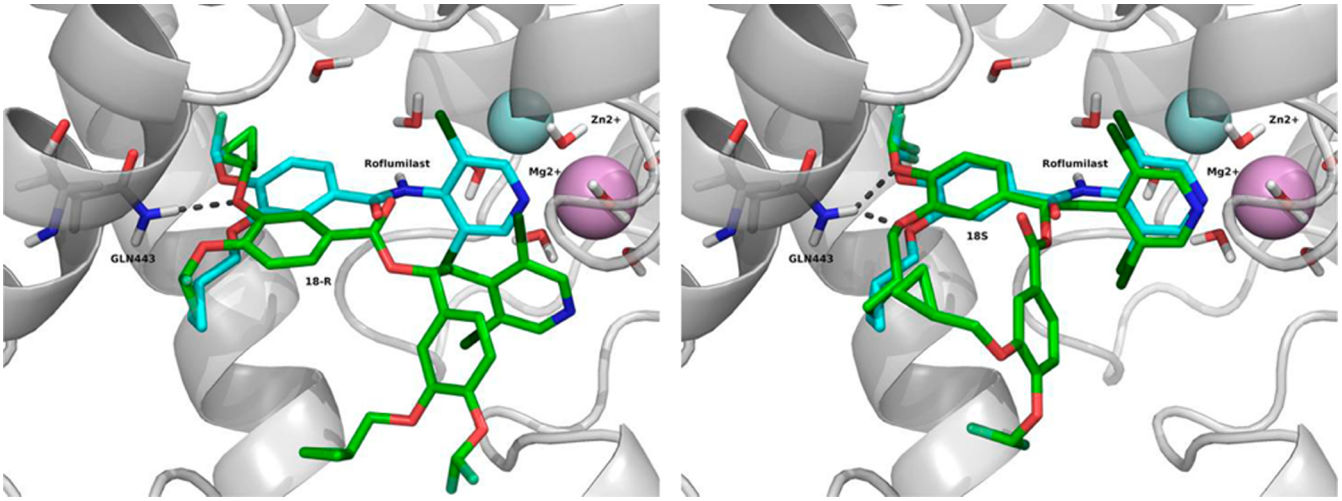


Figure 6. Binding poses of compounds (*R*)-**18** (left) and (*S*)-**18** (right) in the PDE4B catalytic site. The docked ligands (green) are overlaid on the crystallographic binding conformation of roflumilast (light blue).

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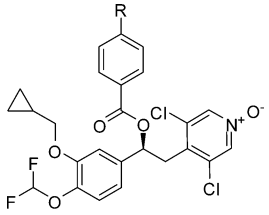
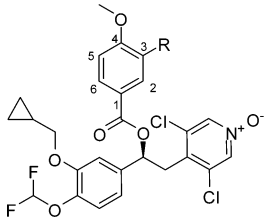
Table 5. Structure and Inhibition Activity in Cell-Free and Cell-Based Assays of Para-Monosubstituted Benzoic Acid Esters of (*S*)-21



compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**26a**	H	0.072 ± 0.03	1.71 (0.85−3.30)
(*S*)-**26b**	Cl	0.16 ± 0.06	1.85 (1.32−2.61)
(*S*)-**26c**	Me	0.10 ± 0.03	0.66 (0.33−1.31)
(*S*)-**26d**	CF_3	0.20 ± 0.06	21.00 (5.12−90.07)
(*S*)-**26e**	CN	0.41 ± 0.28	2.10 (0.67−6.60)
(*S*)-**26f**	OMe	0.14 ± 0.07	0.47 (0.16−1.36)
(*S*)-**36a**	$NHSO_2Me$	0.05 ± 0.03	0.13 (0.05−0.37)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

Table 6. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Disubstituted Benzoic Acid Esters of (*S*)-21 Featuring a Methoxy Substituent at Position 4

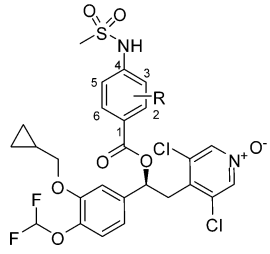


compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**26f**	H	0.14 ± 0.07	0.47 (0.16−1.36)
(*S*)-**26g**	OMe	0.07 ± 0.03	0.12 (0.08−0.17)
(*S*)-**26h**	OCH_2cyclopropyl	0.05 ± 0.02	0.04 (0.02−0.09)
(*S*)-**26i**	OPh	0.21 ± 0.08	1.36 (0.63−2.90)
(*S*)-**44**	OH	0.08 ± 0.04	0.75 (0.48−1.20)
(*S*)-**26j**	OCOMe	0.05 ± 0.02	0.96 (0.30−2.14)
(*S*)-**26k**	NHCOMe	0.08 ± 0.02	0.67 (0.22−2.21)
(*S*)-**32f**	$NHSO_2Me$	0.04 ± 0.01	0.06 (0.03−0.16)
(*S*)-**26l**	SO_2NHMe	0.03 ± 0.01	0.25 (0.12−0.53)
(*S*)-**26m**	SO_2NMe_2	0.03 ± 0.01	1.85 (0.72−4.73)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

substituted derivatives (*S*)-**26g** and (*S*)-**26h** proved to be very active; in particular, compound (*S*)-**26h**, with a cyclopropylmethoxy substituent in the meta position, displayed an excellent inhibitory activity in both cell-free and cell-based assays. The replacement of 3-alkoxy groups by a hydroxy or an acetyloxy group, as in (*S*)-**44** and (*S*)-**26j**, respectively, reduced the PBMCs activity, as did the insertion of an acetamido portion in (*S*)-**26k**; the introduction of the more rigid and lipophilic phenoxy substituent in (*S*)-**26i** resulted in a more pronounced activity decrease in both assays. The combination of a 4-methoxy group with a 3-sulfonamide group strongly enhanced the activity of (*S*)-**32f** (IC_{50} = 0.06 nM) in PBMCs, affording a very potent compound. Reverted N-methylsulfonamide (*S*)-**26l** was well-

Table 7. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Disubstituted Benzoic Acid Esters of (*S*)-21 Featuring a Sulfonamide Substituent at Position 4



compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**36a**	3-H	0.05 ± 0.03	0.13 (0.05−0.37)
(*S*)-**36b**	3-OMe	0.06 ± 0.02	0.38 (0.11−1.5)
(*S*)-**32a**	3-OCH_2cyclopropyl	0.04 ± 0.01	0.06 (0.01−0.38)
(*S*)-**32b**	3-OBn	0.56 ± 0.33	0.99 (0.52−1.88)
(*S*)-**32c**	3-Me	0.05 ± 0.02	0.41 (0.35−0.48)
(*S*)-**36c**	3-CH_2OH	0.03 ± 0.01	1.05 (0.75−1.50)
(*S*)-**32d**	2-OMe	0.06 ± 0.02	0.43 (0.15−1.24)
(*S*)-**32k**	2-OCH_2cyclopropyl	0.26 ± 0.03	1.96 (0.16−24.01)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

tolerated, whereas N-dimethylation in (*S*)-**26m** did not significantly affect the enzymatic activity in the cell-free assay but reduced the cell-based assay activity, probably because of limited cell penetration.

The introduction of a second substituent on 4-sulfonamido analogue (*S*)-**36a** at position 2 or 3 was considered, as shown in Table 7. Small substituents were tolerated or well accepted at both positions 3 and 2, whereas the bulky benzyloxy group at position 3 in (*S*)-**32b** caused a considerable reduction of potency. The cyclopropylmethoxy group, with an intermediate size between that of the methoxy and benzyloxy substituents, was well-accepted at position 3 in (*S*)-**32a**, whereas at position 2 in (*S*)-**32k**, it caused a significant loss of activity in both the cell-free and cell-based assays. The hydroxymethyl group at the 3 position in (*S*)-**36c** caused the highest drop in activity between the cell-free and cell-based assays, whereas the 3-cyclopropylmethoxy group resulted in compound (*S*)-**32a** having the same activity in the cell-free and cell-based assays and showing the best PBMC activity among the 4-sulfonamido derivatives. The cyclopropylmethoxy group at position 3 of the benzoic portion increased the potency of both para-methoxy (**26h**) and para-sulfonamido (**32a**) derivatives. Therefore, an in-depth examination of its role was carried out, and the activity data of the compounds bearing a cyclopropylmethoxy group at position 3 and variable substituents at position 4 are summarized in Table 8. All of the compounds were endowed with high potency in both the cell-free and cell-based assays. Again, (*S*)-**32a** and (*S*)-**26h** emerged as the most potent compounds. From the comparison of (*S*)-**26h** with analogue (*S*)-**26f**, devoid of the cyclopropylmethoxy group at position 3, the contribution of such a group to the potency was clearly evident. Indeed, (*S*)-**26h** was 3-fold more potent than (*S*)-**26f** in the cell-free assay and 11-fold more potent in the cell-based assay (Table 6).

To investigate the class of sulfonamide derivatives further, different compounds featuring this moiety in the meta or ortho position on the benzoic acid portion were then examined (Tables 9 and 10). Among 4-substituted 3-sulfonamides (Table 9),

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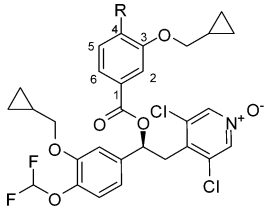
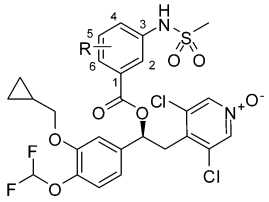
Table 8. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Disubstituted Benzoic Acid Esters of (*S*)-21 Featuring a Cyclopropylmethoxy Substituent at Position 3



compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**20**	$OCHF_2$	0.22 ± 0.15	1.10 (0.67−1.90)
(*S*)-**26h**	OMe	0.05 ± 0.02	0.04 (0.02−0.09)
(*S*)-**26q**	NO_2	0.21 ± 0.12	0.69 (0.45−1.04)
(*S*)-**26t**	NH_2	0.09 ± 0.04	0.11 (0.04−0.30)
(*S*)-**26n**	NMe_2	0.31 ± 0.22	0.63 (0.18−2.20)
(*S*)-**26o**	NHCOMe	0.24 ± 0.12	0.21 (0.11−0.43)
(*S*)-**32a**	$NHSO_2Me$	0.04 ± 0.01	0.06 (0.01−0.38)
(*S*)-**32e**	$NHSO_2$cyclopropyl	0.15 ± 0.07	0.34 (0.19−0.57)
(*S*)-**26u**	OH	0.04 ± 0.01	0.85 (0.40−1.79)
(*S*)-**26v**	CH_2OH	0.08 ± 0.01	0.69 (0.10−4.70)
(*S*)-**26w**	COOH	0.05 ± 0.01	0.30 (0.11−0.84)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

Table 9. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Disubstituted Benzoic Acid Esters of (*S*)-21 Featuring a Sulfonamide Substituent at Position 3

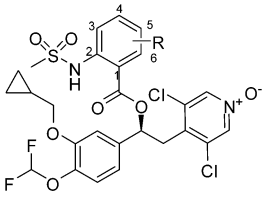


compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**32f**	4-OMe	0.04 ± 0.01	0.06 (0.03−0.16)
(*S*)-**38a**	4-OCH_2cyclopropyl	0.13 ± 0.01	0.12 (0.04−0.41)
(*S*)-**38b**	4-OBn	0.32 ± 0.01	0.53 (0.22−1.25)
(*S*)-**32g**	4-Me	0.03 ± 0.01	0.96 (0.54−1.70)
(*S*)-**38c**	5-OMe	0.17 ± 0.03	0.13 (0.04−0.42)
(*S*)-**32h**	5-OCH_2cyclopropyl	0.06 ± 0.01	0.92 (0.40−2.13)
(*S*)-**32i**	6-OMe	0.04 ± 0.01	0.89 (0.52−1.50)
(*S*)-**26p**	6-OCH_2cyclopropyl	0.40 ± 0.03	4.90 (2.76−8.50)
(*S*)-**32j**	2-OCH_2cyclopropyl	0.18 ± 0.04	1.31 (0.38−4.50)

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression.

methoxy derivative (*S*)-**32f** was the most potent in both assays. Replacement of the cyclopropylmethoxy group with a benzyloxy one in (*S*)-**38b** negatively affected the inhibitory potency, as observed in the 4-sulfonamido series ((*S*)-**32b**, Table 7). Interestingly, compound (*S*)-**38c** was comparably potent in the cell-free and cell-based assays, whereas compound (*S*)-**32h** displayed a 3-fold improved cell-free activity but an 8-fold loss in PBMC potency. A remarkable loss of potency was also found for 6-cyclopropylmethoxy analogue (*S*)-**26p**, whereas the same group was slightly better-tolerated in position 2 ((*S*)-**32j**). In the

Table 10. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Disubstituted Benzoic Acid Esters of (*S*)-21 Featuring a Sulfonamide Substituent at Position 2



compd	R	cell-free IC_{50} (nM)[a]	PBMCs IC_{50} (nM)[b]
(*S*)-**38d**	4-OMe	0.73 ± 0.18	>60
(*S*)-**38e**	5-OMe	0.40 ± 0.02	>60
(*S*)-**32l**	6-OMe	6.83 ± 1.82	>60

[a]Data are the mean ± SD of three to four experiments performed in duplicate. [b]Data are presented as IC_{50} values obtained by nonlinear regression.

3-sulfonamido series, the best results were therefore obtained with the additional substituent in position 4. The limited exploration of 2-sulfonamido derivatives (Table 10) evidently highlighted that such a substituent is not tolerated in position 2, expecially with regard to cell-based activity.

Among the tested derivatives, a number of compounds showing excellent PDE4 potency were identified, and a subset was selected for further characterization (Table 11). In the cell-based assay, all of the selected compounds, having PBMC IC_{50} < 0.2 nM, performed much better than roflumilast in the same assay (PBMC IC_{50} = 3.8 nM; confidence interval: 2.9−5.1).

In Vitro ADME Characterization. Some in vitro ADME properties and experimental $LogD_{7.4}$ of the selected compounds were evaluated to identify PDE4 inhibitors suitable for inhaled administration.

Experimental $LogD_{7.4}$, which is an estimation of lipophilicity and can give an indication on the solubility of the compounds, and half-life in human and rat lung S9, which is a model of the metabolic stability in lung, were chosen as in vitro properties predictive of long retention in the lung in vivo. Plasma protein binding (PPB), which is a measure of the absorbed compound free fraction in the systemic circulation, and Caco-2 permeability, which predicts the oral bioavailability of the swallowed fraction, were taken as properties that can have a role, even if not exhaustive, in limiting unwanted side effects.

As summarized in Table 11, the measured $LogD_{7.4}$ values of the selected compounds were comparable to measured $LogD_{7.4}$ values of inhaled PDE4 inhibitor clinical candidates **4** (AWD-12-281, $LogD_{7.4}$ = 3.97), **5** (CP-325366, $LogD_{7.4}$ = 3.50), and **6** (UK-500,001, $LogD_{7.4}$ = 4.27) as well as to the inhaled corticosteroid fluticasone furoate ($LogD_{7.4}$ = 4.43). As anticipated by the LogD values, the PPB was in general very high in both human (>99%) and rat (>98%) plasma for all of these compounds. The tested compounds showed good stability in rat and especially in human lung S9 fraction (the remaining percent at 1 h incubation in human S9 was about 100%). Concerning the Caco-2 data, it was observed that the presence of the cyclopropylmethoxy substituent proved to be crucial to confer low permeability to compounds (*S*)-**32a** and (*S*)-**38a**.

Compound (*S*)-**32a** proved to be the most interesting derivative, displaying the best combination of high potency, high lung stability, low permeability, and very high PPB, thus satisfying our medicinal chemistry strategy for a drug suitable for

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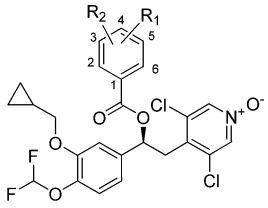
Table 11. ADME Profile of Selected Compounds



			activity			rat ADME data			human ADME data			
							lung S9 stability			lung S9 stability		
compd	R_1	R_2	cell-free IC_{50} (nM)	PBMC IC_{50} (nM)	$LogD_{7.4}$	PPB bound (%)	1 h remain. (%)	$t_{1/2}$ (min)	PPB bound (%)	1 h remain. (%)	$t_{1/2}$ (min)	Caco-2 perm. level[a]
(*S*)-**32a**	4-$NHSO_2CH_3$	3-OCH_2 cyclopropyl	0.04	0.06	4.48	99.20	88	189	99.98	100	>280	low
(*S*)-**38a**	4-OCH_2 cyclopropyl	3-$NHSO_2CH_3$	0.13	0.12	4.23	99.28	76	105	99.87	100	>280	low
(*S*)-**32f**	4-OCH_3	3-$NHSO_2CH_3$	0.04	0.06	3.60	99.41	85	277	99.75	100	>280	high
(*S*)-**38c**	5-$NHSO_2CH_3$	3-OCH_3	0.17	0.13	n.t.	98.38	n.t.		99.81	n.t.		medium
(*S*)-**26g**	4-OCH_3	3-OCH_3	0.07	0.12	4.29	99.85	78	112	99.85	100	>280	high

[a]General absorption classification for permeability values: <10 nm/s, low level; 10−50 nm/s, medium level; and >50 nm/s, high level.

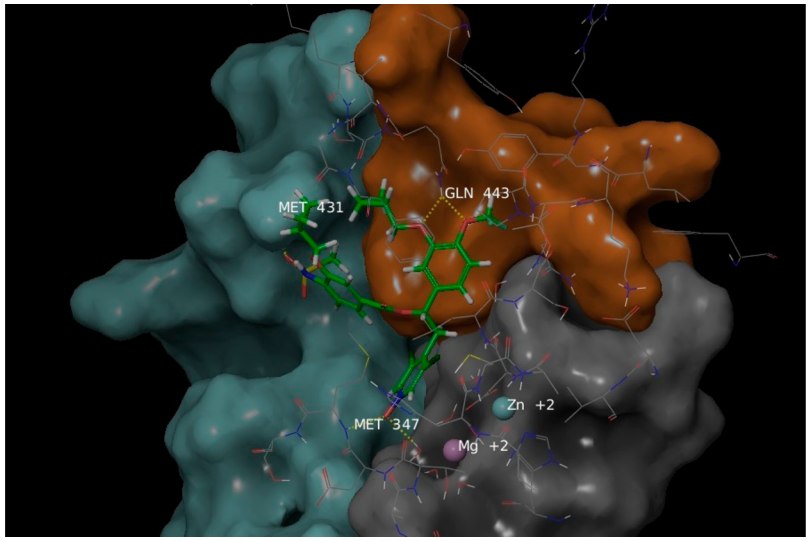


Figure 7. Binding pose of compound (*S*)-**32a** in the PDE4B catalytic site (orange, hydrophobic region; gray, metal region; and turquoise, solvent-exposed region) (PDB ID: 1XMU).

inhaled administration. Therefore, it was selected for further characterization.

In Silico, in Vitro, and in Vivo Characterization of Compound 32a. To evaluate the interactions in the binding site of the PDE4 protein, compound (*S*)-**32a** was docked in the PDE4B catalytic site. Compound (*S*)-**32a** interacts with all three regions of the catalytic binding site (Figure7). The catechol scaffold finds good contacts in the hydrophobic region (orange), and its oxygen atoms form two hydrogen bonds with residue Gln443, consistent with what was observed for roflumilast.[23] The 3,5-dichloro-pyridine 1-oxide ring is placed near the metal region and forms hydrogen bonds with the backbone of the Met347 residue and with a coordinated water molecule. The 3-(cyclopropylmethoxy)-4-(methylsulfonamido)benzoic acid portion is placed in the solvent-exposed region, and a hydrogen bond is formed with the backbone of the Met431 residue.

Given these premises, an extended in vitro characterization of compound **32a** was performed. When tested against the enzymatic activity of bovine PDE1 and PDE6 and human PDE2, PDE3, and PDE5, compound **32a** showed IC_{50} values >1 μM. With an IC_{50} value of 0.04 nM for **32a** in the PDE4 enzymatic assay, our data reveal that **32a** is >20 000-fold selective versus PDE4 in comparison with other PDE isoforms. Binding kinetics experiments were performed using human recombinant catalytic domains of PDE4B and PDE4D isoenzymes and labeled [^{14}C]**32a** as ligand. Binding of [^{14}C]**32a** (100 nM) to both PDE4BCat and PDE4DCat is rapid, reaching saturation in approximately 10 min, with K_{on} values of 1.44×10^6 M^{-1} min^{-1} and 1.69×10^6 M^{-1} min^{-1} for PDE4BCat and PDE4DCat, respectively. We were not able to compete [^{14}C]**32a** off of these enzymes, even when using an excess of unlabeled **32a** (50 μM) with incubation times up to 20 h at room temperature. Although

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we could not calculate an exact K_{off} value for **32a**, the dissociation $t_{1/2}$ can be estimated as >20 h, suggesting a prolonged activity of this compound upon engagement with the PDE4 enzyme. Pulmonary stability of **32a** was confirmed in rat and human lung slices, where the compound proved to be highly stable ($t_{1/2}$ = 172 and 237 min in rat and human, respectively). Interestingly, **32a** proved to be extremely stable when incubated in plasma (100% remaining after 1 h incubation both in rat and human plasma). These in vitro data suggest that this compound is also potentially highly stable under conditions associated with increased hydrolytic and proteolytic activities in the lung parenchyma (i.e., COPD patients). The permeability of **32a** across airway epithelium was studied in Calu-3 cells; negligible transport was measured in this in vitro model of pulmonary absorption, indicating a low potential of the compound for systemic availability.

The in vitro results for compound **32a** are supportive of a compound that is stable, long lasting in the lung, and characterized by minimized systemic exposure.

The in vivo efficacy of **32a** was assessed in a well-known asthma model:[30] ovalbumin (Ova)-induced lung eosinophilia in guinea pigs (GP), an animal species where the in vitro ADME parameters of **32a** are comparable to the ones in human and rat. (The percent remaining at 1 h in both GP plasma and GP lung S9 was 100%, and GP PPB was 99.1%.) When administered intratracheally as dry powder formulation 2 h before Ova challenge, compound **32a** dose-dependently inhibited lung eosinophilia with an efficacious dose (ED_{50}) of 0.016 μmol/kg (Figure 8).

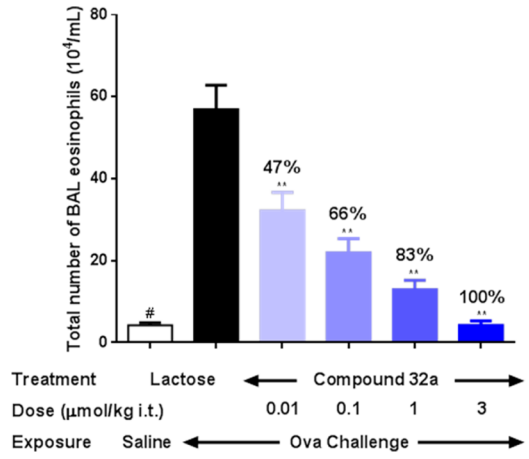

Figure 8. Dose-dependent inhibition of OVA-induced eosinophilia with a 2 h predose with compound **32a**.

The efficacy achieved at the 2 h predosing time point is similar to the one observed after treatment with the steroid fluticasone furoate at a dose of 0.3 μmol/kg (90%). The protective effect was long lasting and was also observed when the compound was administered 24 h before allergen challenge (data not shown).

The in vivo anti-inflammatory activity of compound **32a** indicates that a successful medicinal chemistry strategy can combine a robust anti-inflammatory effect upon topical administration with properties such as an high plasma protein binding and low Calu-3 permeability, with the aim of reducing systemic exposure and providing potential advantages for the safety profile.

CONCLUSIONS

A new class of benzoic acid ester derivatives of (*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-hydroxyethyl)pyridine 1-oxide ((*S*)-**21**) was discovered. To optimize the inhibitory activity toward cAMP-specific phosphodiesterases-4 (PDE4), starting from initial hit (±)-**18** the most relevant SAR results were achieved by the oxidation of the dichloropyridine ring to the N-oxide derivative and by chiral resolution, with the (S) enantiomer displaying higher potency. Subsequently, substitutions at the benzoic acid portion were considered, and the introduction of alkoxy and sulfonamide groups further improved the activity. Among the most potent derivatives, investigation of in vitro ADME properties allowed the selection of a compound, namely, **32a**, with a profile suitable for inhaled administration (slow K_{off}, high PPB, low Calu-3 permeability, and high stability in lung tissue). Anti-inflammatory efficacy upon topical administration of compound **32a** was demonstrated in an in vivo model of allergen-induced pulmonary inflammation.

EXPERIMENTAL SECTION

Chemistry. Reagents and starting materials were commercially available. All solvents and chemicals were used as purchased without further purification. ^{1}H NMR spectra were recorded on a Bruker AC 200 (200 MHz) spectrometer, a Bruker ARX 300 (300 MHz) spectrometer, or a Varian MR-400 (400 MHz) spectrometer equipped with a self-shielded z-gradient coil, 5 mm ^{1}H/^{n}X broad-band probehead for reverse detection, deuterium digital lock channel unit, and quadrature digital detection unit with transmitter offset frequency shift. Chemical shifts are reported as δ downfield in parts per million (ppm) and are referenced to tetramethylsilane (TMS) as the internal standard in the ^{1}H NMR measurements. Coupling constants (J values) are given in hertz (Hz), and multiplicities are reported using the following abbreviation (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; and nd, not determined). The pulse programs were taken from the Varian and Bruker software libraries.

Chiral analyses were performed on a Chiralcel OD-H analytical column or on a Chiralcel OD analytical column.

Preparative HPLC purifications were performed using the following methods:

(1) Waters Corporation UV purification system equipped with a Waters Symmetry Preparative C18, 17 μm, 19 × 300 mm, 30 min gradient of 5−100% solvent B, where solvent A is 90% H_2O, 10% acetonitrile, and 0.05% TFA and solvent B is 10% H_2O, 90% acetonitrile, and 0.05% TFA;

(2) Waters Corporation purification system equipped with a Water X-Terra Preparative, 5.0 μm, 19 × 150 mm, 20 min gradient of 5−100% solvent B, where solvent A is 90% H_2O, 10% acetonitrile, and 0.05% TFA and solvent B is 10% H_2O, 90% acetonitrile, and 0.05% TFA;

(3) Varian Corporation UV purification system equipped with a X-Terra Preparative, 5.0 μm, 19 × 150 mm, Waters column, isocratic elution with H_2O/MeOH = 25:75, flow = 9.0 mL/min.

Chiral separations were performed by chiral HPLC using a semipreparative Chiralcel OD column, 10 μm, 250 × 4.6 mm.

UV purity and m/z of compounds were assessed by ESI+ LC−MS analysis performed on an Acquity UPLC BEH C18, 1.7 μm, 50 × 2.1 mm column with a Micromass ZQ2000 Waters instrument equipped with an ACQ-PDA Waters detector. UV purity was also assessed on an XTerra MS C18, 2.5 μm, 1.6 × 2.5 mm column with an Alliance 2695 Waters instrument equipped with a 2996 PAD Waters detector. All of the compounds tested in biological assays had a purity >95%.

Specific rotation of compounds was measured with a polarimeter (PerkinElmer, model 241) at the sodium D line (589 nm), 20 °C, 1 dm path length. Reactions were monitored by TLC using 0.25 mm Merck silica gel plates (60 F254) or LC−MS analysis; column chromatography

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was performed on Merck silica gel 60 (0.063−0.2 mm). Anhydrous solvents were purchased from Aldrich and used as received. Brine refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of common inorganic salts used in workups are aqueous solutions.

Synthesis of 4-Difluoromethoxy-3-hydroxybenzaldehyde (8a). To a solution of 3,4-dihydroxybenzaldehyde (16.6 g, 120 mmol) and sodium chlorodifluoroacetate (18.3 g, 120 mmol) in DMF (150 mL) and water (3 mL) was added sodium hydroxide (4.8 g, 120 mmol). The mixture was heated at 120 °C and stirred at this temperature for 2 h. The solvent was removed by vacuum distillation, and to the residue was added aqueous HCl (20 mL). The mixture was extracted with Et_2O and washed with brine. The solvent was removed under reduced pressure, and the crude product was purified by chromatography on silica gel (hexane/EtOAc = 80:20) to afford **8** as a white solid (10 g, 52.8 mmol, 44%). LC−MS (ESI+) m/z 189.2 (MH^+).

Synthesis of 3-Cyclopropylmethoxy-4-difluoromethoxybenzaldehyde (9). To a solution of compound **8** (10 g, 53 mmol) in THF (100 mL) was added potassium carbonate (44 g, 105 mmol), and the mixture was cooled to 0 °C. A solution of bromomethylcyclopropane (11 mL, 116.6 mmol) in THF (50 mL) was added, and the reaction was heated to reflux under stirring for 7 h. Fresh bromomethylcyclopropane (5.5 mL, 58.3 mmol) was added, and the heating was continued for a further 7 h. The reaction mixture was cooled to rt, and 2 N sodium hydroxide (100 mL) was added. The aqueous layer was extracted with DCM and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford crude compound **9** (12 g, 50 mmol, 97%), which was used without further purification. LC−MS (ESI+) m/z 243.3 (MH^+).

Synthesis of 3-Cyclopropylmethoxy-4-difluoromethoxybenzoic Acid (10). Compound **9** (12 g, 50 mmol) and sulfamic acid (7.3 g, 75 mmol) were dissolved in glacial acetic acid (50 mL), and a solution of sodium chlorite (8.2 g, 75 mmol) in water (15 mL) was added. The reaction mixture was stirred at rt for 1 h, and then water (300 mL) was added. The precipitated solid was filtered and dried at 40 °C under vacuum, affording compound **10** (12 g, 48 mmol, 97% yield). LC−MS (ESI+) m/z 256.9 (MH^+).

Synthesis of 3-Cyclopropylmethoxy-4-difluoromethoxybenzoyl Chloride (11). To a solution of compound **10** (12 g, 48 mmol) in toluene (100 mL) was added thionyl chloride (25 mL, 344 mmol) dropwise, and the reaction mixture was heated to reflux for 2 h. The mixture was evaporated to dryness, and crude **11** was used without further purification (13.2 g, 48 mmol, quantitative yield).

Synthesis of 3,5-Dichloro-4-methylpyridine (13). A solution of diisopropylamine (70 mL, 500 mmol) in dry THF (500 mL) was cooled to −10 °C, and buthyl lithium (2.5 N in hexane, 210 mL, 525 mmol) was added dropwise under stirring. After 30 min, the solution was cooled to −20 °C, and a solution of 3,5-dichloropyridine **12** (66.6 g, 450 mmol) in THF (200 mL) was added dropwise. The resulting solution was stirred at −10 °C for 30 min, cooled to −70 °C, and added dropwise to a solution of iodomethane (50 mL, 800 mmol) in THF (100 mL). The reaction mixture was allowed to warm to rt, quenched with water (100 mL), and extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The crude product was crystallized from aqueous EtOH than from hexane to afford **13** (49.9 g, 306 mmol, 68%) as a white solid. LC−MS (ESI+) m/z 162.0 (MH^+).

Synthesis of 1-(3-Cyclopropylmethoxy-4-difluoromethoxyphenyl)-2-(3,5-dichloro-pyridin-4-yl)-ethanone (14). A solution of **13** (1.0 g, 6.2 mmol) in dry THF (10 mL) was cooled to −78 °C, and a 1.8 M solution of lithium diisopropylamide in tetrahydrofuran (3.5 mL, 6.3 mmol) was then added dropwise under stirring, keeping the temperature below −70 °C. The resulting solution was stirred for 30 min, and a solution of **11** (0.43 g, 1.6 mmol) in dry THF (10 mL) was then added dropwise, maintaining the temperature below −70 °C. After stirring for 15 min, crushed ice (20 g) was added followed by 200 mL of water. The mixture was extracted with ethyl acetate, and the solvent was dried over Na_2SO_4 and evaporated. The crude product was crystallized from petroleum ether/EtOAc 6:4 to yield **14** as a white solid (130 mg, 0.32 mmol, 20%). LC−MS (ESI+) m/z 402.20 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.53 (s, 2H), 7.47−7.81 (m, 2H), 7.33 (m, 1H), 6.77 (t, J = 75.00 Hz, 1H), 4.65 (s, 2H), 3.97 (d, J = 6.65 Hz, 2H), 1.17−1.44 (m, 1H), 0.60−0.75 (m, 2H), 0.24−0.51 (m, 2H).

Synthesis of 3-Cyclopropylmethoxy-4-difluoromethoxybenzoic Acid (*Z*)-1-(3-Cyclopropylmethoxy-4-difluoromethoxy-phenyl)-2-(3,5-dichloro-pyridin-4-yl)-vinyl Ester (15). A solution of **13** (2.06 g, 12.7 mmol) in dry THF (30 mL) was cooled to −78 °C, and a 1.8 M solution of lithium diisopropylamide in THF (14.8 mL, 26.7 mmol) was added dropwise, keeping the temperature below −70 °C. The resulting solution was stirred for 30 min, a solution of **11** (7.02 g, 25.4 mmol) in dry THF (20 mL) was then added, and the mixture was allowed to warm to rt. After stirring for 30 min, the reaction was quenched with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated, and the resulting oil was purified by flash chromatography on silica gel (petroleum ether/EtOAc from 9:1 to 7:3), yielding **15** as a white solid (2.4 g, 3.8 mmol, 30%). LC−MS (ESI+) m/z 642.2 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.52 (s, 2H), 7.58−7.71 (m, 2H), 7.53 (d, J = 1.32 Hz, 1H), 7.20−7.34 (m, 3H), 7.07 (t, J = 75.00, 75.00 Hz, 1H), 7.04 (s, 1H), 6.98 (t, J = 75.00 Hz, 1H), 4.00 (dd, J = 7.03, 4.83 Hz, 4H), 1.12−1.53 (m, 2H), 0.50−0.70 (m, 4H), 0.24−0.47 (m, 4H).

Synthesis of Racemic 1-(3-Cyclopropylmethoxy-4-difluoromethoxy-phenyl)-2-(3,5-dichloro-pyridin-4-yl)-ethanol ((±)-16). A solution of **9** (5.00 g, 20.64 mmol) and **13** (2.57 g, 15.86 mmol) in 50 mL of dry THF was cooled to −30 °C. Potassium *tert*-butilate (1.96 g, 17.47 mmol) was added portionwise, maintaining the temperature between −30 and −20 °C, and the dark red solution was stirred at −30 °C for 1 h. A saturated aqueous solution of NH_4Cl (50 mL) was then added, maintaining the temperature between −5 and −10 °C (the color turned to yellow). The mixture was extracted with EtOAc, and the organic layer was dried over Na_2SO_4. The solvent was evaporated to dryness, the residue was triturated with petroleum ether/EtOAc 8:2, and the solid was filtered and dried, obtaining (±)-**16** as a white solid (4.83 g, 11.95 mmol, 75%). LC−MS (ESI+) m/z 404.16 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 2H), 7.11 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 1.8 Hz, 1H), 6.88 (dd, J = 8.2, 1.8 Hz, 1H), 7.01 (t, J = 74.8 Hz, 1H), 5.59 (d, J = 4.7 Hz, 1H), 4.91 (dt, J = 8.1, 5.1 Hz, 1H), 3.88 (dd, J = 10.3, 6.7 Hz, 1H), 3.82 (dd, J = 10.3, 7.0 Hz, 1H), 3.28 (dd, J = 12.9, 8.5 Hz, 1H), 3.11 (dd, J = 12.9, 5.6 Hz, 1H), 1.04−1.34 (m, 1H), 0.47−0.68 (m, 2H), 0.20−0.47 (m, 2H).

Synthesis of Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl 3-(Cyclopropylmethoxy)-4-(difluoromethoxy)benzoate ((±)-18). A mixture of (±)-**16** (250 mg, 0.62 mmol), acid **10** (160 mg, 0.62 mmol), EDC (356 mg, 1.86 mmol), and DMAP (0.038 mg, 0.31 mmol) in dry DCM (8 mL) was stirred at rt for 3 days. The reaction mixture was treated with a saturated solution of NH_4Cl and extracted with DCM. The organic phase was dried over Na_2SO_4, and the solvent was removed. The residue was purified by flash chromatography on silica gel (hexane/EtOAc from 90:10 to 80:20) to afford (±)-**18** as an off-white solid (307 mg, 0.476 mmol, 77%). LC−MS (ESI+) m/z 644.1 (MH^+). ^{1}H NMR (300 MHz, $CDCL_3$) δ 8.48 (s, 2H), 7.66 (dd, J = 8.4, 1.9 Hz, 1H), 7.58 (d, J = 2.1 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.08 (dd, J = 8.2, 1.8 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.72 (t, J = 74.8 Hz, 1H), 6.63 (t, J = 74.8 Hz, 1H), 6.30 (dd, J = 10.3, 4.1 Hz, 1H), 3.86−4.00 (m, 4H), 3.79 (dd, J = 13.5, 10.3 Hz, 1H), 3.38 (dd, J = 13.6, 4.3 Hz, 1H), 1.14−1.42 (m, 2H), 0.54−0.75 (m, 4H), 0.28−0.47 (m, 4H).

Resolution of Compound (±)-18: (*S*)-(−)-1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl 3-(cyclopropylmethoxy)-4-(difluoromethoxy)benzoate ((*S*)-18) and (*R*)-(+)-1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl 3-(cyclopropylmethoxy)-4-(difluoromethoxy)benzoate ((*R*)-18). Four-hundred milligrams of (±)-**18** was separated by chiral HPLC on a semipreparative Chiralcel OD column (hexane/*i*PrOH 95:5, flow = 18 mL/min) to afford the two enantiomers.

First-eluting enantiomer: t_R 8.2 min, (*R*)-(+)-**18** (white solid, 128 mg, 32%). $[\alpha]_D^{20}$ = +29.31 (c = 0.5, MeOH). LC−MS (ESI+) m/z 644.1 (MH^+). NMR equal to (±)-**18**.

Second-eluting enantiomer: t_R 9.4 min, (*S*)-(−)-**18** (white solid, 126 mg, 32%). $[\alpha]_D^{20}$ = −27.96 (c = 0.5, MeOH). LC−MS (ESI+) m/z 644.1 (MH^+). NMR equal to (±)-**18**.

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General Procedure for the Synthesis of Compounds 19a−e. *Synthesis of Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl Acetate ((±)-**19a**).* To a mixture of (±)-**16**(0.100 g, 0.247 mmol), EDC (0.142 g, 0.742 mmol), and DMAP (0.030 g, 0.247 mmol) in DCM (10 mL) was added **17a** (0.018 mL, 0.322 mmol), and the reaction was stirred at rt for 4 h. The mixture was diluted with DCM and washed with 1 N HCl, 1 N $NaHCO_3$, and brine, the organic phase was dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel cartridge (petroleum ether/EtOAc from 90:10 to 80:20), affording (±)-**19a** as a white solid (0.092 g, 0.206 mmol, 83%). LC−MS (ESI+) *m*/*z* 446.04 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (s, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 6.97 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.03 (dd, *J* = 9.1, 4.7 Hz, 1H), 3.90 (d, *J* = 6.7 Hz, 2H), 3.54 (dd, *J* = 13.6, 9.2 Hz, 1H), 3.31 (dd, *J* = 13.5, 4.7 Hz, 1H), 1.97 (s, 3H), 1.08−1.34 (m, 1H), 0.48−0.70 (m, 2H), 0.27−0.47 (m, 2H).

Compounds **19b−e** were synthesized following the same procedure starting from corresponding carboxylic acids **17b−e** using DCM or DMF as solvent.

*Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl Butyrate ((±)-**19b**).* Acid **17b** (1 equiv), EDC (1 equiv), DMAP (1 equiv), and DMF, rt, 3 h; purification by trituration with heptane, white solid, 62%. LC−MS (ESI+) *m*/*z* 474.2 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.46 (s, 2H), 7.07−7.24 (m, 1H), 6.80−7.04 (m, 2H), 6.62 (t, *J* = 75.00 Hz, 1H), 6.11 (dd, *J* = 9.54, 4.45 Hz, 1H), 3.88 (d, *J* = 6.99 Hz, 2H), 3.49−3.73 (m, 1H), 3.27 (dd, *J* = 13.99, 4.45 Hz, 1H), 2.11−2.43 (m, 2H), 1.44−1.68 (m, 2H), 1.12−1.38 (m, 1H), 0.82 (t, *J* = 7.31 Hz, 3H), 0.60−0.70 (m, 2H), 0.22−0.50 (m, 2H).

*Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl 4-Phenylbutanoate ((±)-**19c**).* Acid **17c** (1 equiv), EDC (1 equiv), DMAP (1 equiv), and DMF, rt, 3 h; purification by trituration with heptane, white solid, 67%. LC−MS (ESI+) *m*/*z* 550.3 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.17−8.69 (m, 2H), 7.05−7.25 (m, 6H), 6.91−7.02 (m, 2H), 6.62 (t, *J* = 75.00 Hz, 1H), 6.11 (dd, *J* = 9.54, 4.45 Hz, 1H), 3.86 (d, *J* = 6.99 Hz, 2H), 3.46−3.70 (m, 1H), 3.05−3.35 (m, 1H), 2.39−2.67 (m, 2H), 2.13−2.37 (m, 2H), 1.68−2.00 (m, 2H), 1.13−1.34 (m, 1H), 0.57−0.76 (m, 2H), 0.14−0.50 (m, 2H).

*Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl 2-Phenylacetate ((±)-**19d**).* Acid **17d** (1 equiv), EDC (1 equiv), DMAP (1 equiv), and DMF, rt, 3 h; purification by trituration with heptane, white solid, 83%. LC−MS (ESI+) *m*/*z* 522.2 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (s, 2H), 7.04−7.23 (m, 6H), 6.74−6.94 (m, 2H), 6.60 (t, *J* = 75.00 Hz, 1H), 6.10 (dd, *J* = 9.54, 4.45 Hz, 1H), 3.65−3.89 (m, 2H), 3.43−3.62 (m, 3H), 3.05−3.34 (m, 1H), 1.09−1.40 (m, 1H), 0.57−0.77 (m, 2H), 0.11−0.42 (m, 2H).

*Racemic 1-(3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,5-dichloropyridin-4-yl)ethyl Benzoate ((±)-**19e**).* Acid **17e** (1.3 equiv), EDC (3 equiv), DMAP (3 equiv), and DCM, rt, 16 h; purification by flash chromatography on silica gel (petroleum ether/EtOAc from 90:10 to 80:20), white solid, 86%. LC−MS (ESI+) *m*/*z* 508.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (s, 2H), 7.94−8.01 (m, 2H), 7.62−7.71 (m, 1H), 7.48−7.57 (m, 2H), 7.21 (d, *J* = 1.8 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.10 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.30 (dd, *J* = 9.7, 4.4 Hz, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.73 (dd, *J* = 13.8, 9.7 Hz, 1H), 3.45 (dd, *J* = 13.8, 4.4 Hz, 1H), 1.11−1.30 (m, 1H), 0.51−0.62 (m, 2H), 0.29−0.40 (m, 2H)

Synthesis of (*R*)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((*R*)-(+)-20) and (*S*)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((*S*)-(−)-20). To a solution of compound (±)-**18** (150 mg, 0.233 mmol) in DCM (8 mL) was added *m*-chloroperbenzoic acid (77% w/w, 250 mg, 1.12 mmol), and the resulting mixture was stirred at rt for 2 h. The mixture was then diluted with DCM and washed with 1 N NaOH. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by preparative HPLC (method 1) to yield racemic compound (±)-**20** (69 mg, 45%). LC−MS (ESI+) *m*/*z* 660.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.62 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.59 (d, *J* = 1.8 Hz, 1H), 7.04−7.37 (m, 4H), 7.23 (t, *J* = 74.4 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.19 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.85−4.04 (m, 4H), 3.62 (dd, *J* = 14.2, 9.5 Hz, 1H), 3.34 (dd, *J* = 14.2, 4.0 Hz, 1H), 1.12−1.37 (m, 2H), 0.47−0.70 (m, 4H), 0.22−0.47 (m, 4H).

Four-hundred milligrams of (±)-**20** was subjected to chiral HPLC on a semipreparative Chiralcel OD column (hexane/*i*PrOH 50:50). The first-eluting enantiomer, t_R 9.5 min, was collected, evaporated, and triturated with a mixture of hexane/*i*PrOH 50:50 to afford (*R*)-(+)-**20** as a white solid (45 mg, 11%). LC−MS (ESI+) *m*/*z* 660.1 (MH^+). NMR equal to (±)-**20**. $[\alpha]_D^{20}$ = +30.86 (*c* = 0.5, MeOH). The second-eluting enantiomer, t_R 15 min, was collected, evaporated, and triturated with a mixture of hexane/*i*PrOH 50:50 to afford (*S*)-(−)-**20** as a white solid (50 mg, 13%). LC−MS (ESI+) *m*/*z* 660.1 (MH^+). NMR equal to (±)-**20**. $[\alpha]_D^{20}$ = −33.88 (*c* = 0.5, MeOH). NMR in agreement with (±)-**20**.

Synthesis of 1-(3-Cyclopropylmethoxy-4-difluoromethoxy-phenyl)-2-(3,5-dichloro-1-oxy-pyridin-4-yl)-ethanol ((±)-21). Compound (±)-**16** (13.0 g, 32.18 mmol) was dissolved in DCM (250 mL), *m*-chloroperbenzoic acid (77% w/w, 16.5 g, 74 mmol) was added, and the resulting solution was stirred at rt for 2 h. $Na_2S_2O_3$ (25.4 g, 160 mmol) was added, and the mixture was vigorously stirred for 1 h. The solid residue was removed by filtration, the filtrate was washed with 1 N NaOH, the organic phase was dried over Na_2SO_4, and the solvent was evaporated to afford (±)-**21** as a white solid, which was used in the next steps without further purification (10.3 g, 24.5 mmol, 76%). LC−MS (ESI+) *m*/*z* 420.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (s, 2H), 7.11 (d, *J* = 8.2 Hz, 1H), 7.05 (d, *J* = 1.8 Hz, 1H), 6.88 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.01 (t, *J* = 74.8 Hz, 1H), 5.59 (d, *J* = 3.5 Hz, 1H), 4.84 (dd, *J* = 8.2, 5.6 Hz, 1H), 3.89 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.84 (dd, *J* = 10.3, 6.7 Hz, 1H), 3.18 (dd, *J* = 13.2, 8.2 Hz, 1H), 3.02 (dd, *J* = 13.5, 5.6 Hz, 1H), 1.03−1.35 (m, 1H), 0.46−0.67 (m, 2H), 0.24−0.46 (m, 2H).

Synthesis of 3,5-Dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*R*)-2-(6-methoxynaphthalen-2-yl)propanoyloxy)ethyl)pyridine 1-Oxide ((*R,*S***)-22) and 3,5-Dichloro-4-((*R*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*R*)-2-(6-methoxynaphthalen-2-yl)propanoyloxy)ethyl)pyridine 1-Oxide ((*R**,*R***)-22).** Compound (±)-**21** (420 mg, 1.0 mmol) was dissolved in dry DMF (6 mL), and (*R*)-naproxene (231 mg, 1.0 mmol), EDC (290 mg, 1.50 mmol), and DMAP (122 mg, 1.0 mmol) were added. The orange solution was stirred at rt overnight, water (100 mL) was then added, and the precipitate was collected by filtration. The wet solid was dissolved in EtOAc (100 mL), and the solution was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 45:55) to afford 560 mg (0.88 mmol, 88%) of a diastereomeric mixture as a white solid. One-hundred milligrams of this mixture was dissolved in 3 mL of methanol and separated by preparative HPLC (method 3).

First-eluting diastereoisomer: t_R 21 min, (*R**,*S***)-**22**, white solid (35 mg, 35%). LC−MS (ESI+) *m*/*z* 632.1. (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.08 (2H, s, H-pyr), 7.63 (2H, d, *J* = 8.30 Hz, H-ortho naphthyl), 7.51 (1H, d, *J* = 1.46 Hz, H-meta naphthyl), 7.22−7.13 (3H, m, 2 H-ortho meta naphthyl + 1H-meta naphthyl), 6.95 (1H, d, *J* = 8.31 Hz, H-ortho phenyl), 6.90−6.52−6.14 (1H, t, *J* = 75.20 Hz, CHF_2), 6.64 (1H, dd, *J* = 8.30, 1.95 Hz, H-ortho meta phenyl), 6.54−6.52 (1H, m, H-meta phenyl), 5.97 (1H, dd, *J* = 9.76, 4.39 Hz, **CH*-CH_2Ar), 3.93 (3H, s, OCH_3), 3.80 (1H, q, *J* = 14.16, 7.33 Hz, **CH*-CH_3), 3.48−3.24 (3H, m, OCH_2 + H-α of CH_2-Ar), 3.48−3.24 (1H, dd, *J* = 13.67, 4.39 Hz, H-β of CH_2-Ar), 1.48 (3H, d, *J* = 7.32 Hz, CH_3-CH), 1.10−0.94 (1H, m, cyclopropyl CH), 0.58−0.49 (2H, m, cyclopropyl CH_2), 0.21−0.13 (2H, m, cyclopropyl CH_2) (the assignment of absolute configuration by XRD is reported in the Supporting Information).

Second-eluting diastereoisomer: t_R 30 min, (*R**,*R***)-**22**, white solid (45 mg, 45%). (ESI+) *m*/*z* 632.1 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70−7.55 (5H, m, 2H + 3H, 2H-pyr + 2H-ortho naphthyl + 1H-meta naphthyl), 7.24−7.08 (4H, m, 2H-ortho meta naphthyl + 1H-meta

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naphthyl + 1H-ortho phenyl), 6.99−6.61−6.24 (1H, t, J = 75.20 Hz, CHF_2), 6.95 (1H, dd, J = 8.30, 1.95 Hz, H-ortho meta phenyl), 6.90 (1H, d, J = 1.95 Hz, H-meta phenyl), 6.06 (1H, dd, J = 10.74, 4.40 Hz, *CH-CH_2Ar), 3.96 (3H, s, OCH_3), 3.79−3.71 (3H, m, 2H + 1H, OCH_2 + *CH-CH_3), 3.39 (1H, dd, J = 14.16, 10.74 Hz, H-α of CH_2-Ar), 3.00 (1H, dd, J = 14.16, 4.39 Hz, H-β of CH_2-Ar), 1.45 (3H, d, J = 7.32 Hz, CH_3-CH), 1.32−1.17 (1H, m, cyclopropyl CH), 0.70−0.61 (2H, m, cyclopropyl CH_2), 0.39−0.31 (2H, m, cyclopropyl CH_2). m/z = 632.1

Synthesis of 4-((*S*)-2-((*S*)-2-Acetoxy-2-phenylacetoxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((*S,*S**)-24 and 4-((*R*)-2-((*S*)-2-Acetoxy-2-phenylacetoxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((*S***R***)-24).** A mixture of (±)-**21** (19.95 g, 46.43 mmol), (*S*)-acetylmandelic acid (9.22 g, 47.52 mmol), EDC (18 g, 94.24 mmol), and DMAP (2.89 g, 23.69 mmol) in dry DCM (300 mL) was stirred at rt overnight. A 5% aqueous solution of $NaHCO_3$ (200 mL) was added, and the aqueous phase was extracted with DCM. The organic layer was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to give 32 g of a mixture of diastereoisomers that was repeatedly triturated and sonicated with Et_2O to obtain a solid enriched with diastereoisomer (*S**,*S***)-**24**. This solid was crystallized from *i*PrOH and filtered to give 9.65 g of pure (*S**,*S***)-**24** (yield 69.4%). The diastereomeric purity was determined by analytical chiral HPLC performed on a Chiracel OD column (hexane/isopropanol 40:60, flow 0.45 mL/min, t_R 27.2 min). LC−MS (ESI+) m/z 596.2 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (s, 2H), 7.18−7.51 (m, 5H), 7.07 (d, J = 8.8 Hz, 1H), 7.03 (t, J = 74.5 Hz, 1H), 6.74 (d, J = 1.8 Hz, 1H), 6.74 (dd, J = 8.8, 2.1 Hz, 1H), 5.95 (dd, J = 9.7, 4.7 Hz, 1H), 5.86 (s, 1H), 3.72 (dd, J = 10.3, 7.0 Hz, 1H), 3.60 (dd, J = 10.0, 6.7 Hz, 1H), 3.41 (dd, J = 14.1, 10.0 Hz, 1H), 3.24 (dd, J = 14.4, 5.0 Hz, 1H), 2.13 (s, 3H), 1.06−1.28 (m, 1H), 0.46−0.70 (m, 2H), 0.27−0.40 (m, 2H). $[\alpha]_D^{20}$ = +14 (c = 0.5, MeOH).

Mother liquors from triturations were collected and evaporated to give a solid mixture enriched in diastereoismer (*S**,*R***)-**24**, which was crystallized from *i*PrOH to give 6.4 g of pure (*S**,*R***)-**24** (yield 46%) as a white solid. The diastereomeric purity was determined by analytical chiral HPLC performed on a Chiracel OD column (hexane/isopropanol 40:60, flow = 0.45 mL/min, t_R 21.6 min). LC−MS (ESI+) m/z 596.2 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 2H), 7.27−7.45 (m, 5H), 7.20 (d, J = 8.2 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 7.00 (dd, J = 8.2, 1.8 Hz, 1H), 7.08 (t, J = 74.2 Hz, 1H), 5.97 (dd, J = 10.4, 4.3 Hz, 1H), 5.85 (s, 1H), 3.93 (dd, J = 10.3, 7.0 Hz, 1H), 3.89 (dd, J = 10.3, 7.9 Hz, 1H), 3.33 (dd, J = 14.4, 10.9 Hz, 1H), 3.17 (dd, J = 14.1, 4.1 Hz, 1H), 2.07 (s, 3H), 1.14−1.38 (m, 1H), 0.50−0.71 (m, 2H), 0.21−0.47 (m, 2H). $[\alpha]_D^{20}$ = +26 (c = 0.5, MeOH).

Synthesis of (*S*)-1-(3-Cyclopropylmethoxy-4-difluoromethoxy-phenyl)-2-(3,5-dichloro-1-oxy-pyridin-4-yl)-ethanol ((*S*)-(+)-21. Compound (*S**,*S***)-**24** (6.42 g, 10.77 mmol) was suspended in methanol (350 mL), and a saturated solution of $NaHCO_3$ (175 mL) was added. The mixture was vigorously stirred at rt overnight, diluted with DCM (700 mL), and washed with a 5% aqueous solution of $NaHCO_3$ (300 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was triturated with Et_2O and filtered to afford (*S*)-(+)-**21** (3.88 g, 9.24 mmol, 86%) as a white solid with an enantiomeric purity >99%. The enantiomeric purity was determined by analytical chiral HPLC performed on a Chiracel OD column (hexane/isopropanol 30:70, flow = 0.35 mL/min, t_R 22.3 min). LC−MS (ESI+) m/z 420.1 (MH^+). ^{1}H NMR equal to (±)-**21**.

Synthesis of (*R*)-1-(3-Cyclopropylmethoxy-4-difluoromethoxy-phenyl)-2-(3,5-dichloro-1-oxy-pyridin-4-yl)-ethanol ((*R*)-(−)-21). This compound was prepared by hydrolysis of corresponding ester (*S**,*R***)-**24** following the procedure described earlier. The product was purified by two triturations with Et_2O followed by a further trituration with DCM to afford (*R*)-(−)-**21** as a white solid in 88% yield with an enantiomeric purity >99%. The enantiomeric purity was determined by analytical chiral HPLC performed on a Chiracel OD column (hexane/isopropanol 30:70, flow = 0.35 mL/min, t_R 24.0 min). LC−MS (ESI+) m/z 420.1 (MH^+). ^{1}H NMR equal to (±)-**21**.

General Procedure for the Preparation of Compounds (*S*)-26a−s. *Synthesis of (S)-4-(2-(Benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**26a**).* A mixture of (*S*)-**21** (100 mg, 0.238 mmol), acid **25a** (58 mg, 0.476 mmol), EDC (136 mg, 0.714 mmol), and DMAP (32 mg, 0.262 mmol) in DMF (10 mL) was stirred at rt overnight. The mixture was diluted with water and extracted with EtOAc. The organic phase was washed twice with 1 N HCl and dried over Na_2SO_4. The solvent was removed, and the crude product was purified by crystallization from EtOH to afford (*S*)-**26a** as a white solid (100 mg, 0.190 mmol, 80%). LC−MS (ESI+) m/z 524.3 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.25 (s, 2H), 8.08 (d, J = 7.50 Hz, 2H), 7.60−7.71 (m, 1H), 7.47−7.56 (m, 2H), 7.31 (d, J = 1.76 Hz, 1H), 7.15−7.27 (m, 2H), 6.68−6.91−7.12 (t, 1 H, CHF_2), 6.36 (dd, J = 9.48, 4.63 Hz, 1H), 3.98 (d, J = 6.62 Hz, 2H), 3.75 (dd, J = 14.11, 9.70 Hz, 1H), 3.45 (dd, J = 14.11, 4.85 Hz, 1H), 1.27 (m, 1H), 0.52−0.66 (m, 2H), 0.32−0.44 (m, 2H).

Compounds **26b−s** were synthesized following the same procedure starting from corresponding carboxylic acid **25b−s** using DCM or DMF as solvent. The preparation of noncommercially available acids is detailed in the Supporting Information.

*(S)-3,5-Dichloro-4-(2-(4-chlorobenzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((S)-**26b**).* Acid **25b** (1.3 equiv), EDC (3 equiv), DMAP (1 equiv), and DCM, rt, overnight; purification by flash chromatography on silica gel (petroleum ether/EtOAC from 60:40 to 40:60) followed by trituration with EtOH, white solid, 45% yield. LC−MS (ESI+) m/z 558.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 2H), 7.87−8.12 (m, 2H), 7.49−7.72 (m, 2H), 7.22 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 7.09 (dd, J = 8.2, 2.1 Hz, 1H), 7.06 (t, J = 74.8 Hz, 1H), 6.21 (dd, J = 9.1, 4.4 Hz, 1H), 3.93 (d, J = 6.7 Hz, 2H), 3.62 (dd, J = 14.1, 9.4 Hz, 1H), 3.35 (dd, J = 14.1, 4.4 Hz, 1H), 1.06−1.35 (m, 1H), 0.47−0.70 (m, 2H), 0.16−0.45 (m, 2H). $[\alpha]_D^{20}$ = −54.09 (c = 0.5, DCM).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methylbenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26c**).* Acid **25c** (1.3 equiv), EDC (3 equiv), DMAP (1 equiv), and DCM, rt, overnight; purification by trituration with EtOH, white solid, 59% yield. LC−MS (ESI+) m/z 538.3 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 2H), 7.79−7.98 (m, 2H), 7.31−7.43 (m, 2H), 7.21 (d, J = 2.1 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.09 (dd, J = 8.4, 1.9 Hz, 1H), 7.06 (t, J = 74.8 Hz, 1H), 6.21 (dd, J = 9.4, 4.4 Hz, 1H), 3.93 (d, J = 7.0 Hz, 2H), 3.61 (dd, J = 14.2, 9.5 Hz, 1H), 3.34 (dd, J = 14.2, 4.5 Hz, 1H), 2.38 (s, 3H), 1.04−1.38 (m, 1H), 0.45−0.66 (m, 2H), 0.24−0.45 (m, 2H). $[\alpha]_D^{20}$ = −39.92 (c = 0.5, DCM).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-(trifluoromethyl)benzoyloxy)ethyl)-pyridine 1-Oxide ((S)-**26d**).* Acid **25d** (1 equiv), EDC (3 equiv), DMAP (0.5 equiv), and DCM, rt, 24 h; purification by chromatography on silica gel (DCM/EtOAc 80:20) followed by trituration with *i*PrOH, white solid, 51% yield. LC−MS (ESI+) m/z 591.9 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 2H), 8.19 (m, 2H), 7.91 (m, 2H), 7.24 (d, J = 2.1 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.11 (dd, J = 8.2, 1.8 Hz, 1H), 7.07 (t, J = 74.8 Hz, 1H), 6.24 (dd, J = 9.4, 4.4 Hz, 1H), 3.94 (d, J = 7.0 Hz, 2H), 3.65 (dd, J = 14.1, 9.4 Hz, 1H), 3.37 (dd, J = 14.2, 4.5 Hz, 1H), 1.11−1.32 (m, 1H), 0.49−0.65 (m, 2H), 0.26−0.45 (m, 2H). $[\alpha]_D^{20}$ = −30.4 (c = 0.7, MeOH).

*(S)-3,5-Dichloro-4-(2-(4-cyanobenzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((S)-**26e**).* Acid **25e** (1.1 equiv), EDC (1.5 equiv), DMAP (1.1 equiv), and DMF, rt, 24 h, then 70 °C, 2 h; purification by preparative HPLC (method 2), oil, 61% yield. LC−MS (ESI+) m/z 549.3 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02−8.22 (m, 4H), 7.67−7.84 (m, 2H), 7.16−7.25 (m, 1H), 6.93−7.15 (m, 2H), 6.63 (t, J = 75.00 Hz, 1H), 6.16−6.44 (m, 1H), 3.85−4.06 (m, 2H), 3.72 (dd, J = 14.16, 10.25 Hz, 1H), 3.35 (dd, J = 14.16, 4.39 Hz, 1H), 1.06−1.39 (m, 1H), 0.56−0.76 (m, 2H), 0.00−0.52 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxybenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26f**).* Acid **25f** (1.8 equiv), EDC (3 equiv), DMAP (1 equiv), and DCM, rt, 48 h; purification by trituration with MeOH, white solid, 53% yield. LC−MS (ESI+) m/z 554.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 2H), 7.87−8.05 (m, 2H), 7.20 (d, J = 2.1 Hz, 1H), 7.20 (d, J = 8.5 Hz, 1H), 7.08 (dd, J = 8.5, 2.1 Hz, 1H), 7.01−7.08 (m, 2H), 7.06 (t, J = 74.8 Hz, 1H), 6.20 (dd, J = 9.5, 4.3 Hz, 1H), 3.93 (d, J = 7.0 Hz, 2H), 3.84 (s, 3H), 3.60 (dd, J = 13.9, 9.5 Hz, 1H), 3.33 (dd, J

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= 14.1, 4.4 Hz, 1H), 1.04−1.32 (m, 1H), 0.45−0.66 (m, 2H), 0.27−0.45 (m, 2H). $[\alpha]_D^{20}$ = −45.51 (*c* = 0.5, DCM).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3,4-dimethoxybenzoyloxy)ethyl)-pyridine 1-Oxide ((S)-**26g**).* Acid **25g** (4 equiv added portionwise over 6 h), EDC (4 equiv), DMAP (1 equiv), and DCM, rt, 24 h; purification by flash chromatography on silica gel (DCM/EtOAc from 80:20 to 70:30) followed by dissolution in EtOAc, washing with aqueous $NaHCO_3$, evaporation, and treatment with MeOH, white solid, 84% yield. LC−MS (ESI+) *m*/*z* 584.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 2H), 7.63 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.45 (d, *J* = 2.1 Hz, 1H), 7.14−7.27 (m, 2H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.81 (t, *J* = 74.8 Hz, 1H), 6.19 (dd, *J* = 9.8, 4.3 Hz, 1H), 3.88−3.99 (m, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 3.61 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.34 (dd, *J* = 14.4, 4.4 Hz, 1H), 1.14−1.28 (m, 1H), 0.51−0.63 (m, 2H), 0.29−0.41 (m, 2H). $[\alpha]_D^{20}$ = −37.71 (*c* = 0.5, DCM).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-methoxybenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26h**.* Acid **25h** (1.7 equiv), EDC (3 equiv), DMAP (1 equiv), and DCM, rt, overnight; purification by trituration with MeOH, white solid, 74% yield. LC−MS (ESI+) *m*/*z* 624.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.61 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.16−7.23 (m, 2H), 7.02−7.11 (m, 2H), 7.06 (t, 1H), 6.18 (dd, *J* = 9.7, 4.1 Hz, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.85 (s, 3H), 3.84 (d, *J* = 5.9 Hz, 2H), 3.60 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.33 (dd, *J* = 14.4, 4.4 Hz, 1H), 1.05−1.31 (m, 2H), 0.49−0.69 (m, 4H), 0.23−0.45 (m, 4H). $[\alpha]_D^{20}$ = −37.56 (*c* = 0.6, DCM).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxy-3-phenoxybenzoyloxy)-ethyl)pyridine 1-Oxide ((S)-**26i**).* Acid **25i** (1.2 equiv), EDC (2 equiv), DMAP (0.5 equiv), and DCM, rt, 48 h; purification by chromatography on silica gel (DCM/EtOAc 80:20), white solid, 43% yield. LC−MS (ESI +) *m*/*z* 645.9 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (s, 2H), 7.84 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.52 (d, *J* = 2.3 Hz, 1H), 7.33−7.42 (m, 2H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.16−7.21 (m, 2H), 7.12 (m, *J* = 7.3, 7.3 Hz, 1H), 7.03 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.87−6.96 (m, 2H), 7.05 (t, *J* = 74.8 Hz, 1H), 6.15 (dd, *J* = 9.7, 4.4 Hz, 1H), 3.90 (d, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 3.55 (dd, *J* = 14.2, 9.5 Hz, 1H), 3.30 (dd, *J* = 12.9, 4.4 Hz, 1H), 1.09−1.34 (m, 1H), 0.47−0.65 (m, 2H), 0.26−0.43 (m, 2H). $[\alpha]_D^{20}$ = −36.4 (*c* = 0.5, DCM).

*(S)-4-(2-(3-Acetoxy-4-methoxybenzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**26j**).* Acid **25j** (1.2 equiv), EDC (2 equiv), DMAP (1.1 equiv), and DCM, rt, overnight; purification by crystallization from MeOH, white solid, 33,7% yield. LC−MS (ESI+) *m*/*z* 612.4 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 2H), 7.90 (d, *J* = 1.94 Hz, 1H), 7.71 (d, *J* = 1.94 Hz, 1H), 7.18 (d, *J* = 8.26 Hz, 1H), 6.96−7.08 (m, 3H), 6.43−6.62−6.81 (t, 1H, CHF_2), 6.20−6.30 (m, 1H), 3.84−3.96 (m, 5H), 3.60−3.73 (m, 1H), 3.27−3.38 (m, 1H), 2.34 (s, 3H), 1.19−1.36 (m, 1H), 0.66 (d, *J* = 6.80 Hz, 2H), 0.38 (d, *J* = 5.83 Hz, 2H).

*(S)-4-(2-(3-Acetamido-4-methoxybenzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**26k**).* Acid **25k** (1.5 equiv), EDC (1.5 equiv), DMAP (1.5 equiv), and DMF, rt, 1 h; purification by trituration with petroleum ether, white solid, 92% yield. LC−MS (ESI+) *m*/*z* 611.0 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 9.11 (s, 1H), 8.64 (br. s., 1H), 8.24 (s, 2H), 7.77 (dd, *J* = 8.38, 2.21 Hz, 1H), 7.33 (d, *J* = 1.76 Hz, 1H), 7.15−7.23 (m, 1H), 7.05−7.14 (m, 2H), 6.89 (t, *J* = 75.00 Hz, 1H), 6.33 (dd, *J* = 9.48, 4.63 Hz, 1H), 3.97−4.10 (m, 2H), 3.95 (s, 3H), 3.70 (dd, *J* = 14.11, 9.70 Hz, 1H), 3.42 (dd, *J* = 14.11, 4.41 Hz, 1H), 2.13−2.23 (s, 3H), 1.23−1.37 (m, 1H), 0.60 (dd, *J* = 7.94, 1.76 Hz, 2H), 0.33−0.47 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxy-3-(N-methylsulfamoyl)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26l**).* Acid **25l** (1.2), EDC (1.3), DMAP (1.4 equiv), TEA (2.5 equiv), and DCM, rt, 48 h; purification by flash chromatography on silica gel (DCM/MeOH 98:2) followed by preparative HPLC (method 1), evaporation, dissolution in EtOAc, washing with aqueous $NaHCO_3$, and evaporation, white solid, 52% yield. LC−MS (ESI+) *m*/*z* 647.2 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (s, 2H), 8.33 (d, *J* = 2.1 Hz, 1H), 8.19 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.17−7.25 (m, 3H), 7.07 (m, *J* = 8.5, 2.1 Hz, 1H), 7.07 (t, *J* = 74.8 Hz, 1H), 6.20 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.99 (s, 3H), 3.96 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.91 (dd, *J* = 10.3, 6.7 Hz, 1H), 3.63 (dd, *J* = 14.4, 9.7 Hz, 1H), 3.35 (dd, *J* = 14.1, 4.1 Hz, 1H), 2.42 (d, *J* = 4.7 Hz, 3H), 1.15−1.31 (m, 1H), 0.48−0.66 (m, 2H), 0.28−0.44 (m, 2H). $[\alpha]_D^{20}$ = −45.93 (*c* = 0.5, MeOH).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(N,N-dimethylsulfamoyl)-4-methoxybenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26m**).* Acid **25m** (1.2 equiv), EDC (2 equiv), DMAP (1.1 equiv), and DMF, rt, overnight; purification by crystallization from EtOH/hexane, white solid, 80% yield. LC−MS (ESI+) *m*/*z* 683.2 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 2.20 Hz, 1H), 8.07−8.26 (m, 3H), 7.19 (d, *J* = 8.07 Hz, 1H), 6.94−7.11 (m, 3H), 6.63 (t, *J* = 75.00 Hz, 1H), 6.23 (dd, *J* = 10.18, 4.13 Hz, 1H), 4.02 (s, 3H), 3.84−3.96 (m, 2H), 3.63−3.80 (m, 4H), 3.14−3.38 (m, 4H), 1.24−1.40 (m, 1H), 0.58−0.75 (m, 2H), 0.39 (q, *J* = 5.01 Hz, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-(dimethylamino)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26n**).* Acid **25n** (2 equiv), EDC (2 equiv), DMAP (1.2 equiv), and DMF, rt, overnight; purification by preparative HPLC (method 2), white solid, 15% yield. LC−MS (ESI+) *m*/*z* 637.1 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (s, 2H), 7.53−7.71 (m, 1H), 7.40 (m, 1H), 7.09−7.22 (m, 1H), 6.92−7.08 (m, 2H), 6.72−6.89 (m, 1H), 6.61 (t, *J* = 75.00 Hz, 1H), 6.09−6.33 (m, 1H), 3.79−4.03 (m, 4H), 3.55−3.78 (m, 1H), 3.20−3.42 (m, 1H), 2.95 (s, 6H), 1.00−1.46 (m, 2H), 0.66 (d, *J* = 6.84 Hz, 4H), 0.11−0.44 (m, 4H). $[\alpha]_D^{20}$ = −63.33 (*c* = 1, $CHCl_3$).

*(S)-4-(2-(4-Acetamido-3-(cyclopropylmethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**26o**).* Acid **25o** (1 equiv), EDC (3 equiv), DAMP (0.5 equiv), and DCM, rt, 3 days; purification by flash chromatography on silica gel (DCM/EtOAc from 80:20 to 100% EtOAc), white solid, 26% yield. LC−MS (ESI+) *m*/*z* 651.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 8.55 (s, 2H), 8.20 (d, *J* = 8.5 Hz, 1H), 7.59 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.51 (d, *J* = 1.8 Hz, 1H), 7.15−7.24 (m, 2H), 7.07 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.18 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.79−4.09 (m, 4H), 3.60 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.34 (dd, *J* = 14.1, 4.4 Hz, 1H), 2.16 (s, 3H), 1.14−1.37 (m, 2H), 0.49−0.73 (m, 4H), 0.24−0.48 (m, 4H). $[\alpha]_D^{20}$ = −26.22 (*c* = 0.5, MeOH).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-(cyclopropylmethoxy)-5-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26p**).* Acid **25p** (1 equiv), EDC (1 equiv), DMAP (1 equiv), and DCM, rt, overnight; purification by preparative HPLC (method 1), white solid, 10% yield. LC−MS (ESI+) *m*/*z* 687.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.53 (s, 2H), 7.55 (d, *J* = 2.6 Hz, 1H), 7.36 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 7.08−7.17 (m, 3H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.18 (dd, *J* = 8.2, 5.3 Hz, 1H), 3.79−3.98 (m, 4H), 3.53 (dd, *J* = 14.2, 8.7 Hz, 1H), 3.39 (dd, *J* = 17.6, 7.6 Hz, 1H), 2.92 (s, 3H), 0.99−1.32 (m, 2H), 0.42−0.67 (m, 4H), 0.04−0.42 (m, 4H). $[\alpha]_D^{20}$ = −9.90 (*c* = 0.4, MeOH).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-nitrobenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26q**).* Acid **25q** (1 equiv), EDC (3 equiv), DMAP (0.5 equiv), and DCM, rt, overnight; purification by flash chromatography on silica gel (DCM/EtOAc 90:10), light yellow solid, 66% yield. LC−MS (ESI+) *m*/*z* 639.1 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.75 (d, *J* = 1.5 Hz, 1H), 7.69 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.25 (d, *J* = 1.8 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.10 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.07 (t, *J* = 74.8 Hz, 1H), 6.21 (dd, *J* = 9.4, 4.4 Hz, 1H), 4.06−4.18 (m, 2H), 3.84−4.03 (m, 2H), 3.64 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.36 (dd, *J* = 14.1, 4.4 Hz, 1H), 1.08−1.38 (m, 2H), 0.46−0.72 (m, 4H), 0.25−0.46 (m, 4H). $[\alpha]_D^{20}$ = −32.94 (*c* = 0.49, MeOH).

*(S)-4-(2-(4-(Benzyloxy)-3-(cyclopropylmethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**26r**).* Acid **25r** (1.3 equiv), EDC (1.5 equiv), DMAP (1.2 equiv), and DMF, rt, 2 h, 80% yield, used without

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purification. LC−MS (ESI+) m/z 700.5 (MH^+). ^1H NMR (400 MHz, acetone) δ 8.26 (s, 2H), 7.63−7.71 (m, 1H), 7.58 (d, J = 2.21 Hz, 1H), 7.52 (d, J = 7.06 Hz, 2H), 7.28−7.46 (m, 4H), 7.11−7.24 (m, 3H), 6.70−6.90−7.10 (t, 1H, CHF_2), 6.26−6.35 (m, 1H), 5.25 (s, 2H), 3.89−4.06 (m, 4H), 3.66−3.78 (m, 1H), 3.33−3.49 (m, 1H), 1.28 (d, J = 6.62 Hz, 2H), 0.52−0.67 (m, 4H), 0.39 (t, J = 5.07 Hz, 4H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-formylbenzoyloxy)ethyl)pyridine 1-Oxide ((S)-**26s**).* Acid **25s** (1.2 equiv), EDC (2 equiv), DMAP (1.1 equiv), and DMF, rt, 3 h,, light yellow solid, 92% yield, used without purification. LC−MS (ESI+) m/z 622.4 (MH^+). ^1H NMR (400 MHz, $CDCl_3$) δ 10.59 (s, 1H), 8.15 (s, 2H), 7.84−7.96 (m, 1H), 7.65−7.73 (m, 1H), 7.55−7.59 (m, 1H), 7.15−7.23 (m, 1H), 6.96−7.12 (m, 2H), 6.41−6.63−6.85 (t, 1H, CHF_2), 6.20−6.34 (m, 1H), 3.85−4.12 (m, 4H), 3.59−3.76 (m, 1H), 3.26−3.40 (m, 1H), 1.21−1.41 (m, 2H), 0.52−0.77 (m, 4H), 0.29−0.46 (m, 4H).

Synthesis of (S)-4-(2-(4-Amino-3-(cyclopropylmethoxy)-benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)-phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-26t). A mixture of (S)-**26q** (595 mg, 0.931 mmol) and $SnCl_2$ dihydrate (840 mg, 3.72 mmol) in THF (30 mL) was heated at 40 °C for 1 h. Additional $SnCl_2$ dihydrate (840 mg, 3.72 mmol) was added, and the mixture was heated for a further 6 h. The solvent was removed under reduced pressure, and EtOAc and aqueous sat. $NaHCO_3$ were added to the residue. The inorganic precipitate was removed by filtration through a Celite pad, and the biphasic filtrate was separated. The organic layer was washed with brine and dried over Na_2SO_4, the solvent was evaporated under vacuum, and the crude product was first purified by flash chromatography on silica gel (DCM/EtOAc 80:20) and then by trituration with EtOH to afford 200 mg of (S)-**26t** as a light yellow solid (36% yield). LC−MS (ESI+) m/z 608.9 (MH^+). ^1H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 2H), 7.40 (dd, J = 8.4, 1.9 Hz, 1H), 7.29 (d, J = 6.2 Hz, 1H), 7.14−7.23 (m, 2H), 7.04 (dd, J = 8.2, 1.8 Hz, 1H), 7.05 (t, J = 74.8 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 6.14 (dd, J = 9.8, 4.3 Hz, 1H), 5.61 (s, 2H), 3.74−4.04 (m, 4H), 3.55 (dd, J = 13.9, 9.5 Hz, 1H), 3.30 (dd, J = 13.9, 4.4 Hz, 1H), 1.13−1.32 (m, 2H), 0.47−0.69 (m, 4H), 0.15−0.46 (m, 4H). $[\alpha]_D^{20}$ = −42.29 (c = 0.5, MeOH).

Synthesis of (S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-hydroxybenzoyloxy)ethyl)pyridine 1-Oxide ((S)-26u). To a solution of (S)-**26r** (1.093 g, 1.56 mmol) in EtOAc (20 mL) was added a catalytic amount of 5% Pd on $BaSO_4$, and the mixture was hydrogenated in a Parr apparatus at 35 psi for 1 h at rt. The catalyst was filtered off, and the filtrate was evaporated to dryness. The crude product was purifed by crystallization from a mixture of EtOH/petroleum ether to afford (S)-**26u** as a white solid (742 mg, 1.22 mmol, 78%). LC−MS (ESI+) m/z 610.0 (MH^+). ^1H NMR (400 MHz, acetone) δ 8.40−8.56 (m, 1H), 8.26 (s, 2H), 7.62 (dd, J = 8.32, 1.91 Hz, 1H), 7.49−7.57 (m, 1H), 7.29 (d, J = 1.83 Hz, 1H), 7.13−7.22 (m, 2H), 6.90 (m, 2H), 6.30 (dd, J = 9.77, 4.58 Hz, 1H), 3.90−3.99 (m, 4H), 3.71 (dd, J = 14.19, 9.77 Hz, 1H), 3.41 (dd, J = 14.19, 4.58 Hz, 1H), 1.22−1.35 (m, 2H), 0.47−0.66 (m, 4H), 0.29−0.46 (m, 4H).

Synthesis of (S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-(hydroxymethyl)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-26v). To a solution of (S)-**26s** (380 mg, 0.610 mmol) in THF (20 mL) was added $NaBH_4$ (6.93 mg, 0.183 mmol), and the mixture was stirred at rt for 24 h. The reaction mixture was diluted with EtOAc and washed twice with brine; the organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by chromatography on silica gel (DCM/EtOAc from 8:2 to 1:1) to yield (S)-**26v** as a white solid (105 mg, 0.168 mmol, 28%). LC−MS (ESI+) m/z 623.9 (MH^+). ^1H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.61 (dd, J = 7.9, 1.5 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 1.5 Hz, 1H), 7.17−7.25 (m, 2H), 7.08 (dd, J = 8.5, 2.1 Hz, 1H), 7.06 (t, J = 74.8 Hz, 1H), 6.20 (dd, J = 9.5, 4.5 Hz, 1H), 5.18 (t, J = 5.6 Hz, 1H), 4.57 (d, J = 5.9 Hz, 2H), 3.83−4.01 (m, 4H), 3.61 (dd, J = 14.1, 9.7 Hz, 1H), 3.34 (dd, J = 13.8, 4.4 Hz, 1H), 1.05−1.36 (m, 2H), 0.43−0.65 (m, 4H), 0.12−0.46 (m, 4H). $[\alpha]_D^{20}$ = −32.4 (c = 0.7, DCM).

Synthesis of (S)-4-(2-(4-Carboxy-3-(cyclopropylmethoxy)-benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)-phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-26w). To a solution of (S)-**26s** (40 mg, 0.064 mmol) in acetic acid (2 mL) was added a solution of $NaClO_2$ (10 mg, 0.088 mmol) in H_2O (0.5 mL) dropwise, and the resulting mixture was stirred at rt for 3 h and then partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4, and the solvent was removed. The crude product was purified by crystallization from a mixture of EtOH/petroleum ether to afford (S)-**26w** as a white solid (20 mg, 0.031 mmol, yield 50%). LC−MS (ESI+) m/z 639.4 (MH^+). ^1H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 8.22 Hz, 1H), 8.15 (s, 2H), 7.78 (dd, J = 8.07, 1.32 Hz, 1H), 7.60 (d, J = 1.17 Hz, 1H), 7.21 (d, J = 8.22 Hz, 1H), 7.00−7.11 (m, 2H), 6.64 (t, J = 75.00 Hz, 1H), 6.29 (dd, J = 9.98, 4.11 Hz, 1H), 4.13 (d, J = 7.34 Hz, 2H), 3.84−4.01 (m, 2H), 3.63−3.79 (m, 1H), 3.34 (dd, J = 14.38, 4.11 Hz, 1H), 1.33−1.45 (m, 1H), 1.20−1.33 (m, 1H), 0.61−0.84 (m, 4H), 0.31−0.52 (m, 4H).

General Procedure for the Preparation of Intermediates 28a−l. *Synthesis of 3-Cyclopropylmethoxy-4-methanesulfonylamino-benzoic Acid Methyl Ester (**28a**).* To a solution of **27a** (8.86 g, 40.04 mmol) in dry pyridine (80 mL) under a N_2 atmosphere was added methansulfonyl chloride (4.04 mL, 52.19 mmol), and the mixture was stirred at rt for 18 h. The reaction mixture was evaporated to dryness, and the crude product was partitioned between 1 N HCl (500 mL) and DCM (200 mL). The organic layer was dried over Na_2SO_4 and evaporated to yield **28a** (11.7 g, 39.08 mmol, yield 98%). No purification was requried. LC−MS (ESI+) m/z 300.0 (MH^+).

Compounds **28b−l** were synthesized following the same procedure starting from corresponding aniline derivatives **27b−l** and suitable sulfonyl chlorides. In some cases, DCM or chloroform were used as solvent in the mixture with pyridine.

General Procedure for the Preparation of Intermediates 29a−j. *Synthesis of 3-Cyclopropylmethoxy-4-(N-tert-butoxycarbonyl-N-methanesulfonyl)-amino-benzoic Acid Methyl Ester (**29a**).* To a solution of intermediate **28a** (3.0 g, 10.0 mmol) in DCM (150 mL) were added DMAP (1.22 g, 10 mmol) and Boc_2O (2.18 g, 10 mmol), and the mixture was stirred at rt for 1 h. The reaction mixture was washed with 5% aqueous HCl (2 × 50 mL), the organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was triturated with Et_2O and filtered to afford **29a**, which was used in the next steps without further purification (4.0 g, 10.0 mmol, quantitative yield). LC−MS (ESI+) m/z 400.2 (MH^+).

Compounds **29b−j** were synthesized following the same procedure starting from corresponding aniline derivatives **28b−j**.

Hydrolysis Method A: General Procedure for the Preparation of Intermediates 30a, 30c, 30e, and 30g. *Synthesis of 3-Cyclopropylmethoxy-4-(N-tert-butoxycarbonyl-N-methanesulfonyl)-amino-benzoic Acid (**30a**).* Compound **29a** (4.0 g, 10.0 mmol) was dissolved in MeOH (100 mL), 1 N NaOH (15 mL, 15 mmol) was added, and the mixture was stirred at rt for 1 h and heated at 50 °C for a further 2 h. The reaction mixture was diluted with EtOAc and washed with 1 N HCl. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford **30a** (3.5 g, 7.78 mmol, yield 78%). LC−MS (ESI+) m/z 408.0 (MNa^+).

Intermediates **30c**, **30e**, and **30g** were synthesized following the same procedure starting from corresponding esters **29c**, **29e**, and **29g**.

Hydrolysis Method B: General Procedure for the Preparation of Intermediates 30b, 30d, 30f, and 30h−j. *Synthesis of 3-(N-(tert-Butoxycarbonyl)methylsulfonamido)-4-methoxybenzoic Acid (**30f**).* To a solution of intermediate **29f** (0.882 g, 2.454 mmol) in THF (15 mL) was added aqueous 1 N LiOH (2.94 mL, 2.94 mmol),and the reaction was stirred at rt for 24 h. The mixture was acidified with 1 N HCl and extracted with EtOAc. The organic phase was washed with brine and dried over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was triturated with Et_2O to afford **30f** as a white solid (0.487 g, 1.410 mmol, yield 57.5%). LC−MS (ESI+) m/z 367.9 (MNa^+).

Intermediates **30b**, **30d**, and **30h−j** were synthesized following the same procedure starting from corresponding esters **29b**, **29d**, and **29h−j**.

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General Procedure for the Preparation of Intermediates (*S*)-31a−j. *Synthesis of (S)-4-(2-(4-(N-(tert-Butoxycarbonyl)-methylsulfonamido)-3-(cyclopropylmethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**31a**).* A mixture of alcohol (*S*)-**21** (5.85 g, 13.93 mmol), acid **30a** (5.36 g, 13.93 mmol), EDC (8.00 g, 41.78 mmol), and DMAP (0.850 mg, 6.97 mmol) in DCM (250 mL) under a N_2 atmosphere was stirred at rt for 5 h. The reaction mixture was diluted with DCM and washed with 1 N HCl and then with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (from 100% DCM to DCM/EtOAc 75:25), affording (*S*)-**31a** as a white solid (6.69 g, 8.50 mmol, yield 61%). LC−MS (ESI+) *m*/*z* 787.3 (MH^+).

Compounds (*S*)-**31b−j** were synthesized following the same procedure starting from corresponding acid derivatives **30b−j** and using DCM or DMF as solvent.

General Procedure for the Preparation of Compounds (*S*)-32a−j. *Synthesis of (S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32a**).* Compound (*S*)-**31a** (4.6 g, 5.85 mmol) was dissolved in DCM (150 mL), and a solution of 4 M HCl in dioxane (40 mL, 160 mmol) was added dropwise. The reaction was stirred at rt overnight and then evaporated to dryness. The residue was purified by crystallization from EtOH to afford (*S*)-**32a** as a white solid (3 g, 4.36 mmol, 75%). LC−MS (ESI+) *m*/*z* 687.3 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (br s, 1H), 8.55 (s, 2H), 7.58 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.49 (d, *J* = 1.8 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.16−7.25 (m, 2H), 7.07 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.18 (dd, *J* = 9.7, 4.4 Hz, 1H), 3.80−4.12 (m, 4H), 3.61 (dd, *J* = 14.2, 9.8 Hz, 1H), 3.33 (dd, *J* = 14.1, 3.5 Hz, 1H), 3.11 (s, 3H), 1.25−1.42 (m, 1H), 1.12−1.25 (m, 1H), 0.48−0.73 (m, 4H), 0.21−0.48 (m, 4H). $[\alpha]_D^{20}$ = −47 (*c* = 0.4, MeOH).

Compounds (*S*)-**32b−j** were similarly prepared starting from corresponding acids (*S*)-**31b−j**.

*(S)-4-(2-(3-(Benzyloxy)-4-(methylsulfonamido)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**32b**).* From (*S*)-**31b**, 4 M HCl in EtOAc, rt, 3 h, not purified, yellow solid, 71%. LC−MS (ESI+) *m*/*z* 723.6 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.27 (2s, 3H), 7.67−7.78 (m, 2H), 7.51−7.66 (m, 3H), 7.34−7.47 (m, 3H), 7.25−7.32 (m, 1H), 7.12−7.24 (m, 2H), 6.90 (t, *J* = 75.00 Hz, 1H), 6.24−6.43 (m, 1H), 5.22−5.39 (s, 2H), 3.90−4.07 (m, 2H), 3.65−3.80 (m, 1H), 3.37−3.48 (m, 1H), 3.07 (s, 3H), 1.17−1.35 (m, 1H), 0.51−0.67 (m, 2H), 0.30−0.44 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-methyl-4-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32c**).* From (*S*)-**31c**, 4 M HCl in EtOAc, rt, 1 h; purification by crystallization from chloroform/iPr_2O, white solid, 72%. LC−MS (ESI+) *m*/*z* 631.4 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.55 (s, 2H), 7.93 (m, 2H), 7.48 (d, *J* = 7.81 Hz, 1H), 7.00−7.28 (m, 4H), 6.08−6.27 (m, 1H), 3.93 (d, *J* = 6.84 Hz, 2H), 3.60 (m, 4H), 3.31−3.41 (m, 1H), 2.28 (s, 3H), 1.21 (m, 1H), 0.43−0.65 (m, 2H), 0.20−0.39 (m, 2H). $[\alpha]_D^{20}$ = −58 (*c* = 1, $CHCl_3$).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-methoxy-4-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32d**).* From (*S*)-**31d**, 4 M HCl in EtOAc, rt, 2 h; purification by preparative HPLC (method 2), white solid, 35% yield. LC−MS (ESI+) *m*/*z* 647.5 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.76−9.30 (m, 1H), 8.25 (s, 2H), 7.83 (d, *J* = 8.38 Hz, 1H), 7.28 (d, *J* = 1.76 Hz, 1H), 7.12−7.23 (m, 2H), 7.03−7.10 (m, 1H), 6.94 (dd, *J* = 8.38, 2.21 Hz, 1H), 6.90 (t, *J* = 75.00 Hz, 1H), 6.32 (dd, *J* = 9.04, 5.07 Hz, 1H), 3.93−4.05 (m, 2H), 3.87 (s, 3H), 3.64 (dd, *J* = 14.11, 9.26 Hz, 1H), 3.35−3.45 (m, 1H), 3.11 (s, 3H), 1.19−1.34 (m, 1H), 0.52−0.67 (m, 2H), 0.32−0.47 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(4-(cyclopropanesulfonamido)-3-(cyclopropylmethoxy)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((S)-**32e**).* From (*S*)-**31e**, 4 M HCl in EtOAc, rt, 1.5 h; purification by crystallization from EtOH, white solid, 45%. LC−MS (ESI+) *m*/*z* 713.6 (MH^+). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15 (s, 2H), 7.66 (m, 2H), 7.45 (m, 1H), 7.14−7.25 (m, 2H), 6.92−7.13 (m, 2H), 6.63 (t, *J* = 75.00 Hz, 1H), 6.12−6.39 (m, 1H), 3.91 (d, *J* = 6.84 Hz, 4H), 3.70 (dd, *J* = 13.67, 10.25 Hz, 1H), 3.31 (dd, *J* = 13.92, 4.15 Hz, 1H), 2.55 (m, 1H), 1.12−1.39 (m, 4H), 1.00 (dd, *J* = 8.06, 2.20 Hz, 2H), 0.56−0.84 (m, 4H), 0.18−0.52 (m, 4H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxy-3-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32f**).* From (*S*)-**31f**, 4 M HCl in EtOAc, rt, 2 h; purification by crystallization from EtOH, white solid, 72%. LC−MS (ESI+) *m*/*z* 647.5 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 2.05 Hz, 1H), 8.15 (s, 2H), 7.83 (dd, *J* = 8.66, 1.91 Hz, 1H), 7.17 (d, *J* = 8.22 Hz, 1H), 7.09 (m, 1H), 7.02 (dd, *J* = 8.22, 1.76 Hz, 1H), 6.96 (d, *J* = 8.51 Hz, 1H), 6.83 (m, 1H), 6.44−6.63−6.82 (t, 1 H, CHF_2), 6.27 (dd, *J* = 10.12, 3.96 Hz, 1H), 3.87−3.99 (m, 5H), 3.69 (dd, *J* = 14.09, 10.27 Hz, 1H), 3.30 (dd, *J* = 13.79, 4.11 Hz, 1H), 2.98 (s, 3H), 1.22−1.34 (m, 1H), 0.60−0.70 (m, 2H), 0.39 (q, *J* = 4.89 Hz, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methyl-3-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32g**).* From (*S*)-**31g**, 4 M HCl in EtOAc, rt, 1 h; purification by crystallization from EtOH, white solid, 72%. LC−MS (ESI+) *m*/*z* 631.4 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.53 (s, 2H), 7.91 (d, *J* = 1.46 Hz, 1H), 7.76 (dd, *J* = 7.81, 1.46 Hz, 1H), 7.32−7.45 (m, 1H), 7.14−7.26 (m, 2H), 6.91−7.12 (m, 2H), 6.21 (dd, *J* = 9.52, 4.15 Hz, 1H), 3.92 (d, *J* = 6.84 Hz, 2H), 3.47−3.73 (m, 1H), 3.37 (d, *J* = 4.39 Hz, 1H), 3.00 (s, 3H), 2.38 (s, 3H), 1.11−1.28 (m, 1H), 0.48−0.65 (m, 2H), 0.25−0.42 (m, 2H). $[\alpha]_D^{20}$ = −38.67 (*c* = 1, $CHCl_3$).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-5-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32h**).* From (*S*)-**31h**, 4 M HCl in dioxane, DCM, rt, 2 h; purification by preparative HPLC (method 2), white solid, 45%. LC−MS (ESI+) *m*/*z* 687.5 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.64−8.92 (br s, 1H), 8.27 (s, 2H), 7.59−7.72 (m, 1H), 7.28−7.38 (m, 2H), 7.13−7.25 (m, 3H), 6.92 (t, *J* = 75.00 Hz, 1H), 6.33 (dd, *J* = 9.70, 4.41 Hz, 1H), 3.99 (dd, *J* = 6.84, 1.98 Hz, 2H), 3.91 (d, *J* = 7.06 Hz, 2H), 3.74 (dd, *J* = 14.55, 9.70 Hz, 1H), 3.43 (dd, *J* = 14.11, 4.41 Hz, 1H), 3.05 (s, 3H), 1.16−1.36 (m, 2H), 0.52−0.71 (m, 4H), 0.29−0.47 (m, 4H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-methoxy-5-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**32i**).* From (*S*)-**31i**, 4 M HCl in dioxane, DCM, rt, 5 days; purification by flash chromatography on silica gel (from 100% EtOAc to EtOAc/MeOH 95:5) followed by preparative HPLC (method 1), white solid, 27%. LC−MS (ESI+) *m*/*z* 647.3 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.54 (s, 2H), 7.56 (d, *J* = 2.6 Hz, 1H), 7.40 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.17 (d, *J* = 2.3 Hz, 1H), 7.15 (d, *J* = 8.9 Hz, 1H), 7.05 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.07 (t, *J* = 74.8 Hz, 1H), 6.20 (dd, *J* = 8.9, 4.8 Hz, 1H), 3.94 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.89 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.78 (s, 3H), 3.51 (dd, *J* = 14.1, 9.1 Hz, 1H), 3.31 (dd, *J* = 14.1, 4.7 Hz, 1H), 2.92 (s, 3H), 1.13−1.35 (m, 1H), 0.49−0.66 (m, 2H), 0.26−0.43 (m, 2H). $[\alpha]_D^{20}$ = −9.90 (*c* = 0.4, MeOH).

*(S)-3,5-Dichloro-4-(2-(2-(cyclopropylmethoxy)-3-(methylsulfonamido)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)pyridine 1-Oxide ((S)-**32j**).* From (*S*)-**31j**, 4 M HCl in dioxane, DCM, rt, overnight; purification by preparative HPLC (method 1), white solid, 31%. LC−MS (ESI+) *m*/*z* 687.2 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.56 (s, 2H), 7.57 (ddd, *J* = 7.9, 1.5, 0.6 Hz, 1H), 7.50 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.16−7.25 (m, 3H), 7.05−7.11 (m, 1H), 7.07 (t, *J* = 74.8 Hz, 1H), 6.19 (dd, *J* = 9.1, 4.7 Hz, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.59 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.49−3.59 (m, 2H), 3.33 (dd, *J* = 14.1, 4.7 Hz, 1H), 3.10 (s, 3H), 1.14−1.27 (m, 1H), 0.95−1.12 (m, 1H), 0.49−0.67 (m, 2H), 0.24−0.46 (m, 4H), 0.01−0.16 (m, 2H). $[\alpha]_D^{20}$ = −39.46 (*c* = 0.5, MeOH).

Synthesis of (*S*)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-(cyclopropylmethoxy)-4-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((*S*)-32k). To a solution of **28k** (150 mg, 0.5 mmol) in MeOH (10 mL) was added 6 N NaOH (0.167 mL, 1.0 mmol),and the reaction was heated at 80 °C for 16 h. After addition of 6 N NaOH (0.167 mL, 1.0 mmol), the mixture was heated for a further 16 h. MeOH was removed

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under reduced pressure, and the remaining aqueous solution was acidified with 2 N HCl and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under vacuum. The crude residue (130 mg) was dissolved in DCM (10 mL), (*S*)-**21** (0.230 g, 0.547 mmol) was added followed by EDC (0.087 g, 0.456 mmol) and DMAP (0.056 g, 0.456 mmol), and the reaction was stirred at rt overnight. 1 N HCl was added, and the organic phase was separated, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by preparative HPLC (method 1), affording (*S*)-**32k** as a white solid (0.095 g, 0.138 mmol, 30.3%). LC−MS (ESI+) *m/z* 687.2 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.54 (s, 2H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.15 (d, *J* = 1.5 Hz, 1H), 7.12 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.87−6.93 (m, 1H), 6.76−6.86 (m, 2H), 7.05 (t, *J* = 74.8 Hz, 1H), 6.16 (dd, *J* = 8.5, 5.3 Hz, 1H), 3.74−4.01 (m, 4H), 3.53 (dd, *J* = 13.8, 8.8 Hz, 1H), 3.10 (s, 3H), 1.11−1.37 (m, 2H), 0.44−0.66 (m, 4H), 0.24−0.44 (m, 4H). $[\alpha]_D^{20}$ = −7.24 (*c* = 0.5, MeOH).

Synthesis of (*S*)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-methoxy-6-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((*S*)-32l). To a solution of KOH (764 mg, 11.57 mmol) in EtOH (5 mL) was added compound **28l** (300 mg, 1.157 mmol), and the reaction was heated to reflux for 8 h. Additional KOH (764 mg, 11.57 mmol) was added, and the heating was continued for 8 h. The solvent was evaporated under vacuum, and the residue was dissolved in water and acidified with concentrated HCl to pH 2. The precipitate was collected by filtration, washed with water, and dried to afford 250 mg of acid intermediate. To a solution of this acid (100 mg, 0.408 mmol), EDC (196 mg, 1.021 mmol), and DMAP (41.6 mg, 0.340 mmol) in dry DCM (10 mL) was added (*S*)-**21** (143 mg, 0.340 mmol), and the resulting solution was stirred at rt for 3 h. The solvent was evaporated, and the residue was partioned between EtOAc and sat. $NaHCO_3$. The organic phase was washed with 1 N HCl and brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography on silica gel (DCM/MeOH/aq. 33% NH_4OH 95:5:0.5) followed by trituration with iPr_2O to afford (*S*)-**32l** as a white solid (85 mg, 0.131 mmol, 34%). LC−MS (ESI+) *m/z* 647.14 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (br s, 1H), 8.52 (s, 2H), 7.44 (t, *J* = 8.2 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 2.1 Hz, 1H), 7.00−7.07 (m, 2H), 7.00 (d, *J* = 8.5 Hz, 1H), 7.05 (t, *J* = 74.8 Hz, 1H), 6.09 (dd, *J* = 8.2, 6.2 Hz, 1H), 3.92 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.87 (dd, *J* = 10.3, 7.0 Hz, 1H), 3.74 (s, 3H), 3.52 (dd, *J* = 13.8, 8.5 Hz, 1H), 3.31−3.37 (m, 1H), 2.83 (s, 3H), 1.08−1.38 (m, 1H), 0.49−0.70 (m, 2H), 0.19−0.43 (m, 2H). $[\alpha]_D^{20}$ = −22.96 (*c* = 0.5, DCM).

General Procedure for the Preparation of Intermediates 34a−c. *Synthesis of 4-(N-(tert-Butoxycarbonyl)-methylsulfonamido)benzoic Acid (**34a**).* A mixture of compound **33a** (445 mg, 1.46 mmol), Boc_2O (350 mg, 1.60 mmol), and DMAP (196 mg, 1.60 mmol) in DCM (30 mL) was stirred at rt for 2 h. The reaction mixture was diluted with DCM and washed with 1 N HCl; the organic phase was dried over Na_2SO_4, and the solvent was removed under vacuum. The residue was dissolved in MeOH (60 mL), and 10% Pd/C (60 mg) was added. The mixture was hydrogenated in a Parr apparatus at 20 psi for 1 h. The catalyst was filtered off, and the filtrate was evaporated to dryness, affording **34a** (450 mg, 98%), which was used without purification. LC−MS (ESI+) *m/z* 316.2 (MH^+).

Compounds **34b−c** were synthesized following the same protocol and starting from corresponding intermediates **33b−c**.

Preparation of Intermediates 35a−c. Compounds (*S*)-**35a−c** were synthesized following the protocol described for the preparation of compound (*S*)-**31a** starting from corresponding acids **34a−c**.

Preparation of Compounds 36a−c. Compounds (*S*)-**36a−c** were synthesized following the protocol described for the preparation of compound (*S*)-**32a** starting from corresponding Boc-protected intermediates (*S*)-**35a−c**.

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-(methylsulfonamido)benzoyloxy)-ethyl)pyridine 1-Oxide ((S)-**36a**).* From (*S*)-**35a**, 4 M HCl in dioxane, DCM, rt, 3 days; purification by trituration with EtOH, white solid, 70%. LC−MS (ESI+) *m/z* 617.0 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (br s, 1H), 8.55 (s, 2H), 7.94 (m, 2H), 7.27 (m, 2H), 7.20 (d, *J* = 1.8 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 7.07 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.19 (dd, *J* = 9.4, 4.4 Hz, 1H), 3.93 (d, *J* = 7.0 Hz, 2H), 3.60 (dd, *J* = 14.2, 9.5 Hz, 1H), 3.33 (dd, *J* = 14.4, 4.7 Hz, 1H), 3.09 (s, 3H), 1.09−1.38 (m, 1H), 0.45−0.71 (m, 2H), 0.21−0.45 (m, 2H). $[\alpha]_D^{20}$ = −45.06 (c = 0.5, MeOH).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-methoxy-4-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**36b**).* From (*S*)-**35b**, 4 M HCl in EtOAc, rt, 3 h; purification by crystallization from EtOAc, white solid, 57%. LC−MS (ESI+) *m/z* 647.5 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 2H), 7.33−7.41 (m, 1H), 7.21−7.26 (m, 1H), 7.17 (m, 1H), 6.81−6.89 (m, 1H), 6.76−6.80 (m, 1H), 6.70 (m, 2H), 6.29 (t, *J* = 75.00 Hz, 1H), 5.87−5.99 (m, 1H), 3.48−3.71 (m, 5H), 3.24−3.43 (m, 1H), 2.90−3.02 (m, 1H), 0.81−1.03 (m, 1H), 0.23−0.44 (m, 2H), −0.16−0.18 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(hydroxymethyl)-4-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**36c**).* From (*S*)-**35c**, 4 M HCl in dioxane, DCM, rt, overnight; purification by flash chromatography on silica gel (DCM/MeOH 98:2), white solid, 78%. LC−MS (ESI+) *m/z* 646.9 (MH^+). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.54 (s, 2H), 8.04 (d, *J* = 2.1 Hz, 1H), 7.89 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.17−7.23 (m, 2H), 7.03−7.10 (m, 1H), 7.06 (t, *J* = 74.8 Hz, 1H), 6.21 (dd, *J* = 9.2, 4.3 Hz, 1H), 5.52 (s, 1H), 4.63 (s, 2H), 3.93 (d, *J* = 7.0 Hz, 2H), 3.61 (dd, *J* = 14.1, 9.4 Hz, 1H), 3.35 (dd, *J* = 14.4, 4.7 Hz, 1H), 3.10 (s, 3H), 1.13−1.37 (m, 1H), 0.48−0.72 (m, 2H), 0.24−0.43 (m, 2H). $[\alpha]_D^{20}$ = −40.42 (*c* = 0.5, MeOH).

General Protocol for the Preparation of Compounds (*S*)-38a−e by Sulfonylation of the Corresponding Aniline Intermediates. *Synthesis of (S)-3,5-Dichloro-4-(2-(4-(cyclopropylmethoxy)-3-(methylsulfonamido)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl) Pyridine 1-Oxide ((S)-**38a**).* Compound (*S*)-**37a** (40 mg, 0.06 mmmol) was dissolved in $CHCl_3$ (2 mL), and the solution was cooled to 0 °C. Methanesulfonyl chloride (8 mg, 0.07 mmol) and dry pyridine (0.5 mL) were added, and the reaction was stirred at 0−10 °C for 4 h. Aqueous 1 M HCl (2 mL) was added, and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by crystallization from EtOH to afford (*S*)-**38a** as a white solid (31 mg, 0.045 mmol, 68%). LC−MS (ESI+) *m/z* 687.2 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.12−8.37 (m, 3H), 7.95 (s, 1H), 7.87 (dd, *J* = 8.60, 1.98 Hz, 1H), 7.31 (d, *J* = 1.76 Hz, 1H), 6.68−7.25 (m, 4H), 6.34−6.30 (m, 1H), 3.92−4.16 (m, 4H), 3.68−3.78 (m, 1H), 3.44 (d, *J* = 4.41 Hz, 1H), 3.00−3.05 (s, 3H), 1.17−1.46 (m, 2H), 0.61 (dd, *J* = 12.57, 7.72 Hz, 4H), 0.40 (t, *J* = 4.63 Hz, 4H).

Compounds (*S*)-**38b−e** were synthesized in a similar way starting from corresponding aniline intermediates (*S*)-**37b−e**.

*(S)-4-(2-(4-(Benzyloxy)-3-(methylsulfonamido)benzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((S)-**38b**).* From (*S*)-**37b**, $CHCl_3$, rt, 2 h; purification by preparative HPLC (method 2), white solid, 89%. LC−MS (ESI+) *m/z* 723.6 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.19−8.31 (m, 3H), 8.08 (s, 1H), 7.87 (dd, *J* = 8.60, 1.98 Hz, 1H), 7.55 (d, *J* = 7.06 Hz, 2H), 7.12−7.47 (m, 7H), 6.67−6.91−7.11 (t, 1 H, CHF_2), 6.33 (dd, *J* = 9.26, 4.41 Hz, 1H), 5.23−5.39 (m, 2H), 3.99 (dd, *J* = 7.06, 3.97 Hz, 2H), 3.72 (dd, *J* = 14.11, 9.70 Hz, 1H), 3.42 (dd, *J* = 14.11, 4.41 Hz, 1H), 2.99 (s, 3H), 1.27 (br s, 1H), 0.60 (dd, *J* = 7.94, 1.32 Hz, 2H), 0.32−0.45 (m, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-methoxy-5-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**38c**).* From (*S*)-**37c**, $CHCl_3$, rt, 2 h; purification by preparative HPLC (method 2), white solid, 66%. LC−MS (ESI+) *m/z* 646.9 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.69−8.86 (m, 1H), 8.28 (s, 2H), 7.68 (m, 1H), 7.29−7.40 (m, 2H), 7.18−7.25 (m, 1H), 7.16 (dd, *J* = 5.29, 2.21 Hz, 2H), 6.92 (t, *J* = 75.00 Hz, 1H), 6.29−6.36 (m, 1H), 3.99 (dd, *J* = 7.06, 2.21 Hz, 2H), 3.87 (s, 3H), 3.69−3.79 (m, 1H), 3.39−3.47 (m, 1H), 3.05 (s, 3H), 1.21−1.34 (m, 1H), 0.60 (dd, *J* = 8.16, 1.54 Hz, 2H), 0.38 (d, *J* = 5.73 Hz, 2H). $[\alpha]_D^{20}$ = −59.33 (*c* = 1, $CHCl_3$).

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*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxy-2-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**38d**).* From (*S*)-**37d**, DCM, rt, 2 h; purification by preparative HPLC (method 2), beige solid, 35%. LC−MS (ESI+) *m/z* 647.5 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 10.23−10.41 (m, 1H), 8.18−8.37 (m, 3H), 7.11−7.39 (m, 4H), 6.92 (t, *J* = 75.00 Hz, 1H), 6.67−6.84 (m, 1H), 6.19−6.42 (m, 1H), 3.85−4.09 (m, 5H), 3.68−3.83 (m, 1H), 3.37−3.53 (m, 1H), 3.12 (s, 3H), 1.20−1.37 (m, 1H), 0.60 (d, *J* = 7.94 Hz, 2H), 0.38 (d, *J* = 4.41 Hz, 2H).

*(S)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(5-methoxy-2-(methylsulfonamido)-benzoyloxy)ethyl)pyridine 1-Oxide ((S)-**38e**).* From (*S*)-**37e**, DCM, rt, 2 h; purification by crystallization from EtOH, beige solid, 70% yield. LC−MS (ESI+) *m/z* 647.2 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 9.62 (br s, 1H), 8.29 (s, 2H), 7.77 (d, *J* = 3.09 Hz, 1H), 7.60 (d, *J* = 8.82 Hz, 1H), 7.36 (d, *J* = 1.32 Hz, 1H), 7.16−7.31 (m, 3H), 6.92 (t, *J* = 75.00 Hz, 1H), 6.36 (dd, *J* = 9.70, 4.85 Hz, 1H), 4.00 (dd, *J* = 6.84, 1.10 Hz, 2H), 3.91 (s, 3H), 3.81 (dd, *J* = 14.33, 9.92 Hz, 2H), 3.47 (dd, *J* = 14.11, 4.41 Hz, 2H), 2.94 (s, 3H), 1.21−1.39 (m, 1H), 0.52−0.66 (m, 2H), 0.52−0.66 (m, 2H).

Synthesis of (*S*)-1-*tert*-Butyl 2-(5-formyl-2-methoxyphenyl)-pyrrolidine-1,2-dicarboxylate (40). To a solution of **39** (2.1 g, 14 mmol) in DMF (20 mL) were added N-Boc-L-proline (3 g, 14 mmol), EDC (3 g, 15 mmol), and DMAP (2 g, 16 mmol). The mixture was stirred at rt for 24 h was then partitioned between an aqueous saturated solution of K_2CO_3 and iPr_2O. The organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum to give **40** as an oil (4.8 g, 98%), which was used without purification. LC−MS (ESI+) *m/z* 350.2 (MH^+).

Synthesis of (*S*)-3-(1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carbonyloxy)-4-methoxybenzoic Acid (41). Compound **40** (4.8 g, 13.7 mmol) was dissolved in acetic acid (50 mL), and sulfamic acid (1.7 g, 17 mmol) was added. A solution of sodium chlorite (3 g, 33 mmol) in water (20 mL) was added dropwise, and the reaction was stirred at rt for 30 min. The mixture was partitioned between water and EtOAc. The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was dissolved in $CHCl_3$ and precipitated with hexane. The solid was filtered and triturated in iPr_2O/hexane 1:1 to afford **41** as a white solid (3.8 g, 10.4 mmol, 76%). LC−MS (ESI+) *m/z* 366.2 (MH^+).

Synthesis of 4-((*S*)-2-(3-((S)-1-(*tert*-Butoxycarbonyl)-pyrrolidine-2-carbonyloxy)-4-methoxybenzoyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-Oxide ((*S*)-42). A mixture of alcohol (*S*)-**21** (0.3 g, 0.71 mmol), acid **41** (0.3 g, 0.82 mmol), DMAP (0.15 g, 1.2 mmmol), and EDC (0.3 g, 1.5 mmol) in DMF (2.5 mL) was stirred at rt overnight. 1 M HCl was added, and the mixture was extracted with EtOAc. The organic layer was washed with 0.5 M K_2CO_3, dried over Na_2SO_4, and evaporated to dryness to obtain crude (*S*)-**42**, which was used in the next step without purification (520 mg, 0.677 mmol, 95%). LC−MS (ESI+) *m/z* 767.4 (MH^+).

Synthesis of 3,5-Dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(4-methoxy-3-((*S*)-pyrrolidine-2-carbonyloxy)benzoyloxy)ethyl)pyridine 1-Oxide Hydrochloride ((*S*)-43). Crude (*S*)-**42** (0.5 g, 0.6 mmol) was suspended in 4 M HCl in EtOAc (2 mL) and stirred at rt for 2 h. The solvent was removed under reduced pressure, and the residue was purified by crystallization from hexane/EtOAc 2:1 to yield (*S*)-**43** as a white solid (380 mg, 0.57 mmol, 95%). LC−MS (ESI+) *m/z* 703.4 (MH^+).

Synthesis of (*S*)-3,5-Dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-hydroxy-4-methoxybenzoyloxy)ethyl)pyridine 1-Oxide ((*S*)-44). To a solution of (*S*)-**43** (0.12 g, 0.18 mmol) in MeOH was added a saturated solution of aqueous $NaHCO_3$, and the reaction mixture was stirred at rt for 2 h. 1 M HCl was added, and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, and the solvent was evaporated under vacuum. The crude was purified by crystallization from EtOH/hexane 1:1 to yield (*S*)-**44** as a white solid (90 mg, 0.16 mmol, 88%). LC−MS (ESI+) *m/z* 570.1 (MH^+). ^{1}H NMR (400 MHz, acetone) δ 8.25 (s, 2H), 7.94 (s, 1H), 7.47−7.66 (m, 2H), 7.12−7.36 (m, 3H), 7.09−6.90−6.71 (t, 1H, CHF_2), 7.03 (d, *J* = 8.38 Hz, 1H), 6.31 (dd, *J* = 9.48, 4.63 Hz, 1H), 3.89−4.01 (m, 5H), 3.71 (dd, *J* = 13.89, 9.48 Hz, 1H), 3.42 (dd, *J* = 14.11, 4.41 Hz, 1H), 1.27 (br s, 1H), 0.53−0.67 (m, 2H), 0.34−0.46 (m, 2H). $[\alpha]_D^{20}$ = −41.04 (*c* = 1, $CHCl_3$).

Biology and ADME. *In Vitro Determination of PDE4 Inhibitory Activity in the Cell-Free Assay.* PDE4 activity was determined in U937 human monocytic supernatants cells lysate (ECACC, UK). Cells were cultured and harvested, and the supernatant fraction was prepared essentially as described by Torphy.[31] All of the materials used for cell culture were from Gibco (Monza, Italy). U937 cells were grown at 37 °C and 5% CO_2 in RPMI-1640 with GlutaMAX-I medium supplemented with 10% fetal bovine serum and 100 μg/mL of pen/strep. Cells were harvested and washed twice by centrifugation (150*g*, 8 min) in cold PBS. Washed cells were resuspended in cold Krebs−Ringer−Henseleit buffer at a final concentration 20 × 10^6 cells/mL and sonicated. After centrifugation at 15000*g* for 20 min, the supernatants were pooled, divided into aliquots, and stored at −80 °C. PDE4 activity was determined in cells supernatants by assaying cAMP disappearance from the incubation mixtures. The concentration of the test compounds ranged between 10^{-12} and 10^{-6} M. Reactions were stopped by enzyme heat inactivation (2.5 min at 100 °C), and residual cAMP content was determined using the LANCE cAMP Detection kit from PerkinElmer (Milan, Italy) following the manufacturer's instructions. IC_{50} values were determined from concentration−response curves by nonlinear regression analysis using the Solver tool in the Excel program (Microsoft, Milan, Italy) and a four-parameters logistic equation.

In Vitro Determination of PDE4 Inhibitory Activity in the PBMCs Assay. The assay was based on the known inhibitory activity exerted by PDE4 inhibitors on LPS-induced TNF-α release.[32] PDE4 inhibitors were solubilized in DMSO at 5 mM (by sonication for 30 min and incubation for 30 min at 37 °C), diluted in RPMI culture medium, and added to wells at a range of concentrations (final DMSO concentration = 0.2%). Human peripheral blood mononuclear cells (PBMCs) were purchased from Lonza (Basel, CH). PBMCs were washed, resuspended in RPMI 1640 medium (w/o phenol red) supplemented with 10% FBS, 2 mM glutamine, 100 U penicillin, and 100 μg/mL streptomycin (Invitrogen), plated in 96-well tissue culture plates at a density of 10^5 cells/well, and grown in an atmosphere of 95% air and 5% CO_2 at 37 °C. Cells were treated with different concentrations of PDE4 inhibitors (10^{-15} to 10^{-6} M, final DMSO concentration = 0.2%), stimulated with lipolysaccharide (LPS) from *Escherichia coli* at a final concentration of 3 ng/mL, and incubated for 18 h in RPMI (w/o phenol red) supplemented with 10% FBS. Human TNFα in the supernatant was assayed using a paired antibody quantitative ELISA kit (Bender MedSystems GmbH, Vienna, Austria). IC_{50} values were determined from concentration−response curves by nonlinear regression analysis using GraphPad Prism v.6 (GraphPad Software, La Jolla, CA, USA) and a four-parameter logistic equation.

Rat and Human Lung S9 Stability. Test compounds were incubated in duplicate at a concentration of 1 μM with lung S9 fraction (0.5 mg protein/mL) in Dulbecco's buffer (pH 7.4) at 37 °C in the presence of 1 mM NADPH. At different time points (0, 15, 30, and 60 min), 50 μL aliquots were taken, added to 80 μL of ice-cold acetonitrile and 20 μL of 1 μM warfarin in acetonitrile (injection check) to stop the reaction, and centrifuged. The supernatants were analyzed by LC−MS/MS for unchanged compound.

Test compounds were incubated with lung S9 fraction in Dulbecco's buffer in the absence of NADPH and in Dulbecco's buffer alone for 0 and 60 min as controls. 7-Ethoxyresorufin at a concentration of 1 μM was incubated with the S9 fraction as a positive control for phase I activity. Control samples were processed the same as the test-compound samples.

Plasma Protein Binding. Five micromolar solutions of standards and compounds were prepared in plasma from a 500 μM stock solution. Plasma solutions (200 μL) were equilibrated with 200 μL of phosphate buffer (pH 7.4) for 5 h at 37 °C under slow rotation in a Dianorm equilibrium dialysis apparatus. After the incubation period, the solutions were taken from the cells and extracted with ACN/MeOH (with Verapamil 100 nM as IS). Forty microliters of dialyzed plasma was added to 40 μL of blank PBS and extracted with 320 μL of the solvent solution. Solutions were then vortexed and centrifuged at 3000 rpm for 25 min, and the supernatants were analyzed by LC−MS/MS.

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Caco-2 Membrane Permeability Assay. Caco-2 cells (ECACC) were cultured in DMEM, 10% FCS, 1% NEAA, 10 mM Hepes buffer, 50 U/mL penicillin, and 50 μg/mL streptomycin. For transport studies, 200 000 cells/well were seeded on Millicell 24-well cell culture plates. After 24 h of incubation at 37 °C and 5% CO_2, the medium was changed with Enterocyte Differentiation Medium (Becton Dickinson), which allows Caco-2 cells to establish a differentiated enterocyte monolayer within 3 days. The transport across the Caco-2 monolayer was determined by adding a 10 μM solution of compound in DMEM (1% final concentration of DMSO) to the apical side; after 2 h of incubation at 37 °C, the basolateral side, the apical side, and the starting solutions were analyzed and quantified by LC−MS/MS. The experiment was performed using buffers at different pH (6.5 apical vs 7.4 basolateral) to mimic physiological conditions better. The P_{app}, expressed in nanometers per second, was calculated as follows: $P_{app} = J/C_o$, where J = flux (dX/dt per A) and C_o = donor concentration (μM) at $t = 0$; dX/dt = change in mass (X, nmol) per time (t, sec) and A = filter surface area (cm^2). The rank order of apparent permeability of the test compound was compared with that of known reference compounds tested in the same experiment, including caffeine (as a probe for transcellular transport) and cimetidine (as a P-gp substrate). The general absorption classification for P_{app} values in Caco-2 assay were <10 nm/s, low level; 10−50 nm/s, medium level; and >50 nm/s, high level.

PDEs Enzyme Assays. PDE1 was purified from bovine brain.[33] PDE2, PDE3, and PDE5 were purified from human platelets.[34] PDE6 was purified from bovine retina.[35] The radiometric assay method is a modification of the two-step method of Thompson and Appleman.[36] Compound **32a** was tested atfive concentrations (1% final DMSO concentration) in duplicate with a starting concentration of 30 μM and a 1:10 serial dilution against human PDEs.

$LogD_{7.4}$ Measurement. $LogD_{7.4}$ values were measured in the *n*-octanol/buffer partition system at room temperature (25 ± 3 °C) via the shake-flask method.[37] Test compounds, after equilibrating overnight between presaturated partition phases, were analyzed in each phase via LC−UV after dilution with CH_3OH. The $LogD_{7.4}$ values are the means of at least three independent partition experiments employing different *n*-octanol/buffer volume ratios. The chosen buffer was 50 mM MOPS (3-morpholinopropanesulfonic acid), pH 7.4, adjusted to 0.15 M ionic strength with KCl. LC−UV elution conditions were optimized for each analyte. The column used was a Phenomenex Kinetex C18 100 Å 50 × 2.1 mm, 5 μm particle size. Solvent A was water (0.1% formic acid). Solvent B was acetonitrile (0.1% formic acid). Flow rate was 0.8 mL/min. The standard deviations of the $LogD_{7.4}$ values were all ≤±0.04.

Binding Kinetics. Recombinant human PDE4BCat and PDE4DCat enzymes were prepared from baculovirus-infected Sf9 cells. The assay buffer for K_{on} and K_{off} experiments was 30 mM Tris, pH 7.4, 50 mM NaCl, 5 mM MgCl2, 0.01% Tween 20, and 0.01% BSA. K_{on} experiments were conducted by mixing PDEs or uninfected protein with 100 nM [^{14}C]**32a** for 0, 2.5, 5, 10, 20, 30, 45, 60, and 90 min at room temperature. After the incubation, the reactions were placed into a centrifugal concentrator (Vivaspin 500, 10 kDa cut off), centrifuged for 30 min at 15 000*g*, and subsequently washed three times with 500 μL of assay buffer. The retained proteins were mixed with scintillation fluid and counted. K_{off} experiments were conducted by mixing PDE proteins with 1 μM [^{14}C]**32a** for 30 min at room temperature. The reactions were then centrifuged in Vivaspins for 30 min at 15 000*g* and washed five times with assay buffer. Aliquots of the retained protein and filtrates were mixed with scintillation fluid and counted. The [^{14}C]**32a** -bound proteins were divided into appropriate aliquots and mixed with **32a** (50 μM) for 0, 5, 10, 15, 20, 30, 45, 60, and 90 min and 20 h at room temperature. After incubation, the reactions were centrifuged in Vivaspins, washed, and counted as described for the K_{on} experiments.

Rat and Human Lung Slices Stability. Fresh lung slices from both species were preincubated for 1 h in preincubation medium (DMEM/F-12, 10% fetal calf serum, penicillin/streptomycin, ITS, and glutamine) at 37 °C in a O_2/CO_2 (95%/5%) humidified atmosphere; at the end of the 1 h preincubation period, the test compounds were incubated for 2 h with liver slices and lung slices of both species (three replicates with human slices and four replicates with rat slices) at 1 μM in MEM-EARLE medium without phenol red and L-glutamine but with sodium bicarbonate. Testosterone was incubated under the same conditions to assess the metabolic viability of the fresh slices (n = 2). The concentration of each test compound was assessed by LC−MS/MS after an incubation period of 0, 20, 40, 60, 90, and 120 min.

Calu-3 Membrane Permeability. Calu-3 cells were maintained at 37 °C, 5% CO_2, and 90−98% relative humidity in 75 cm^2 culture flasks with MEM Medium. The cells were passaged once a week using a trypsin/EDTA solution. About 2.5 × 10^6 cells were seeded per flask. The cells at a grown density of 1 × 10^5 Calu-3 cells per 1 cm^2 on transwell filters (polyester, with a permeation area of 1.12 cm^2 and pore diameter of 0.4 μm^2).

For the transport experiments, Calu-3 cells were seeded on Transwell filter inserts, which were placed into 12-well flat-bottom culture plates. The inserts (apical compartments) were supplied with 0.5 mL and the outer wells (basal compartments) with 1.5 mL of MEM culture medium. The cells were cultured at 37 °C, 5% CO_2, and 90−98% relative humidity in MEM culture medium for 3 days. The cells were cultured for a further 11 to 27 days until they formed confluent monolayers. The transport across the monolayer was determined by adding three different concentrations (1.5, 3.5, and 7 μM) to the apical side. During incubation, samples was taken from the receiver compartment at five time points, 0, 30, 60, 90, and 120 min, and the test compounds in the samples were quantified by LC−MS/MS.

Plasma Stability. Test compounds were dissolved in DMSO at a concentration of 5 mM and then added to plasma to reach a final concentration of 5 μM. Test compounds were incubated for 120 min at 37 °C. At t = 0 and after 15, 30, 60, 90, and 120 min incubation, sample aliquots were taken, treated with a 2-fold volume of chilled acetonitrile, and centrifuged. The supernatants were analyzed by LC−MS/MS.

In Vivo Pharmacology. *ED_{50} Determination of Inhibition of Ovalbumin-Induced Lung Eosinophilia in Guinea Pigs.* Male Dunkin−Hartley guinea pigs were actively sensitized with ovalbumin (OVA, grade III; Sigma). On days 1 and 11, 20 μg of Ova in 5% aluminum hydroxide adjuvant suspended in 0.5 mL of saline was injected intraperitoneally. On day 20 after the first sensitization, animals were challenged with a 1 h aerosol of OVA (10 μg mL^{-1}) dissolved in saline to provoke an influx of eosinophils into the airways. The sham control group was exposed to an aerosol of saline. The dose−response curve and the duration of action of compound **32a** were investigated by administering the drug 2 and 24 h before antigen challenge, respectively. Sham animals and control Ova group received lactose as vehicle. For each time point, eight drug- and vehicle-treated animals were used. Intratracheal administration of compound **32a** was performed by placing the micronized drug blended with lactose into a dry powder insufflator (Penn Century, Philadelphia, PA, USA). The tip of the insufflator was then inserted into the lower part of the trachea of animals under isoflurane anesthesia (4% in O_2), and the powder was blown into the airways during the inspiration phase with 4 mL of air. The control groups received a corresponding amount of lactose (10 mg/kg body wt). Bronchoalveolar lavage (BAL) collection was carried out by instilling 5 mL of saline into the lungs via a tracheal cannula in terminally anaesthetised animals. Total cell counts of the BAL fluid samples were performed using a Neubaur hemocytometer. Cytospin smears of BAL samples were prepared by centrifugation at 200 rpm for 5 min at room temperate and stained using a DiffQuik stain system (Dade Behring). Differential cell count was performed using oil-immersion microscopy. A dose-response curve was constructed for the inhibition of Ova-induced lung eosinophilia at 2 h predosing, and half-maximal effect doses (ED_{50}) were estimated from the fitted curve.

Molecular Modeling. PDB crystal structures were prepared with the Protein Preparation Wizard in Maestro.[38] The binding poses of compounds (*R*)-**18**, (*S*)-**18**, and (*S*)-**32a** in the PDE4B catalytic site (PDB ID: 1XMU) were obtained by applying the following docking procedure. For each ligand, five preliminary poses were generated in Glide[39] with the standard precision (SP) mode using the formation of hydrogen bonds with key residue Gln443 in the hydrophobic region as a constraint, the poses were refined with the accurate extra-precision (XP) method, and the docking pose with the best XP GScore value was selected for each ligand.

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■ ASSOCIATED CONTENT

Supporting Information

XRD single-crystal analysis of compound (*R**,*S***)-**22**; chemical correlation details depicted in Scheme 5; synthesis and characterization of intermediates **25i**, **25o−s**, **27h**, **27j**, **27k**, **33a−c**, and (*S*)-**37a−e**; and characterization of intermediates **28b−l**, **29b−j**, **30b−e**, **30g−j**, **31b−l**, **34b−c**, and **35a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

ACN, acetonitrile; AcOH, acetic acid; ADME, absorption, distribution, metabolism, and excretion; AMP, adenosine monophosphate; BAL, bronchoalveolar lavage; BOC, *tert*-butoxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; BSA, bovine serum albumin; BuLi, butyl lithium; cAMP, cyclic adenosine monophosphate; cat, catalytic domain; compd, compound; COPD, chronic obstructive pulmonary disease; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide); EDTA, ethylendiaminetetraacetic acid; ESI, electrospray ionization; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethyl alcohol; FBS, fetal bovine serum; FCS, fetal calf serum; GP, guinea pig; HPLC, high-performance liquid chromatography; iPr_2NH, diisopropylamine; $iPrO_2$, diisopropyl ether; *i*PrOH, isopropyl alcohol; ITS, insulin, transferrin, selenium; LARBS, low-affinity rolipram binding site; LC−MS, liquid chromatography−mass spectrometry; LDA, lithium diisopropylamide; LPS, lipopolysaccharides; *m*CPBA, meta-chloroperoxybenzoic acid; MeI, iodomethane; MEM, minimum essential medium; MEM-EARLE, minimum essential medium with Earle's salts; MeOH, methyl alcohol; $MeSO_2Cl$, methansulphonyl chloride; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NEAA, nonesssential amino acids; NMR, nuclear magnetic resonance; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; PDE, phosphodiesterase; PDE4, phosphodiesterase-4; Pgp, P-glycoprotein; PPB, plasma protein binding; PPh_3, triphenylphosphine; RPMI, Roswell Park Memorial Institute; t_R, retention time; S_9, lung S_9 fraction; *t*BuOK, potassium *tert*-butoxide; TEA, triethylamine; THF, tetrahydrofuran; TLC, thin-layer chromatography; TNF-α, tumor necrosis factor alpha; XRD, X-ray diffraction

■ REFERENCES

(1) Page, C. P.; Spina, D. Phosphodiesterase inhibitors in the treatment of inflammatory diseases. *Handb. Exp. Pharmacol.* **2011**, *204*, 391−414.
(2) Sanz, M. J.; Cortijo, J.; Morcillo, E. J. PDE4 inhibitors as new anti-inflammatory drugs: Effects on cell trafficking and cell adhesion molecule expression. *Pharmacol. Ther.* **2005**, *106*, 269−297.
(3) Houslay, M. D.; Schafer, P.; Zhang, K. Y. Keynote review: Phosphodiesterase-4 as a therapeutic target. *Drug. Discovery Today* **2005**, *10*, 1503−1510.
(4) Hatzelmann, A.; Morcillo, E. J.; Lungarella, G.; Adnot, S.; Sanjar, S.; Beume, R.; Schudt, C.; Tenor, H. The preclinical pharmacology of roflumilast − a selective oral phosphodiesterase4 inhibitor in development for chronic obstructive pulmonary disease. *Pulm. Pharmacol. Ther.* **2010**, *23*, 235−256.
(5) Horowski, R.; Sastre-y-Hernandez, M. Clinical effects of neurotropic selective cAMP phosphodiesterase inhibitor rolipram in depressed patients: Global evaluation of the preliminary reports. *Curr. Ther. Res.* **1985**, *38*, 23−29.
(6) Beghè, B.; Rabe, F.; Fabbri, L. M. Phosphodiesterase-4 inhibitor therapy for lung diseases. *Am. J. Respir. Crit. Care Med.* **2013**, *188*, 271−278.
(7) Compton, C. H.; Gubb, J.; Niemann, R.; Edelson, J.; Amit, O.; Bakst, A.; Ayres, J. C.; Creemers, J. P.; Schultze-Werninghaus, G.; Brambilla, C.; Barnes, N. C. Cylomilast, a selective phosphodiesterase-4 inhibitor for treatment of patients with chronic obstructive pulmonary disease: a randomized, dose-ranging study. *Lancet* **2001**, *358*, 265−270.
(8) Calverley, P. M.; Sanchez-Toril, F.; McIvor, A.; Teichmann, P.; Bredenbroeker, D.; Fabbri, L. M. Effect of 1-year treatment with Roflumilast in severe chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **2007**, *176*, 154−161.
(9) Rennard, S. I.; Schachter, N.; Strek, M.; Rickard, K.; Amit, O. Cilomilast for COPD: Results of a 6-month, placebo-controlled study of a potent, selective inhibitor of phosphodiesterase 4. *Chest* **2006**, *129*, 56−66.
(10) Page, C. P.; Spina, D. Selective PDE inhibitors as novel treatments for respiratory disorders. *Curr. Opin. Pharmacol.* **2012**, *12*, 275−286.
(11) Kranz, M.; Wall, M.; Evans, B.; Miah, A.; Ballantine, S.; Delves, C.; Dombroski, B.; Groos, J.; Schneck, J.; Villa, J. P.; Neu, M.; Somers, D. O. Identification of PDE4B over 4D subtype-selective inhibitors revealing an unprecedented binding mode. *Bioorg. Med. Chem.* **2009**, *17*, 5336−5341.
(12) Robichaud, A.; Stamatiou, P. B.; Jin, S. L. C.; Lachance, N.; MacDonald, D.; Laliberte, F.; Liu, S.; Huang, Z.; Conti, M.; Chan, C. C. Deletion of phosphodiesterase 4D in mice shortens alpha2-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis. *J. Clin. Invest.* **2002**, *110*, 1045−1052.
(13) Souness, J. E.; Rao, S. Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterase. *Cell. Signalling* **1997**, *9*, 227−236.
(14) Burgin, A. B.; Magnusson, O. T.; Singh, J.; Witte, P.; Staker, B. R.; Bjornsson, J. M.; Thorsteinsdottir, M.; Hrafnsdottir, S.; Hagen, T.; Kiselyov, A. S.; Stewart, L. J.; Gurney, M. E. Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. *Nat. Biotechnol.* **2010**, *28*, 63−70.
(15) Gross, P. M.; Wall, K. M.; Pang, J J.; Shaver, S. W.; Wainman, D .S. Microvascular specialization promoting rapid interstial solute dispersion in nucleus tractus solitaries. *Am. J. Physiol.* **1990**, *259*, R1131−R1138.
(16) Okuda, S.; Honda, M.; Ito, Y.; Aihara, E.; Kato, S.; Mitsufuji, S.; Yoshikawa, T.; Takeuchi, K. Phosphodiesterase isozymes involved in regulating acid secretion in the isolated mouse stomach. *J. Physiol. Pharmacol.* **2009**, *60*, 183−190.
(17) Kuss, H.; Hoefgen, N.; Johanssen, S.; Kronbach, T.; Rundfeldt, C. In vivo efficacy in airway disease models of N-(3,5-dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide (AWD 12−281), a selective phosphodiesterase 4 inhibitor for inhaled administration. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 373−385.
(18) Duplantier, A. J.; Bachert, E. L.; Cheng, J. B.; Cohan, V. L.; Jenkinson, T. H.; Kraus, K. G.; McKechney, M. W.; Pillar, J. D.; Watson, J. W. SAR of a series of 5,6-dihydro-(9H)-pyrazolo[3,4-c]-1,2,4-

triazolo[4,3-alpha]pyridines as potent inhibitors of human eosinophil phosphodiesterase. *J. Med. Chem.* **2007**, *50*, 344−349.
(19) (a) Phillips, P.; Bennetts, M.; Banner, K.; Ward, J.; Wessels, D.; Fuhr, R. The PDE4 inhibitor UK-500 001 does not significantly inhibit airway responses to allergen and histamine. *Eur. Respir. J.* **2007**, 491s. (b) Vestbo, J.; Tan, L.; Atkinsons, G.; Ward, J. The UK-500,001 Global Study Team. A controlled trial of 6-weeks' treatment with a novel inhaled phosphodiesterase type-4 inhibitor in COPD. *Eur. Respir. J.* **2009**, *33*, 1039−1044.
(20) Tralau-Stewart, C. J.; Williamson, R. A.; Nials, A. T.; Gascoigne, M.; Dawson, J.; Hart, G. J.; Angell, A. D. R.; Solanke, Y. E.; Lucas, F. S.; Wiseman, J.; Ward, P.; Ranshaw, L. E.; Knowles, R. G. GSK256066, an exceptionally high-affinity and selective inhibitor of phosphodiesterase 4 suitable for administration by inhalation: In vitro, kinetic, and in vivo characterization. *J. Pharmacol. Exp. Ther.* **2011**, *337*, 145−154.
(21) Pages, L.; Gavaldà, A.; Lehner, M. D. PDE4 inhibitors: A review of current developments (2005−2009). *Expert Opin. Ther. Pat.* **2009**, *19*, 1501−1519.
(22) (a) Singh, D.; Petavy, F.; Macdonald, A. J.; Lazaar, A. L.; O'Connor, B. J. The inhaled phosphodiesterase 4 inhibitor GSK256066 reduces allergen challenge responses in asthma. *Respir. Res.* **2010**, *1*, 26−34. (b) Lazaar, A. L.; Mistry, S.; Barrett, C.; Lulic-Burns, Z. The IPC 101939 Study Team. A 4-week randomized study of the safety and tolerability of the inhaled PDE4 inhibitor GSK256066 in COPD patients. *Am. J. Respir. Crit. Care Med.* **2010**, *181*, A4444.
(23) Card, G. L.; England, B. P.; Suzuki, Y.; Fong, D.; Powell, B.; Lee, B.; Luu, C.; Tabrizizad, M.; Gillette, S.; Ibrahim, P. N.; Artis, D. R.; Bollag, G.; Milburn, M. V.; Kim, S. H.; Schlessinger, J.; Zhang, K. Y. J. Structural basis for the activity of drugs that inhibit phosphodiesterases. *Structure* **2004**, *12*, 2233−2247.
(24) Gleeson, M. P. Plasma Protein Binding Affinity and Its Relationship to Molecular Structure: An In-silico Analysis. *J. Med. Chem.* **2007**, *50*, 101−112.
(25) *ACD/LogD release 12.00*; Advanced Chemistry Development, Inc.: Toronto, Canada.
(26) Medley, H.; Orozoco, S.; Allen, A. Efficacy and safety profile of fluticasone furoate administered once daily in the morning or evening: A randomized, double-blind, double-dummy, placebo-controlled trial in adult and adolescent patients with persistent bronchial asthma. *Clin. Ther.* **2012**, *34*, 1683−1695.
(27) (a) Delcanale, M.; Amari, G.; Armani, E. Derivatives of 1-phenyl-2-pyridinyl alkyl alcohols as phosphodiesterase inhibitors. PCT Int. Appl. WO2008006509, February 12, 2009. (b) Amari, G.; Aquino, G.; Armani, E. Synthesis, in vitro and in vivo characterization of novel enolesters as PDE4 inhibitors. Frontiers in Medicinal Chemistry Meeting, Stockholm, Sweden, June 19−21, 2011.
(28) Bethke, T. D.; Boehmer, G. M.; Hermann, R.; Hauns, B.; Fux, R.; Moerike, K.; David, M.; Knoerzer, D.; Wurst, W.; Gleiter, C. H. Dose-proportional intraindividual single- and repeated-dose pharmacokinetics of roflumilast, an oral, once-daily phosphodiesterase 4 inhibitor. *J. Clin. Pharmacol.* **2007**, *47*, 26−36.
(29) Hatzelmann, A.; Schudt, C. Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 267−279.
(30) Franova, S.; Joskova, M.; Sadlonova, V.; Pavelcikova, D.; Mesarosova, L.; Novakova, E.; Sutovska, M. Experimental model of allergic asthma. *Adv. Exp. Med. Biol.* **2013**, *756*, 49−55.
(31) Torphy, T. J; Zhou, H. L.; Cieslinski, L. B. Stimulation of beta adrenoceptors in a human monocyte cell line (U937) up-regulates cyclic AMP-specific phosphodiesterase activity. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1195−1205.
(32) Cooper, N.; Teixeira, M. M.; Warneck, J.; Miotla, J. M.; Wills, R. E.; Macari, D. M.; Gristwood, R. W.; Hellewell, P. G. A comparison of the inhibitory activity of PDE4 inhibitors on leukocyte PDE4 activity in vitro and eosinophil trafficking in vivo. *Br. J. Pharmacol.* **1999**, *126*, 1863−1871.
(33) Gietzen, K.; Sadorf, I.; Bader, H. A model for the regulation of the calmodulin-dependent enzymes erythrocyte Ca2+-transport ATPase and brain phosphodiesterase by activators and inhibitors. *Biochem. J.* **1982**, *207*, 541−548.
(34) Schudt, C.; Winder, S.; Muller, B.; Ukena, D. Zardaverine as a selective inhibitor of phosphodiesterase isozymes. *Biochem. Pharmacol.* **1991**, *42*, 153−162.
(35) Baehr, W.; Devlin, M. J.; Applebury, M. L. Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments. *J. Biol. Chem.* **1979**, *254*, 11669−11677.
(36) Thompson, W. J.; Appleman, M. M. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry* **1971**, *10*, 311−316.
(37) (a) Leo, A.; Hansch, C.; Elkins, D. Partition coefficients and their uses. *Chem. Rev.* **1971**, *71*, 525−616. (b) Sangster, J. *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*; Wiley: New York, 1997.
(38) *Maestro*, version 9.2; Schrödinger, LLC: New York, 2011.
(39) *Glide*, version 5.7; Schrödinger, LLC: New York, 2011.