Article

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Discovery and optimization of thiazolidinyl- and pyrrolidinyl- derivatives as inhaled PDE4 inhibitors for respiratory diseases

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ABSTRACT. Phosphodiesterase 4 (PDE4) is a key cAMP-metabolising enzyme involved in the pathogenesis of inflammatory disease and its pharmacological inhibition has been shown to exert therapeutic efficacy in chronic obstructive pulmonary disease (COPD). Herein we describe a drug discovery program aiming at the identification of novel classes of potent PDE4 inhibitors suitable for pulmonary administration. Starting from a previous series of benzoic acid esters, we explored the chemical space in the solvent-exposed region of the enzyme catalytic binding pocket. Extensive structural modifications led to the discovery of a number of heterocycloalkyl esters as potent *in vitro* PDE4 inhibitors. (*S**,*S***)-**18e** and (*S**,*S***)-**22e**, in particular, exhibited optimal *in vitro* ADME and pharmacokinetics properties and dose-dependently counteracted acute lung eosinophilia in an experimental animal model. The optimal biological profile as well as the excellent solid state properties suggest that both compounds have the potential to be effective topical agents for treating respiratory inflammatory diseases.

INTRODUCTION.

Phosphodiesterases (PDEs) are intracellular enzymes responsible for the degradation of cyclic nucleotides (cAMP and/or cGMP) to their inactive forms through the cleavage of a phosphodiester bond. PDEs have been classified in 11 families based on sequence homogeneity, inhibitor sensitivity and biochemical properties. This large number of PDE splicing variants serve to fine-tune intracellular levels of cyclic nucleotides which are key intracellular second messengers regulating a wide range of cellular processes.[1]

PDE4 family, which consists of 4 subtypes (PDE4A, PDE4B, PDE4C and PDE4D) sharing a highly conserved catalytic domain, has received particular attention due to the fact that represents the most prominent cAMP-metabolising enzyme in immune cells, including neutrophils, monocytes, macrophages and T cells, the main inflammatory cells involved in the pathophysiology of lung inflammation underlying asthma and chronic obstructive pulmonary diseases (COPD). In particular, PDE4 plays a key role in regulating the biosynthesis of several pro-inflammatory mediators and in modulating chemotaxis of leukocytes to sites of inflammation.[2] Moreover, PDE4 regulates the function

of structural lung cells such as fibroblasts, airway smooth muscle, airway epithelium, vascular endothelium and airway sensory nerves.[2]

These potential beneficial actions of PDE4 inhibitors have been proven in various experimental models of airway inflammation, thus generating a considerable interest in utilizing PDE4 inhibitors to target inflammation underlying pulmonary diseases such as COPD and asthma.[3,4]

Prototypical PDE4 inhibitors, designed for oral administration (Figure 1), suffered from the high prevalence of gastrointestinal side effects such as nausea and emesis, which greatly limited their clinical application.[4] Furthermore, the disappointing results of the second generation PDE4 inhibitor cilomilast **(1)** (Figure 1) Phase III clinical programme raised major concerns about the therapeutic utility of this class of compounds in COPD. Indeed, it is likely that the highest tolerated dose of cilomilast (i.e. 15 mg bid) was on the leading edge of the dose–response curve and that the low therapeutic ratio of this compound prevented efficacy from being realized.[5]

The latest generation of oral PDE4 inhibitors such as roflumilast[6] **2** and apremilast[7] **3** seems to have a better therapeutic index than cilomilast. Indeed, cilomilast is 10-fold more potent in inhibiting PDE4D in comparison with the other PDE4A, PDE4B, and PDE4C isoforms[8] and such subtype selectivity has been associated with emesis-inducing effects of PDE4 inhibitors.[9] Unlike cilomilast, both roflumilast and apremilast do not preferentially inhibit PDE4D subtype and this might contribute to their improved therapeutic ratio when compared with cilomilast and possibly other predecessors.

Roflumilast has been licensed in EU and US for once-daily treatment of severe and very severe COPD associated with chronic bronchitis and frequent exacerbations as add-on to standard of care, making PDE4 inhibitors a reliable anti-inflammatory therapy for COPD,[10] while apremilast has recently been approved for the treatment of adult patients with active psoriatic arthritis. Furthermore, among oral PDE4 inhibitors, tetomilast **(4)** has been reported to be under clinical development in COPD, although nausea and vomiting are frequent side effects, but the results have not been published yet.[11]

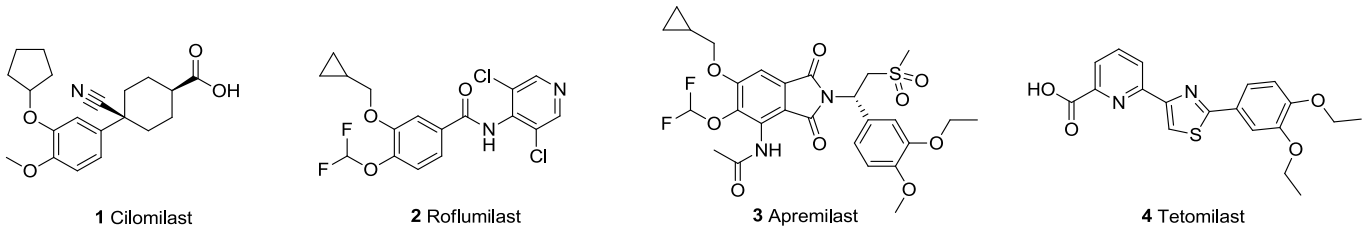


Figure 1. Structures of oral PDE4 inhibitors: cilomilast **(1),** roflumilast **(2)**, apremilast **(3)** and tetomilast **(4)**

However, even roflumilast is fraught with dose-limiting adverse effects like diarrhea, nausea, weight loss, back pain, headache and psychiatric adverse events, resulting in 9% to 16% of patients discontinuing therapy. At this stage, it is still unclear whether the dose limiting side effects of roflumilast allow the use of doses that provide full therapeutic efficacy in COPD patients.[12] Indeed, there are no published clinical data on the use of roflumilast at doses higher than 500 μg/day in this patient population.

This has prompted the search for PDE4 inhibitors with a better therapeutic ratio.

In the respiratory field, a logical way to improve the therapeutic index is to deliver drugs directly into the lung via the inhalation route. This route of delivery could provide prolonged inhibition of the target at the therapeutic site of action with limited systemic exposure and consequently lower potential for target-related side effects. However, only compounds specifically designed for topical treatment can be successfully administered using the inhalation route. Therefore, a successful medicinal chemistry strategy aimed at identifying an inhaled PDE4 inhibitor with limited side effects potential should be directed to obtain: 1) potent anti-inflammatory effects with convincing activity after topical administration; 2) long persistence in the lung but no significant accumulation; 3) minimal systemic exposure; 4) production of inactive metabolites.

Some inhaled PDE4 inhibitors that progressed to the clinic have been reported so far, these include AWD 12-281 **5**[13], tofimilast **6**[14] and UK-500,001 **7**[15] (Figure 2). These drugs proved to be potent *in vitro* PDE4 inhibitors and showed efficacy in preclinical pathology models but no positive effects were reported in the clinic in patients suffering from moderate to severe COPD and have been discontinued.

GSK-256066 **8**[16,17] a highly potent and selective inhaled PDE4 inhibitor, demonstrated potent and long-lasting anti-inflammatory effects in preclinical in vivo models. In a phase 2a study in mild asthmatics, **8** (GSK-256066) exhibited inhibition of forced expiratory volume (FEV1) decrease both in the late asthmatic response (LAR) and in the early asthmatic response (EAR) after 1 week of treatment. Nevertheless, **8** is no longer reported in the company's pipeline. To date, CHF 6001 **9**[18,19] is the only example of inhaled PDE4 inhibitor currently under active clinical development for COPD and its efficacy and safety have been proved in a phase 2a study in mild asthmatics where **9** (CHF 6001) significantly attenuated allergen challenge response.[20]

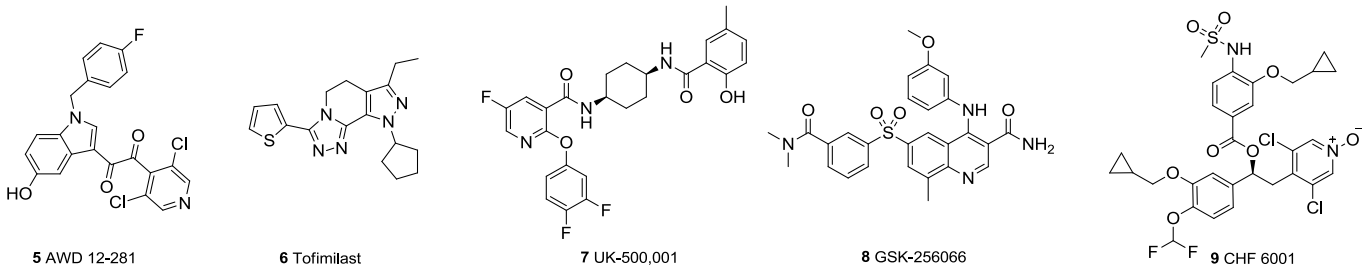


Figure 2. Structures of representative inhaled PDE4 inhibitors in clinical development.

More recently, Astra Zeneca and Merck reported the design and discovery of highly potent and pre-clinically efficacious inhaled PDE4 inhibitors with low emetic potential[21,22] as candidates for treatment of COPD but no clinical development has been reported to date (Figure 3).

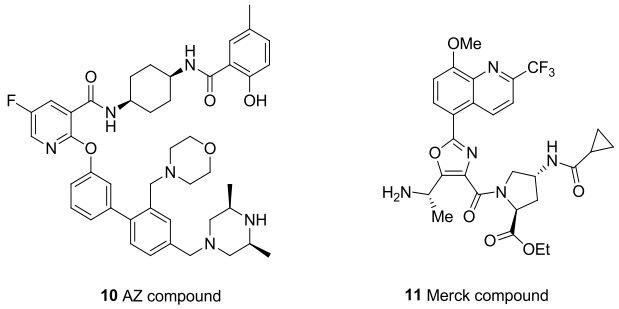


Figure 3. Structures of Astra Zeneca (**10**) and Merck (**11**) inhaled PDE4 inhibitors

Following a structure-based design we have synthetized and characterized a novel classes of inhaled PDE4 inhibitors. These efforts have led to the identification of two potent compounds that effectively

counteracted allergen-induced airway inflammation and showed a pharmacokinetic profile suitable for lung delivery, when tested upon intratracheal administration in the rat.

Rationale We previously disclosed a class of highly potent benzoic acid esters as PDE4 inhibitors, of which **9** is the most representative example.[23] This paper describes the search for a structurally differentiated backup series to access previously unexplored region of the chemical space and to prepare for possible unpredictable non-mechanism-based, chemotype-specific liabilities.

New compounds were designed on the basis of the abovementioned series of esters showing potent PDE4 inhibitory activity.[23] In particular, the crystal structure of the human PDE4B catalytic domain in complex with roflumilast[24] was used as starting structure to perform molecular docking experiments of the benzoic ester series. Analyzing the binding pose of the unsubstituted inhibitor (*S*)-**14a**[23] (Scheme 1) it was observed that a deep pocket was available for the insertion of substituents not only in the *para* and *meta* positions, as seen for compound **9** (CHF6001) and its close analogues,[23] but also in the *ortho* position of the phenyl ring (Figure 4). Nonetheless, when *ortho* substitution was addressed in our former program, we observed a decrease in the cell-based potency or even a complete loss of activity. We envisaged that alternative central cores could provide the scaffolding of exit *ortho*-vectors in the appropriate geometrics to achieve potency while avoiding any cellular drop-off. We therefore focused our efforts on new structural classes of *ortho*-substituted compounds.

In additon to this, since we aimed at potential candidates for pulmonary administration, our strategy targeted new scaffolds whose features could be able to minimize the systemic exsposure due to the swallowed dose. Foreasmuch as previous benzoate esters[23] were poorly metabolized *in vitro*, we focused on obtaining molecules endowed with high intrinsic Clearance, a predictive tool for high systemic Clearance, in addition to low free fraction in human plasma and reduced gut wall permeability. With this purpose we pursued the replacement of benzoyl group gradually increasing the three-dimensional morphology of the compound (Aryl → Heteroaryl → Heterocycloalkyl) to probe if it was feasible maintaining potency while, at the same time, achieving an effectively turned-over series.

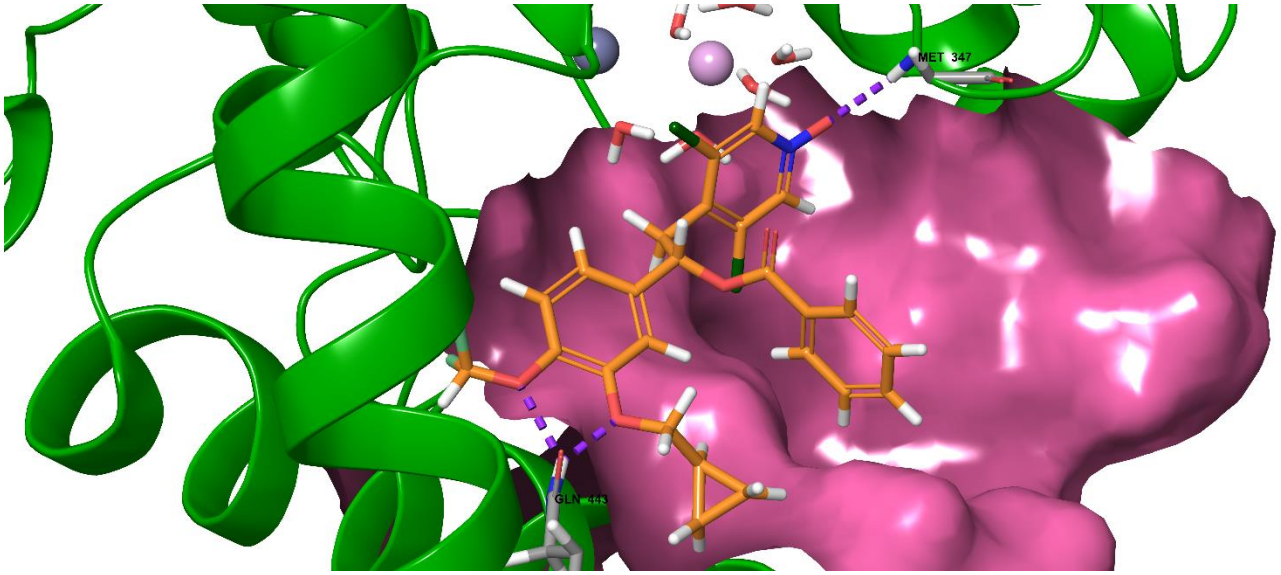
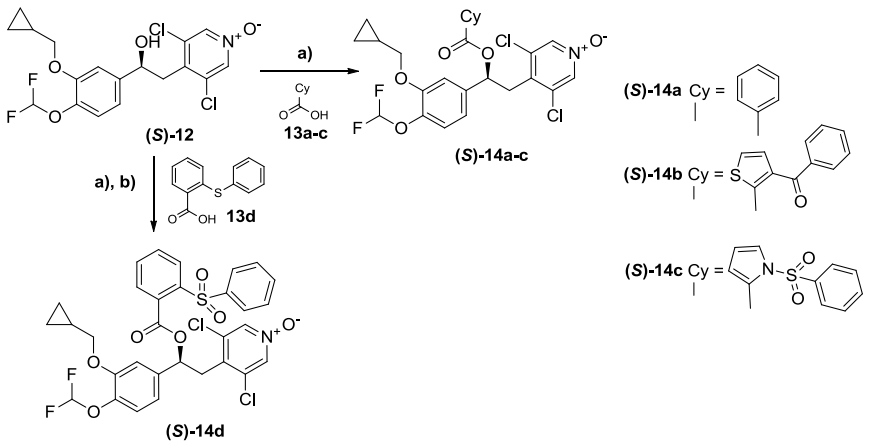


Figure 4. Docking pose of (*S*)-**14a** in the catalytic site of the PDE4B crystal structure (PDB ID: 1XMU). A pocket for the insertion of substituents on the phenyl ring of the ligand is available in the solvent exposed region of the catalytic binding site (grey).

At first we synthesized a new series of ester derivatives where structural modifications were achieved functionalizing the *ortho* position of aromatic rings with sulfonyl and carbonyl substituents. Encouraging results from these compounds prompted us to expand our exploration towards the replacement of the aryl esters with 5- and 6-membered heterocycloalkyl rings, investigating the steric and stereochemical requirements for optimal activity. Newly synthesized compounds were at first assessed in a cell-free assay and structure-activity relationships of the most promising classes were further investigated. Our efforts were focused on obtaining derivatives able to retain their activity in the cell-based assay, overcoming any potency drop-off likely to occur in a cellular system, to identify a suitable lead compound whose optimization could be profitably developed. In addition we looked for a candidate able to exert sustained in vivo efficacy in animal models of inflammation through its persistence in the pulmonary district along with high stability in human lung.

Chemistry. All compounds described in this paper were synthesized starting from common intermediates (*S*)-**12** and (*R*)-**12,** which were prepared as described in ref. 23.

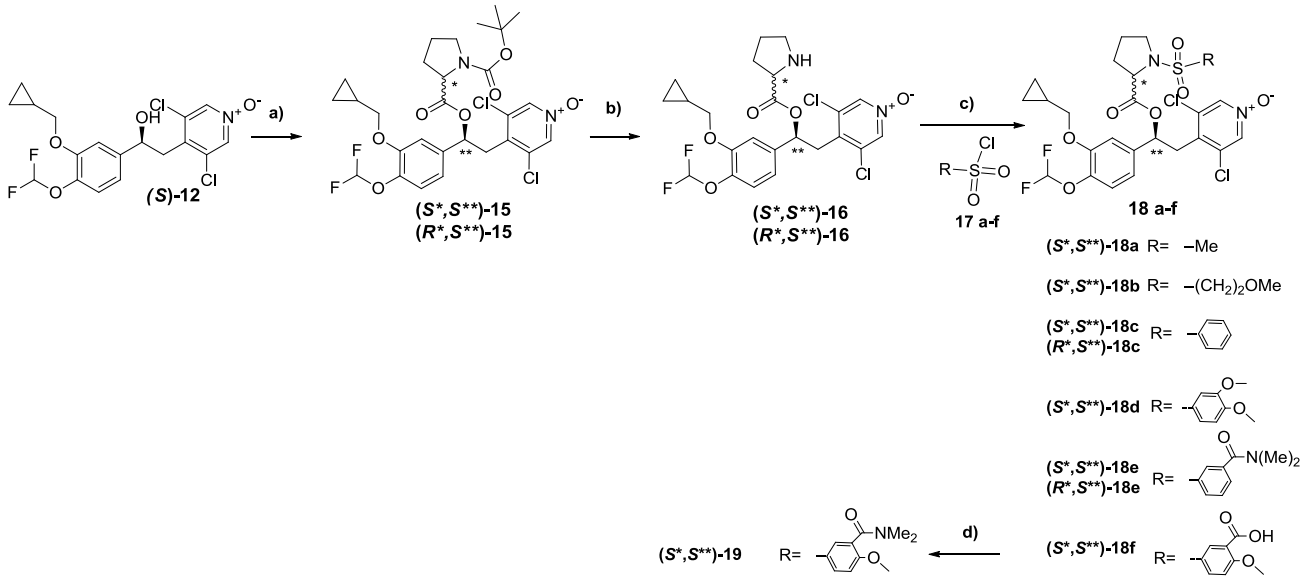
Scheme 1. Preparation of Compounds 14[a]



[a]Reagents and conditions: (a) EDC, DMAP, DCM or DMF, rt; (b) mCPBA, DCM, rt, 48h.

The synthesis of aryl esters (*S*)-**14a-d** is shown in Scheme 1. Esters (*S*)-**14a-c** were obtained by coupling the intermediate (*S*)-**12** with suitable carboxylic acids using EDC and DMAP as condensing agents, while ester (*S*)-**14d** was achieved performing a further oxidation step using mCPBA. Aryl acids **13a-d** where either commercially available or prepared as detailed in the Supporting Information.

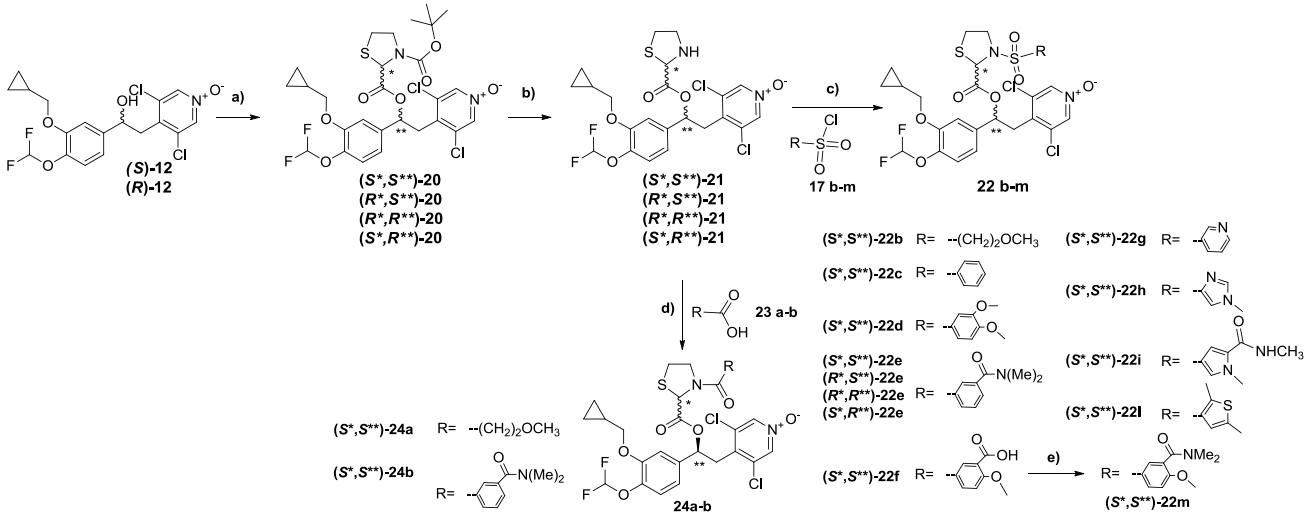
Scheme 2. Preparation of Compounds 18 and 19[a]



[a]Reagents and conditions: a) (*S*)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid or (*R*)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid, EDC, DMAP, DMF, rt, 48 h; b) HCl/Dioxane 4 M, rt, 8 h c) Py or Py/DCM, rt; d) dimethylamine 2M in THF, CDI, DMF, rt.

The synthetic pathway for the synthesis of pyrrolidinyl derivatives **18a-f** and **19** is depicted in Scheme 2. Chiral alcohol (*S*)-**12** was condensed with either (*R*)- or (*S*)- enantiomerically pure Boc-protected proline, then removal of tert-butoxycarbonyl group under acidic conditions led to the versatile intermediates (*S**,*S***)-**15** and (*R**,*S***)-**15** which underwent N-sulfonylation to afford sulfonamides **18a-f**. Further amidation of **18f** using CDI as condensing agent afforded amide (*S**,*S***)-**19**.

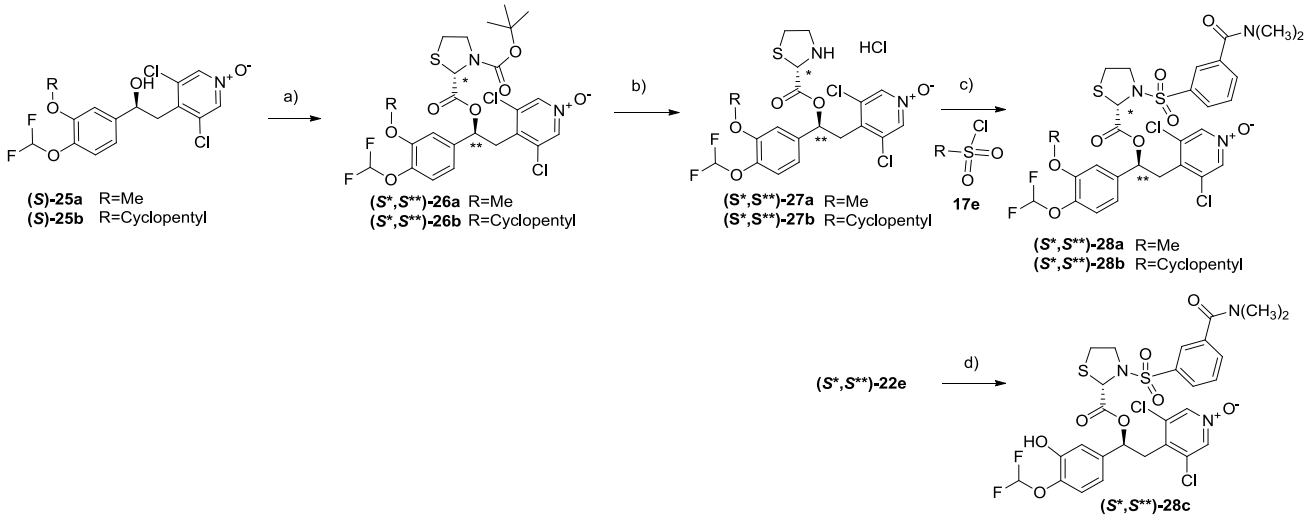
Scheme 3. Preparation of Compounds 22 and 24[a]



[a]Reagents and conditions: a) (*S*)-3-(tert-butoxycarbonyl)thiazolidine-2-carboxylic acid or (*R*)-3-(tert-butoxycarbonyl)thiazolidine-2-carboxylic acid, EDC, DMAP, DMF, rt; b) HCl/AcOEt 5 M, rt; c) Py, rt; d) EDC, DMAP, DCM or DMF, rt; e) alkylamine, CDI, DMF, rt.

An analogous synthetic route was followed for N-thiazolidinyl-sulfones **22b-m** (Scheme 3). Thiazolidinyl esters **20** were prepared by coupling either alcohol (*S*)-**12** or (*R*)-**12** with the suitable thiazolidine-carboxylic acid isomer in the presence of EDC and DMAP followed by Boc cleaveage. The subsequent sulfonylation in pyridine gave sulfonamides **22b-m**. Carboxylic acid **22f** was further converted into amides **22m** reacting the appropriate amine with CDI in DMF. Alternatively, amidation of intermediate **21** with acids **23a-b** afforded compounds **24a-b**. Sulfonyl chlorides **17** and carboxylic acids **23** were either commercially available or prepared as described in Supporting Information.

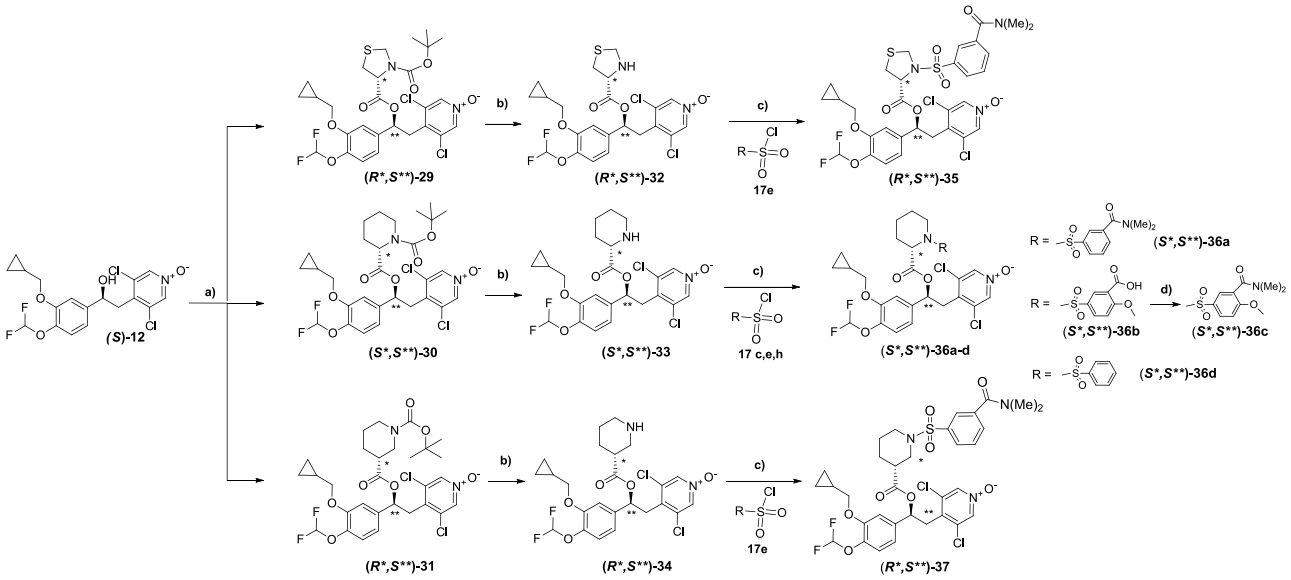
Scheme 4. Preparation of Compounds 28[a]



[a]Reagents and conditions: a) (*S*)-3-(tert-butoxycarbonyl)thiazolidine-2-carboxylic acid, EDC, DMAP, DCM, rt; b) HCl/AcOEt 4 M, rt; c) Py, DCM, rt; d) TFA, rt, overnight.

A synthetic pathway for the preparation of compounds **28** bearing a differently decorated catecholic moiety is summarized in Scheme 4. Enantiomerically pure alcohols (*S*)-**25a-b,** obtained as described in Supporting Information, were coupled with the suitable thiazolidine-carboxylic acid isomer in the presence of EDC and DMAP, the subsequent Boc cleavage led to esters (*S**,*S***)-**27a-b**. Sulfonamides (*S**,*S***)-**28a-b** were finally achieved via sulfonylation as described in Scheme 3. A modified procedure was followed to obtain compound (*S**,*S***)-**28c** which was achieved via acidic cleavage of the catecholic ether of thiazolidinyl ester (*S**,*S***)-**22e,** prepared as described in Scheme 3.

Scheme 5. Preparation of Compounds 35, 36 and 37[a]



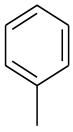
[a]Reagents and conditions: a) (*R*)-3-(tert-butoxycarbonyl)thiazolidine-4-carboxylic acid or (*R*)-1-(tert-butoxycarbonyl)piperidine-3-carboxylic acid or (*S*)-1-(tert-butoxycarbonyl)piperidine-2-carboxylic acid, EDC, DMAP, DMF, rt; b) HCl/Dioxane 4 M, rt; c) Py, rt; d) dimethylamine 2 M in THF, CDI, DMF, rt.

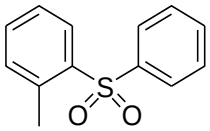
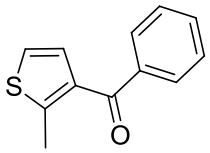
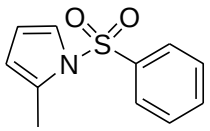
Sulfonamides **35, 36** and **37** were obtained by sulfonylation of the corresponding heterocycloalkylamino intermediates **32, 33, 34** according to Scheme 5. Intermediates **32, 33, 34** were prepared by coupling of (*S*)-**12** with suitable N-Boc heterocycloalkyl carboxylic acids followed by acidic deprotection. (*S**,*S***)-**36b** underwent further amidation with dimethylamine to afford (*S**,*S***)-**36c**.

RESULTS AND DISCUSSION

SAR Analysis.

Table 1. Structure and Inhibitory Activity in Cell-Free and Cell-Based Assays of Aryl Esters

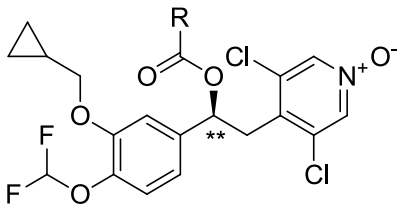
Compd.	R	Cell-free IC_{50} (nM) ±SD[a]
(*S*)-14a[c]		0.072

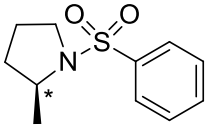
(*S*)-14d		0.323±0.085
(*S*)-14b		0.320±0.094
(*S*)-14c		0.825±0.043

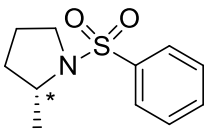
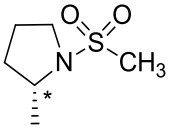
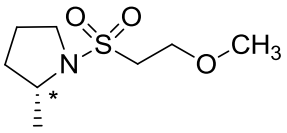
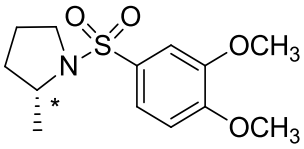
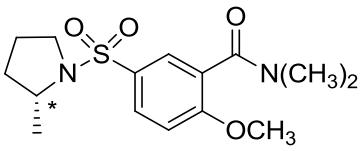
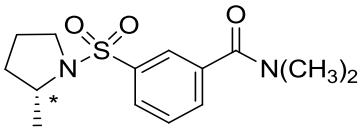
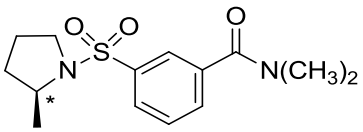
[a] Data are the mean ± SD of three to four experiments performed in duplicate. [b] Data are presented as IC_{50} values followed by their 95% confidence intervals obtained by nonlinear regression. [c]compound (*S*)-**26a** ref. 23

Our initial hypothesis, based on additional available space in the solvent-exposed region of the catalytic binding pocket of PDE4, was confirmed by the potency achieved by the first series of analogues. The phenyl ester (*S*)-**14d** and its thienyl (*S*)-**14b** and pyrrolyl (*S*)-**14c** bioisosteric analogues, carrying a phenyl-sulfonyl or a phenyl-carbonyl substituent in the position adjacent to the ester function, showed limited reduction of PDE4 inhibitory potency compared to unsubstituted derivative (*S*)-**14a**.

Table 2. Structure and inhibitory activity in cell-free and cell-based assays of pyrrolidinyl esters featuring a substituent on the Nitrogen Atom.



Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)
(*R*,S*)-18c**		0.06±0.011	0.4 (0.12 – 1.3)

(*S,*S***)-18c**		0.109±0.045	0.34 (0.130 – 0.900)
(*S,*S***)-18a**		0.5038±0.080	4.2 (1.500 – 12.000)
(*S,*S***)-18b**		0.0442±0.006	0.31 (0.21 – 0.46)
(*S,*S***)-18d**		0.0324±0.027	0.36 (0.260 – 0.510)
(*S,*S***)-19**		0.0351±0.020	0.042 (0.014-0.130)
(*S,*S***)-18e**		0.0378±0.022	0.032 (0.014 – 0.070)
(*R,*S***)-18e**		0.0533±0.027	0.38 (0.200 – 0.700)

[a] Data are the mean ± SD of three to four experiments performed induplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression.

To verify if a subtle geometrical variation in the arrangement of the ester portion could improve the inhibitory potency, our exploration was then focused on the synthetically easily accessible saturated analogues of (*S*)-**14c**. Therefore a series of pyrrolydinyl analogues was examined, in which ring unsaturation led to a heterocycloalkanyl core containing an additional stereogenic centre. Both diastereomers featuring the pyrrolydinyl moiety ((*R**,*S***)-**18c**, (*S**,*S***)-**18c**) were endowed with higher potency than their corresponding aromatic analogue (*S*)-**14c**, irrespective of their stereochemistry (Table 2). We then considered mono or bis substitutions at the *para* and *meta* position of the aryl sulfonyl ring. Concurrently we prepared both diastereomers of the *meta*-dimethylamido derivative to further study the impact of stereochemistry on potency: (*S**,*S***)-**18e** and (*R**,*S***)-**18e**.

They exerted similar potency in the cell-free test but the *R* configuration at the heterocycloalkyl ring caused a 10-fold drop in the cell-based activity. This finding prompted us to select the stereochemical configuration of (*S**,*S***)-**18e** as the one able to provide the best inhibition of LPS-induced TNF-α release in the *in vitro* model of PBMCs. Preparation of (*S**,*S***)-**18d** and (*S**,*S***)-**19** allowed us to infer that, in this series, the *m*-dimethylaminocarbonyl substituent, coupled with *S,S* stereochemistry, is able to enhance by 10-fold the potency in PBMCs. Replacement of the phenyl ring with a methyl group in (*S**,*S***)-**18a** was detrimental, whereas compound (*S**,*S***)-**18b** with a longer methoxyethyl substituent showed interesting PDE4 inhibitory activity.

The potent inhibitor (*S**,*S***)-**18e** was crystallized in the PDE4B catalytic site. Its binding mode is similar to that of roflumilast in the PDB 1XMU crystal structure. Indeed, the catecholic oxygens form two hydrogen bonds with the side chain of Gln443 residue in the hydrophobic pocket of the binding site, while the pyridine N-oxide ring is located in the metal region and forms a hydrogen bond with the backbone NH group of Met347 residue. The phenylsulfonyl-proline fragment is positioned in the solvent-exposed region of the catalytic site and the carbonyl group of the dimethylaminocarbonyl substituent interacts with a water molecule, which is indirectly linked to the water molecules coordinated to the metals (Figure 5). The high PDE4 inhibitory potency of compound (*S**,*S***)-**18b** may be related to its ability to undertake a hydrogen bond with the same water molecule bound to the amide substituent of (*S**,*S***)-**18e**. (Figure 6). The wide surface of the solvent exposed region accommodates structurally different fragments, which in most cases are able to maintain high binding energy values.

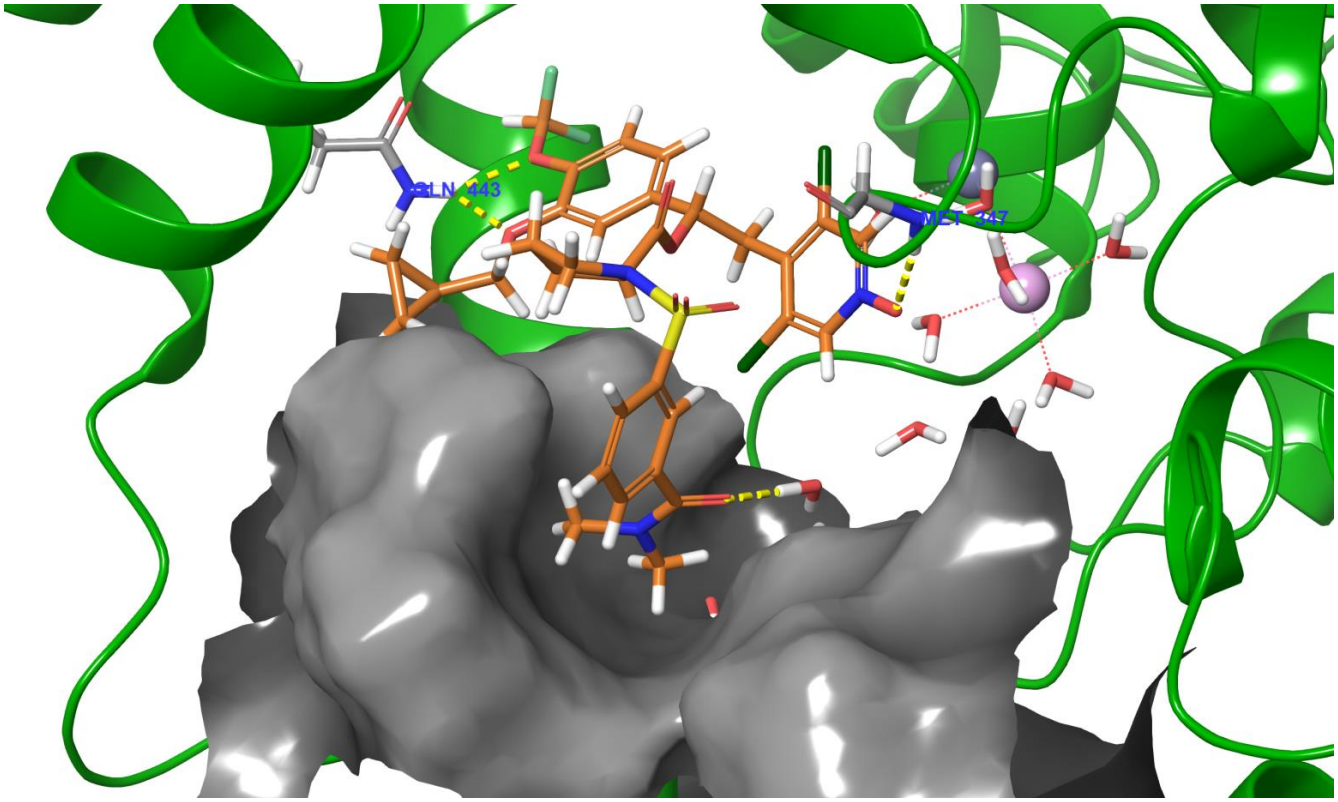


Figure 5. Crystal structure of compound (*S**,*S***)-**18e** bound to the PDE4B catalytic binding site. Hydrogen bonds are represented as yellow dashed lines.

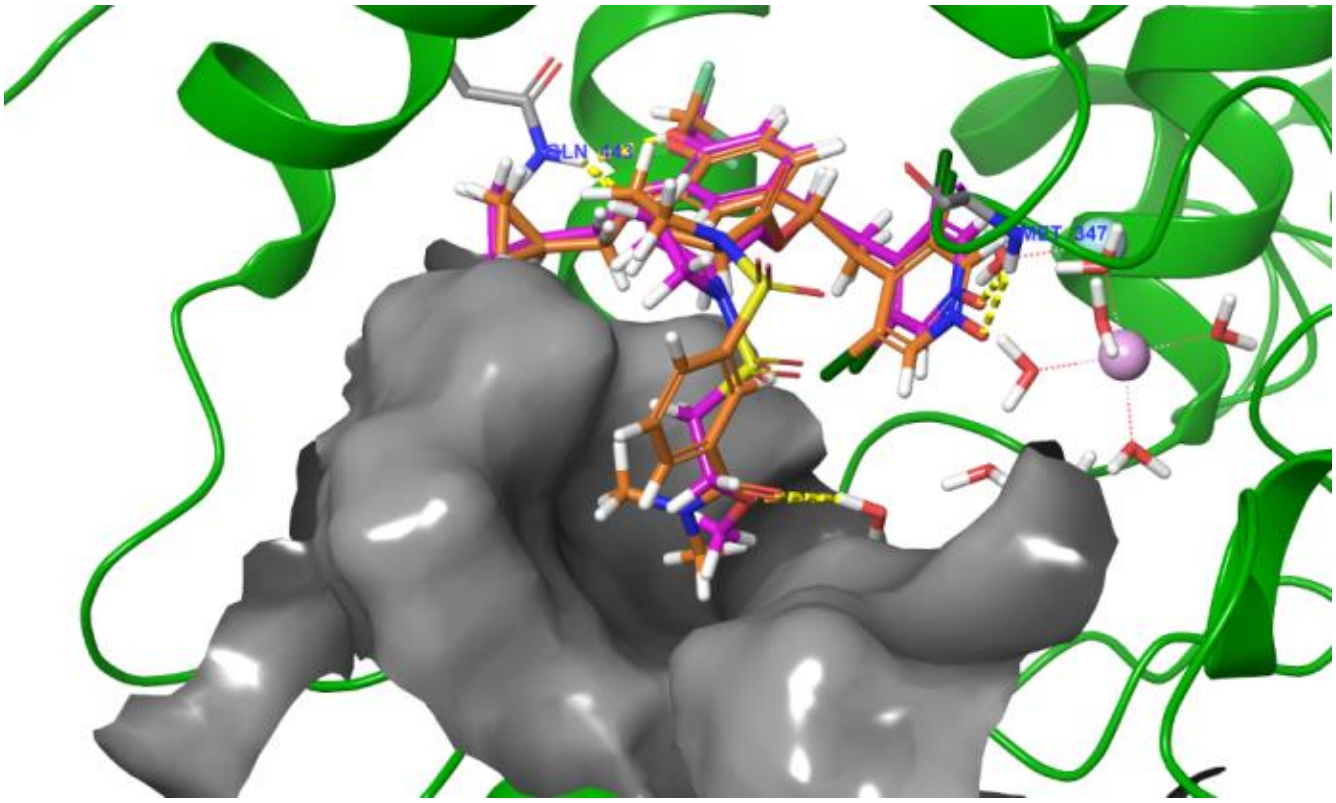
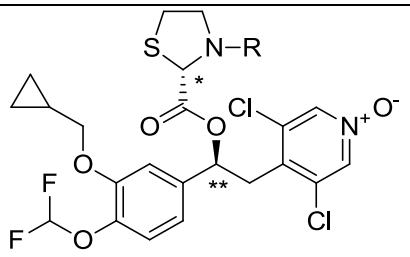
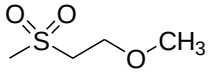
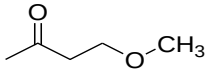
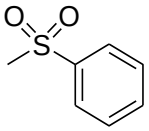
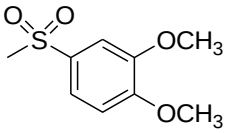
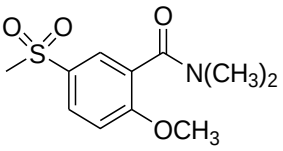
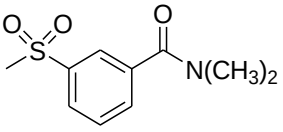
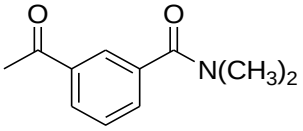


Figure 6. Overlay of the docking binding pose for compound (*S**,*S***)-**18b** (magenta colour) on the structure of (*S**,*S***)-**18e** (orange colour) bound in the PDE4B catalytic site. Hydrogen bonds are represented as yellow dashed lines.

Table 3. Structure and inhibitory activity in cell free and cell based assays of thiazolidinyl esters featuring -a substituent on the Nitrogen Atom.

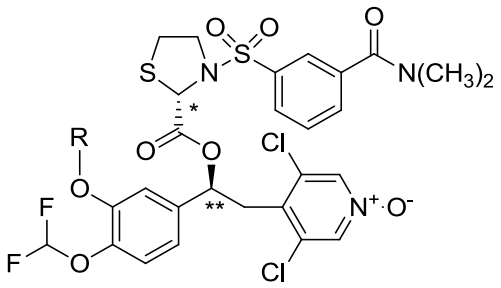


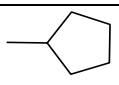
Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)
(*S,*S***)-22b**		0.043±0.02	0.078 (0.049 – 0.120)
(*S,*S***)-24a**		0.028±0.01	0.860 (0.360 – 2.000)
(*S,*S***)-22c**		0.176±0.131	0.260 (0.140-0.460)
(*S,*S***)-22d**		0.041±0.015	0.057 (0.010-0.280)
(*S,*S***)-22m**		0.026±0.010	0.022 (0.011-0.041)
(*S,*S***)-22e**		0.053±0.007	0.019 (0.012 – 0.030)
(*S,*S***)-24b**		nd	0.570(0.150 – 2.270)

[a] Data are the mean ± SD of three to four experiments performed induplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression. Nd: not determined.

To further investigate the class of saturated heterocycles, we explored the SAR of a set of bioisosteric compounds where we incorporated the thiazolidinyl core thus replacing the pyrrolydinyl one (Table 3). An aliphatic sulfonyl substituent was well accepted in (*S**,*S***)-**22b**, increasing the PBMCs potency of this compound by 10-fold with respect to its pyrrolydinyl analogues (*S**,*S***)-**18a** and (*S**,*S***)-**18b**, whereas the aliphatic carboxamide in (*S**,*S***)-**24a** caused a drop in activity in the cell-based assay. Successively an in-depth examination of the role of the N-aryl-sulfonyl ring decorations was carried out. The unsubstitued benzensulfonylamide derivative (*S**,*S***)-**22c** served as baseline compound and showed limited potency. The dimethoxy (*S**,*S***)-**22d** proved to be very active and, with the introduction of the amido groups, the inhibitory potency was slightly further enhanced ((*S**,*S***)-**22m,** (*S**,*S***)-**22e**). Replacement of the N-sulfonylamido with an N-carboxamido substituent as in (*S**,*S***)-**24b** is detrimental for cell-based activity, as already proved by the aliphatic derivative (*S**,*S***)-**24a**.

Table 4. Structure and inhibitory activity in cell free and cell based assays of (*S,*S***)-22e analogues featuring different R at the phenolic Oxygen.**

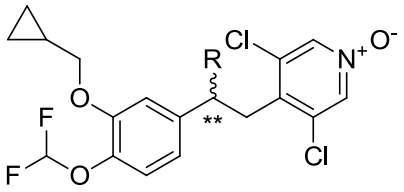


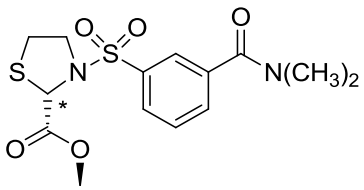
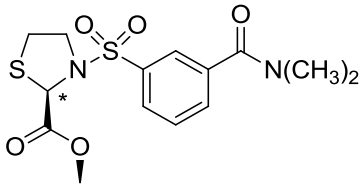
Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)
(*S,*S***)-22e**		0.053±0.007	0.019 (0.010 – 0.030)
(*S,*S***)-28c**	H	0.183±0.119	1.950 (1.310 – 2.890)
(*S,*S***)-28a**	CH_3	0.014±0.0004	0.032 (0.0026 – 0.04)
(*S,*S***)-28b**		0.021±0.023	0.040 (0.023 – 0.070)

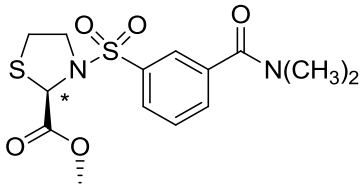
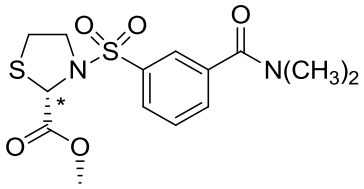
[a] Data are the mean ± SD of three to four experiments performed in duplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression.

(*S**,*S***)-**22e** emerged as one of the most potent compounds in the thiazolidinyl series and was chosen as reference compound to investigate the impact of the alkylation of the catecholic hydroxyl group on the inhibitory activity, as shown in Table 4. The contribution of cyclopropylmethyl group was clearly evident in (*S**,*S***)-**28c**, where O-dealkylation led to a 3-fold less potent derivative in the cell-free assay and 100-fold less potent in the cell-based assay, likely because of limited cell penetration. Conversely, the alkyl moiety replacement in (*S**,*S***)-**28a** and (*S**,*S***)-**28b**, where methyl and cyclopentyl were chosen by analogy with rolipram and cilomilast, respectively, restores the inhibitory activity to the nanomolar range.

Table 5. Structure and inhibitory activity in cell free and cell based assays of the four diastereomer of (*S,*S***)-22e.**



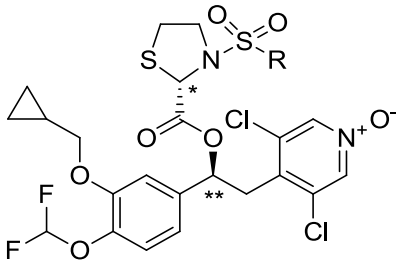
Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)
(*S**,*S***)-22e		0.053±0.007	0.019 (0.012 – 0.030)
(*R**,*S***)-22e		0.047±0.022	0.047 (0.010 – 0.230)

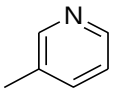
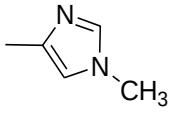
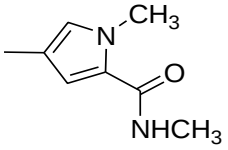
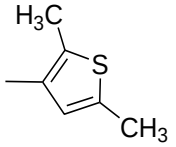
(*R,*R***)-22e**		6.397±1.041	nd
(*S,*R***)-22e**		3.006±0.482	nd

[a] Data are the mean ± SD of three to four experiments performed in duplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression. Nd: not determined.

We subsequently examined the effect of the stereochemical configuration in thiazolidinyl derivatives against activity (Table 5). With this aim the three diastereoisomers of (*S**,*S***)-**22e** were synthesized. (*R**,*S***)-**22e** maintained comparable affinity and biological activity to the ones exerted by its epimer (*S**,*S*****)-22e**. Conversely stereocenter inversion of the alcoholic portion ((*R**,*R***)-**22e**, (*S**,*R***)-**22e**) caused a remarkable loss of potency: (*R**,*R***)-**22e** and (*S**,*R***)-**22e** showed 50 and 150-fold drop of activity compared to their respective enantiomers, thus meaning that there is a stereochemical preference for the interaction with PDE4, confirming previous findings,[23] while the configuration of the asymmetric carbon centre on the thiazolidinyl core is not relevant for the interaction with PDE4. Accordingly in the thiazolidinyl series the *R* configuration at the thiazolidine carbon seems to affect less PBMC potency than in the pyrrolidinyl series.

Table 6. Structure and inhibitory activity in cell free and cell based assays of thiazolidinyl esters featuring a heteroarylsulfonyl Group on the Nitrogen Atom.

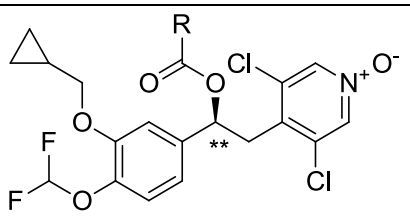


Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)
(*S**,*S***)-**22g**		0.061±0.016	0.070 (0.040-0.120)
(*S**,*S***)-**22h**		0.020±0.009	0.028 (0.009-0.085)
(*S**,*S***)-**22i**		0.025±0.006	0.030 (0.020-0.050)
(*S**,*S***)-**22l**		0.176±0.080	0.170 (0.080-0.360)

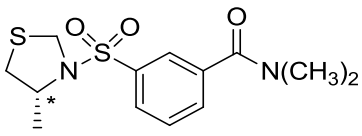
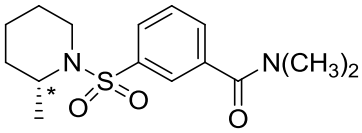
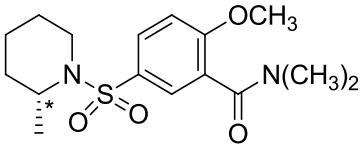
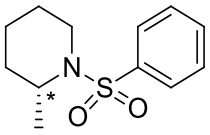
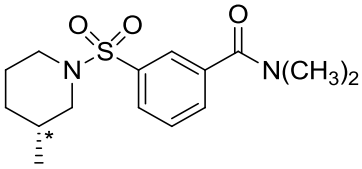
[a] Data are the mean ± SD of three to four experiments performed induplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression.

In the thiazolidinyl series the replacement of the terminal phenyl ring with a set of heteroaryl bioisosteres was considered, as shown in Table 6. The introduction of pyridine in (*S**,*S***)-**22g** and five-member heteroaryls in (*S**,*S***)-**22h** and (*S**,*S***)-**22i** led to compounds endowed with higher potency than the naked phenyl (*S**,*S***)-**22c**. In contrast to what observed for the N-phenylsulfonyl series, no further contribution to the of potency was achieved by heteroaryl ring decoration in (*S**,*S***)-**22i**. The decrease of potency for thienyl derivative (*S**,*S***)-**22l** observed in both assays may be related to the steric hindrance exerted by the methyl groups.

Table 7. Structure and inhibitory activity in cell free and cell based assays of heterocycloalkyl esters featuring an arylsulfonyl Substituent on the Nitrogen Atom.



Compd.	R	Cell-free IC_{50} (nM)	PBMCs IC_{50} (nM)

(*R,*S***)-35**		0.055±0.032	0.035 (0.017-0.073)
(*S,*S***)-36a**		0.056±0.013	0.012 (0.003-0.050)
(*S,*S***)-36c**		0.031±0.0004	0.015 (0.005-0.050)
(*S,*S***)-36d**		0.094±0.041	0.203 (0.100-0.540)
(*R,*S***)-37**		0.031±0.011	0.120 (0.030-0.480)

[a] Data are the mean ± SD of three to four experiments performed in duplicate. [b] Data are presented as IC50 values followed by their 95%confidence intervals obtained by nonlinear regression.

Finally, as part of our core scaffold modifications, we extended our investigations to additional saturated cyclic systems which deserved a more extensive SAR analysis. We therefore performed a heterocycloalkyl swap introducing a small subset of 5- and 6-atoms cyclic bioisosteres, while maintaining almost unmodified the N-arylsulfonyl group (Table 7). No substantial changes in terms of potency were observed for the thioprolinyl analogue in (*R**,*S***)-**35** and piperidines (*S**,*S***)-**36a** and (*S**,*S***)-**36c**, suggesting that neither the absence nor the position of the sulfur atom seems to affect their biological activity. Preparation of (*S**,*S***)-**36d** confirmed that the contribution of the amide decoration on the phenyl ring is fundamental to achieve the wanted potency range, especially in the cellular system. Comparable activity to 2-substitued analogues is displayed by compounds (*R**,*S***)-**37** where the nitrogen was moved from position 2 to 3.

***In vitro* ADME Characterization.**

Table 8. ADME profile of selected compounds.

Compd.	CACO-2 permeability			hPPB[b]	Hepatic microsomes[c]	Hepatocytes[d]	hLung S9 stability	hPlasma stability
	AB (nm/sec)	BA (nm/sec)	Caco-2 perm. Level[a]	bound (%)	Clearance (μL/min/mg)	Clearance (μL/min/10^6 cell)	60 min remain. (%)	60 min remain. (%)
(*S**,*S***)-18e	4.8	101.8	Low	99.65	590.56	95.50	71.1	108.40
(*S**,*S***)-19	5	124.9	Low	99.68	365.35	56.67	71.6	101.06
(*S**,*S***)-22m	2.3	76.7	Low	99.83	360.36	47.56	71.2	98.78
(S*,S**)-22e	4.2	31.8	Low	99.79	287.41	82.45	70.2	97.96
(*S**,*S***)-22d	2.5	8.2	Low	99.60	200.19	53.76	50.7	100.60
(*S**,*S***)-22i	12.6	182.7	Low/Medium	99.84	202.10	47.62	63.5	100.25
(*S**,*S***)-22h	2.2	39.5	Low	98.50	281.17	66.86	77.7	101.10
(*R**,*S***)-35	28.0	44.6	Medium	99.83	267.87	25.91	52.1	93.99
(*S**,*S***)-36a	217.4	734	High	99.88	362.74	64.57	72.5	104.50
(*S**,*S***)-36c	131.8	684.3	High	99.77	243.50	33.16	59.7	88.22
(*S*)-32k[e]	nd	nd	nd	nd	35.22	nd	nd	nd
(*S*)-26p[e]	nd	nd	nd	nd	50.94	nd	nd	nd
(*S*)-38e[e]	nd	nd	nd	nd	71.24	nd	nd	nd

[a] General absorption classification for permeability values: <10 nm/s, low level; 10-50 nm/s, medium level; >50 nm/s, high level. [b] hPPB = human plasma protein binding. [c] Compounds were incubated with human liver microsomes at 1 μM for 60 min. [d] Compounds were incubated with human hepatocytes at 1 μM for 90 min. [e] Ref.23. Nd = not determined.

Through a funnel-like process, a subset of 10 compounds displaying excellent inhibitory activity on PDE4 and in the PBMC assay was chosen for further characterization and their *in vitro* ADME profiles are captured in Table 8. The selection was based on the criteria that both IC_{50} values on the free enzyme and in cell-based test should be less than or equal to 0.05 nM. Almost all compounds met the desired criteria to be progressed further as candidates for inhaled administration, showing i) adequate metabolic stability in human lung S9 preparation, ii) low *in vitro* permeability in the Caco2 assay, except for the piperidinyl-derivatives (*S**,*S***)-**36a** and (*S**,*S***)-**36c** that were susceptible to high efflux, and iii)

human plasma protein binding values higher than 99%, excluding (*S**,*S***)-**22h**. The aforementioned ADME profile strongly reduces the likelihood that the free fraction in the systemic circulation, absorbed through the lung compartment or by the GI tract as a consequence of the swallowed fraction, could have an impact on araising unwanted side effects. Additionally, the selected compounds were stable in human plasma and showed favorable intrinsic Clearance (CL_{int}) values, allowing us to infer that such metabolic liability is likely to translate into the desired sustained hepatic turnover, thus furnishing the sought-after prerogatives for a minimized systemic exposure. A comparison between *in vitro* Clearance data for the heterocycloalkyl series herein described and the benzoic ester series, whose values of Clearance in hepatic microsomes for three representative compounds (*S*)-**32k**, (*S*)-**26p** and (*S*)-**38e**[23] are listed in Table 8, clearly highlighted that we were able to reach the goal of obtaining effectively turned over compounds while maintaining potency.

Since physico-chemical properties are critical for an inhalation drug, the choice to progress compounds (*S**,*S***)-**18e** and (*S**,*S***)-**22e** to further *in vivo* investigations relied on their ability to better perform in solid state studies. In particular, highly crystalline, anhydrous, not hygroscopic forms were identified for both (*S**,*S***)-**18e** and (*S**,*S***)-**22e** by means of proper crystallisation screening and experiments (data not shown). Both compounds were successfully micronised by jet mill with acceptable yields, good physico-chemical integrity and allowing to obtain a particle size suitable for inhalation. They were then blended with lactose at strengths suitable for animal administration.

In Vitro* and *In Vivo* Characterization of Compounds (*S**,*S)-18e and (*S**,*S***)-22e.**

(*S**,*S***)-**18e** and (*S**,*S***)-**22e**, administered as dry-powder formulation 2 hours before the aerosolized ovalbumin (OVA) challenge, were screened in a dose-response study in Brown Norway rat OVA-induced airway inflammation model via intratracheal (IT) dosing (Figure 7). Both compounds displayed a dose-dependent pattern and markedly inhibited inflammatory cells infiltration in the bronchoalveolar lavage (BAL), exerting a 50% inhibition of eosinophils recruitment at 0.1 μmol/kg, while at 1 μmol/kg (*S**,*S***)-**22e** produced a higher efficacy in reducing cell infiltration than (*S**,*S***)-**18e**. Estimated values of ED_{50} of 0.10 and 0.04 μmol/kg for (*S**,*S***)-**18e** and (*S**,*S***)-**22e**, respectively, were calculated from the dose-response curves of inhibition of eosinophils recruitment.

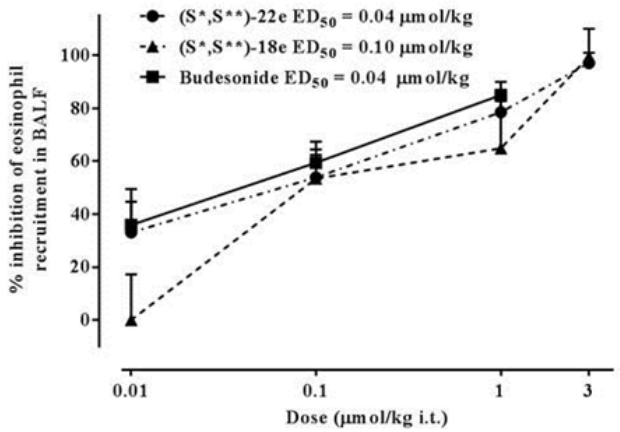
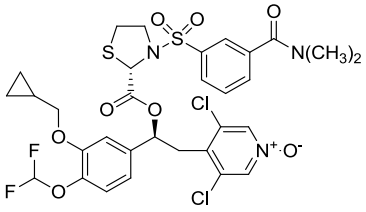
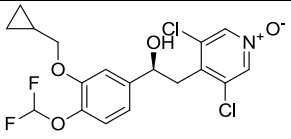
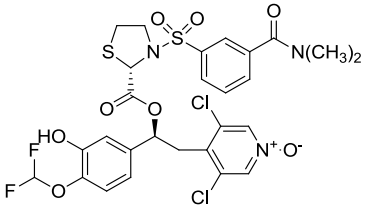
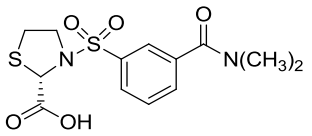
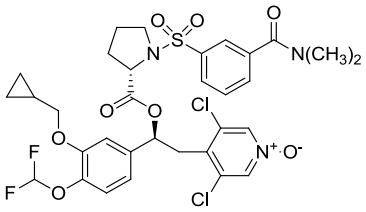
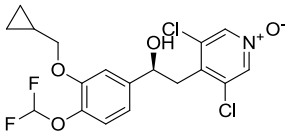
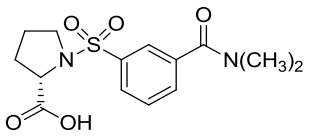


Figure 7. Dose-dependent inhibition of OVA-induced recruitment of eosinophils in BALF (broncho alveolar lavage fluid) of rats treated with compound (*S*,S***)-**18e** and (*S*,S***)-**22e**. The reference antinflammatory compound budesonide is reported for comparison.

Table 9. Structure and Inhibitory Activity in Cell-Based Assay of Metabolites obtained after 60 min incubation of (*S*,S*)-22e (compounds 38-40) and (*S*,S***)-18e (compounds 32, 35) with human hepatocytes at a concentration of 10 μM.**

Compd.	% of total drug-related material	Structure	PBMC IC_{50} nM [CI]
(*S*,S*)-22e**	51		0.019 [0.003-0.020]
38	12		479.6 [288.1-798.7]
39	11		1.95 [1.31-2.89]

40			>1µM
(*S,*S***)-18e**	78		0.032
38	9		479.6 [288.1-798.7]
41			>1µM

The *in vitro* metabolite profiles of (*S**,*S***)-**18e** and (*S**,*S***)-**22e** were studied using human hepatocytes and the main metabolites detected are depicted in Table 9. For (*S**,*S***)-**18e** two metabolites were identified in a percentage higher than 10% (**38** and **39**) and assessed in the PBMCs assay, exhibiting a 50000-fold and a 300-fold lower potency than the parent molecule, respectively. Considering that alcohol **38** stems from parent compound ester hydrolysis, the carboxylic acid fragment **40**, whose presence in vivo in an amount comparable to the alcoholic fragment can be arguably assumed, was prepared and tested as well, proving to be inactive with an IC_{50} lower than 1 µM. Comparably to (*S**,*S***)-**22e**, compound (*S**,*S***)-**18e** generates a substantial amount of alcohol **38**. Its corresponding acid **41** showed to be unable to inhibit TNF-α release in our cellular model (IC_{50} > 1 µM).

Table 10. Pharmacokinetic parameters of (*S,*S***)-18e and (*S**,*S***)-22e determined from lung tissues following IT administration as dry powder in rats at 1 µmole/kg.**

Parameter	**(*S**,*S***)-22e**	**(*S**,*S***)-18e**

C_{max} (nmol/g)	16.06	16.24
T_{max} (h)	0.250	0.083
T_{last} (h)	24	24
$T_{1/2}$ (h)	4.39	5.18
AUC_{last} (hr·nmol/g)	65.27	49.65
MRT_{last}(h)	5.56	4.52

Table 11. Pharmacokinetic parameters of (*S*,S*)-18e and (*S*,S***)-22e determined from plasma following IT administration as dry powder in rats at 1 µmole/kg.**

Parameter	**(*S*,S***)-22e**	**(*S*,S***)-18e**
C_{max} (pmol/mL)	9.80	20.65
T_{max} (h)	0.083	0.083
T_{last} (h)	8	8
$T_{1/2}$ (h)	nc	nc
AUC_{last} (hr·pmol/mL)	10.38	33.27
MRT_{last}(h)	1.33	1.08

(*S*,S***)-**18e** and (*S*,S***)-**22e** exhibited ideal in vivo pharmacokinetic profiles for an inhaled agent. Lung levels, plasma levels and pharmacokinetic parameters following IT treatment with (*S*,S***)-**18e** and (*S*,S***)-**22e** in Sprague-Dawley rats are shown in Figure 8, Table 10 and Table 11. (*S*,S***)-**18e** and (*S*,S***)-**22e** showed a lung half-life of around 5 h which is in line with the corresponding mean residence time (MRT) (Table 10). Both compounds displayed prolonged lung retention (0.128 and 0.220 nmol/g concentration at 24 hours, for (*S*,S***)-**18e** and (*S*,S***)-**22e** , respectively), indicating that both molecules could deliver a long-lasting protective effect. Additionally, being optimized for inhalation, a key pharmacokinetic measure of success was having a high exposure ratio, at the same T_{last}, between lung and plasma. In fact the exposures calculated until 8 hours clearly indicated that (*S*,S***)-**18e** and (*S*,S***)-**22e** were mainly distributed in lung compared to plasma (2261 and 5152-fold ratio for (*S*,S***)-**18e** and (*S*,S***)-**22e**, respectively**)** (Tables 10 and 11).

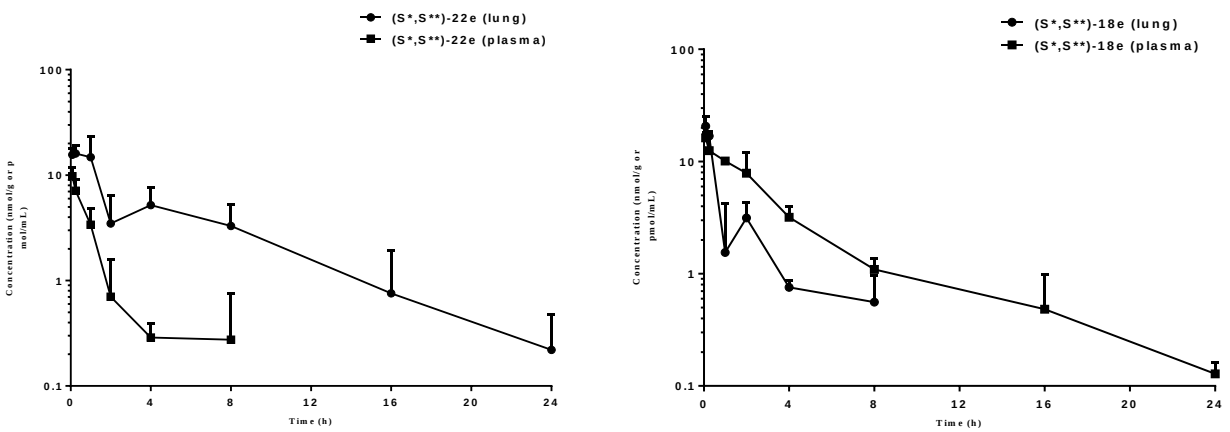


Figure 8. Lung levels (nmol/g) and plasma levels (pmol/ml) of (*S**,*S***)-**22e** (left) and (*S**,*S***)-**18e** (right) after IT administration as dry powder in Sprague-Dawley rats at 1 μmole/kg.

Table 12. Isoform Selectivity Profile of (*S,*S***)-18e and (*S**,*S***)-22e**

Target	*(S*,S**)* 18e[a]	*(S*,S**)* 22e[a]
hPDE1A	25.6 μM	7.40 μM
hPDE2A	>30 μM	>30 μM
hPDE3A	>30 μM	6.21 μM
hPDE4B	8.81×10^{-5} μM	2.78×10^{-5} μM
hPDE5A	13.9 μM	4.61 μM
Bov PDE6	>30 μM	5.78 μM
hPDE7A	3.46 μM	0.66 μM
hPDE8A1	17.0 μM	3.56 μM
hPDE9A2	>30 μM	>30 μM
hPDE10A2	4.03 μM	0.81 μM

hPDE11A4	>30 μM	>30 μM

[a] Data are given as mean IC_{50} values (μmol/l) calculated from concentration-inhibition curves by nonlinear regression analysis from 2 independent experiments

(*S*,S***)-**18e** and (*S*,S***)-**22e** were subsequently evaluated in safety assessment studies. There was no evidence of inhibition of hERG potassium channels current at a concentration of 1 μM (data not shown). Both (*S*,S***)-**18e** and (*S*,S***)-**22e** proved to be highly selective inhibitors for PDE4 (>10,000 versus PDE1, 3, 5, 7, 8, 10 and > 100,000 vs PDE2, 6, 9, 11) (Table 12). The propensity of (*S**,*S***)-**18e** and (*S**,*S***)-**22e** to bind biological target proteins unrelated to PDE family enzymes was evaluated by interrogating a panel of 400 key receptor, enzyme, and ion channel proteins at 1μM concentration (Eurofins selectivity panels). The panel identified only three hits showing > 50% binding at the concentration of 1 μM for (*S*,S***)-**18e** (rat Acyl CoA-Cholesterol Acyltransferase (50%), human CYP3A4 (73%) and human monoamine oxidase A (76%)), and five hits showing > 50% binding at the concentration of 1 μM for (*S*,S***)-**22e** (human CYP2C19 (69%), human CYP3A4 (84%), human monoamine oxidase A (94%), human UDP glucuronyltransferase 1A1 e rat imidazoline I2 receptor (60%)).

CONCLUSIONS

Following a structure-based design we identified a novel class of PDE4 inhibitors endowed with potent anti-inflammatory properties and suitable for topical pulmonary administration. Starting from a previously described series of benzoic esters, we explored the chemical space in the solvent-exposed region of the PDE4 catalytic pocket, focusing our efforts on new structural classes of *ortho*-substituted compounds. Our SAR efforts led to the discovery of pyrrolidinyl- and thiazolidinyl- esters as potent *in vitro* PDE4 inhibitors. The optimal *in vitro* ADME profiles (high plasma protein binding, high CL_{int}, low Caco2 permeability level, high lung S9 stability) together with their ability to better perform in solid state studies (highly crystalline, anhydrous, not hygroscopic) led to the selection of compounds (*S*,S***)-**18e** and (*S*,S***)-**22e** for progression to in vivo preclinical studies. Both compounds effectively counteracted OVA-induced airway inflammation in rat upon IT administration as dry

powders displaying a lung pharmacokinetic profile consistent with a long-lasting effect. For both compounds the risk of potential systemic side-effects is minimised by the extensive liver metabolism to inactive metabolites, by their low plasmatic levels when administered intratracheally and by the excellent selectivity profile when counterscreened towards more than 400 off-targets, including other human PDE isoforms. (*S**,*S***)-**18e** and (*S**,*S***)-**22** were therefore identified as compounds from these series having the potential to provide clinical benefits in conditions associated with pulmonary inflammation, including asthma and COPD.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded on a Bruker ARX 300 (300 MHz) spectrometer or on a Varian AS400 (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, quadrature digital detection unit with transmitter offset frequency shift. Chemical shifts are reported as δ downfield in parts per million (ppm) and referenced to tetramethylsilane (TMS) as the internal standard in the ^{1}H 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, nd=not determined). The pulse programs were taken from the Varian and Bruker software libraries.

UV Purity and m/z of compounds were assessed by ESI+ LC-MS analysis, performed on an Alliance Waters HPLC Kinetex 2.6u C18 100A 100 x 4.6 mm column with Waters Quattro Micro API equipped with a UV detector set at 245 and 254 nm (Method A), or on an Acquity Waters UPLC Kinetex 1.7u XB-C18 100A 100 x 2.1 mm (Phenomenex) column with Waters ZQ equipped with a UV detector set at 254 nm (Method B) or on an Acquity Waters UPLC BEH C18 1.7 µm 50x2.1 mm column with Waters ZQ interfaced with 2996 PDA detector (Method C) or on an Acquity UPLC Kinetex C18 1.7 um 50x2.1 mm (Phenomenex) column with ZQ interfaced with 2996 PDA detector (Method D). All the compounds tested in biological assays have purity >95%.

Specific rotation of compounds was measured with a Polarimeter Perkin Elmer (model 241 or 341) at sodium D-line (589 nm), 25 °C, 1 dm path lenght.

Diastereoisomeric ratio by LC/UV/MS, when indicated, is estimated to be affected by an

experimental error of ± 1%. Alternatively diastereoisomeric ratio is determined by ^{1}H NMR and it is estimated to be >95:5 when a single diastereoisomer was detected using NMR analysis.

Preparative HPLC purifications were performed using the following methods:

Method 1): Waters Corporation purification system equipped with a XTerra Prep MS C18 Column (5 µm, 19 x 150 mm, Waters), 11 minutes gradient of 0-100% solvent B, where solvent A is water:MeCN:HCOOH 95:5:0.05 and solvent B is water:MeCN:HCOOH 5:95:0.05;

Method 2): Waters Corporation purification system equipped with a Waters Symmetry Prep C18 17um 19x300, 30 minutes gradient of 5-100% solvent B, where solvent A is 90% H_2O, 10% acetonitrile, 0.05% TFA; and solvent B is 10% H_2O, 90% acetonitrile, 0.05% TFA;

Reactions were monitored by TLC using 0.25 mm Merck silica gel plates (60 F254) or LC/MS analysis; column chromatography 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.

(*S*)-4-(2-(3-benzoylthiophene-2-carbonyloxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-oxide (*S*)-14b.

(*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-hydroxyethyl)pyridine-1-oxide (*S*)-**12** (100 mg, 0.24 mmol), 5-benzoylthiophene-2-carboxylic acid (**13b**, 111mg, 0.48 mmol), EDC (137 mg, 0.71 mmol) and DMAP (35 mg, 0.29 mmol) were dissolved in DMF (1.5 mL) at rt for 5 h; the reaction is quenched with H_2O the precipitate is filtered, washed with H_2O, dissolved in ethyl acetate and washed with HCl 1N, Na_2CO_3 saturated solution and brine, dried over Na_2SO_4 and evaporated under vacuum; The crude was purified by preparative HPLC (Method 1) condition to give 80 mg of a beige solid (0.126 mmol, yield 53%). *m/z* 634.5 [MH]+; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.51 (s, 2 H), 8.12 (d, *J*=4.9 Hz, 1 H), 7.64 - 7.61 (m, 3 H), 7.45 - 7.57 (m, 2 H), 7.33 (d, *J*=1.0 Hz, 1 H), 7.08 - 7.14 (m, 1 H), 7.05 (t, *J*=75.0 Hz, 1 H), 6.93 - 7.00 (m, 1 H), 6.74 - 6.81 (m, 1 H), 5.91 - 6.03 (m, 1 H), 3.86 (d, *J*=7.1 Hz, 2 H), 3.26 - 3.31 (m, 1 H), 3.01 - 3.13 (m, 1 H), 1.14 - 1.29 (m, 1 H), 0.49 - 0.64 (m, 2 H), 0.24 - 0.41 (m, 2 H).

(*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(1-(phenylsulfonyl)-1H-pyrrole-2-carbonyloxy)ethyl)pyridine 1-oxide (*S*)-14c.

A mixture of crude 1-(phenylsulfonyl)-1H-pyrrole-2-carboxylic acid (**13c**, 234 mg, purity 30%, approximately 0.28 mmol, obtained as described in Supporting Information), (*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-hydroxyethyl)pyridine 1-oxide (*S*)-**12** (117 mg, 0.279 mmol), EDC (161 mg, 0.838 mmol) and DMAP (34.1 mg, 0.279 mmol) in DCM (15 mL) was stirred at rt for 5 h. The mixture was diluted with DCM and washed with 0.5N HCl, sat. Na_2CO_3 and finally with brine; the organic phase was dried over sodium sulfate and the solvent removed under vacuum. The crude was purified by preparative HPLC (Method 2) to afford 45 mg of (*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(1-(phenylsulfonyl)-1H-pyrrole-2-carbonyloxy)ethyl)pyridine 1-oxide (0.069 mmol, 25% yield). *m/z* 653.07 $[MH]^+$, [αD] = -25.83 (c 0.6, DCM). 1H NMR (300 MHz, DMSO-d_6) δ ppm 8.47 (s, 2 H), 7.87 (dd, 1 H), 7.78 - 7.85 (m, 2 H), 7.68 - 7.78 (m, 1 H), 7.51 - 7.64 (m, 2 H), 7.28 (dd, 1 H), 7.16 (d, 1 H), 7.11 (d, 1 H), 6.98 (dd, 1 H), 7.06 (t, 1 H), 6.52 (t, 1 H), 6.01 (dd, 1 H), 3.89 (d, 2 H), 3.44 (dd, 1 H), 3.22 (dd, 1 H), 1.09 - 1.30 (m, 1 H), 0.47 - 0.63 (m, 2 H), 0.26 - 0.41 (m, 2 H).

(*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-(phenylsulfonyl)benzoyloxy)ethyl)pyridine 1-oxide (*S*)-14d.

(*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-hydroxyethyl)pyridine 1-oxide (*S*)-**12** (456 mg, 1.086 mmol), 2-(phenylthio)benzoic acid (**13d**, 300 mg, 1.303 mmol, obtained as described in Supporting Information), EDC (312 mg, 1.628 mmol), DMAP (265 mg, 2.171 mmol) were dissolved in dry DCM (12 mL) and the mixture was stirred at r.t. overnight. Thus, the mixture was treated with HCl 1M (30 mL) and extracted with CH_2Cl_2 (2x 100 mL). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude residue (500 mg) that was purified by flash chromatography on silica gel eluting with DCM/MeOH from 100% DCM to 97/3 DCM/MeOH, obtaining (*S*)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-(phenylthio)benzoyloxy)ethyl)pyridine 1-oxide (380 mg, 0.601 mmol, 55.3 % yield) as a white solid. *m/z* 632.02 $[MH]^+$. (*S*)-3,5-dichloro-4-(2-

(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(2-(phenylthio)benzoyloxy)ethyl)pyridine 1-oxide (340 mg, 0.538 mmol) was dissolved in dry DCM (8 mL). The solution was cooled at 0 °C and mCPBA (301 mg, 1.344 mmol) was added and the mixture was kept at rt for two days. The mixture was washed several times with sat. $NaHCO_3$, the organic phase was dried over sodium sulfate and the solvent was removed to afford 326 mg of crude. This crude was purified by flash chromatography on silica gel eluting with DCM/MeOH 97/3 to afford 220 mg of the final compound (*S*)-**14d** (0.33 mmol, 62% yield). *m/z* 664.02 $[MH]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 8.05 - 8.15 (m, 1 H), 7.73 - 7.89 (m, 4 H), 7.62 - 7.72 (m, 1 H), 7.49 - 7.62 (m, 3 H), 7.21 (d, 1 H), 7.19 (d, 1 H), 7.06 (dd, 1 H), 7.09 (t, 1 H), 6.22 (dd, 1 H), 3.92 (d, 2 H), 3.62 (dd, 1 H), 3.32 (dd, 1 H), 1.09 - 1.29 (m, 1 H), 0.48 - 0.64 (m, 2 H), 0.28 - 0.42 (m, 2 H).

Procedure for the preparation of sulfonamides 18. *Synthesis of 3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-1-(methylsulfonyl)pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide, (S*,S**)-**18a***

3,5-Dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-pyrrolidine-2-carbonyloxy)ethyl)-pyridine 1-oxide (*S**,*S***)-**16** (50 mg, 0.1 mmol) was dissolved in pyridine (0.5 mL) and DCM (2 mL); methanesulfonyl chloride **17a** (0.5 mL, 6.5 mmol) was added, and the reaction was stirred at rt for 2 h to achieve completion. The reaction mixture was diluted with aqueous HCl 1N, and extracted with ethyl acetate. The organic phase dried over Na_2SO_4 and concentrated under vacuum to give 20 mg of (*S**,*S***)-**18a** as a white solid (0.03 mmol, 30% yield). *m/z* 596.44 $[MH]^+$, t_R = 5.68, Diastereomeric Ratio= 85:15 (method A); ^{1}H NMR (400 MHz, acetone-d_6) δ ppm 8.26 (s, 2 H), 7.14 - 7.28 (m, 2 H), 7.01 - 7.09 (m, 1 H), 6.92 (t, *J*=75.0 Hz, 1 H), 6.13 (dd, *J*=9.48, 4.63 Hz, 1 H), 4.38 (dd, *J*=8.60, 3.75 Hz, 1 H), 3.99 (d, *J*=6.62 Hz, 3 H), 3.45 (t, *J*=6.39 Hz, 3 H), 3.29 - 3.38 (m, 1 H), 2.88 (s, 3 H), 2.27 - 2.41 (m, 1 H), 1.76 - 2.00 (m, 3 H), 1.29 (d, *J*=6.62 Hz, 1 H), 0.61 (d, *J*=6.62 Hz, 2 H), 0.39 (d, *J*=3.97 Hz, 2 H).

Sulfonamides **18b-f** were prepared following the same procedure starting from corresponding enantiomerically pure pyrrolidine **16** and sulfonyl chloride **17.**

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-1-(2-methoxyethylsulfonyl)pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (S,S**)-**18b.***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**16** (200 mg, 0.361 mmol), pyridine (5mL), 2-methoxyethanesulfonyl chloride **17b** (86 mg, 0.542 mmol), rt for 3h, purification by preparative HPLC (Method 1), 37 mg of a pale yellow solid (0.06 mmol, 17% yield). *m/z* 639.14 $[MH]^+$, t_R = 3.67 min, Diastereomeric Ratio ≥ 99:1 (method C); $[\alpha_D]$ = -23.88 (c = 0.5; DCM). ^{1}H NMR (300 MHz, acetone-d_6) δ ppm 8.54 (s, 2 H), 7.18 (d, 1 H), 7.12 (d, 1 H), 6.97 (dd, 1 H), 7.07 (t, 1 H), 5.99 (dd, 1 H), 4.30 (dd, 1 H), 3.92 (d, 2 H), 3.58 - 3.68 (m, 2 H), 3.31 - 3.50 (m, 5 H), 3.29 (s, 3 H), 3.24 (dd, 1 H), 2.07 - 2.25 (m, 1 H), 1.59 - 2.01 (m, 3 H), 1.05 - 1.36 (m, 1 H), 0.46 - 0.69 (m, 2 H), 0.21 - 0.46 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-1-(phenylsulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**18c.***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**16** (50 mg, 0.1 mmol), benzenesulfonyl chloride **17c** (28 mg, 0.16 mmol), pyridine (2 mL), rt for 4h, 40 mg of the desired product (0.06 mmol, 63% yield). *m/z* 657.3 $[MH]^+$, t_R = 4.09 min, Diastereomeric Ratio= 2:98 (method B); ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.61 (s, 2 H), 7.69 - 7.82 (m, 3 H), 7.60 - 7.68 (m, 2 H), 7.19 (d, *J*=7.9 Hz, 1 H), 7.14 (d, *J*=1.8 Hz, 1 H), 7.08 (t, *J*=75.0 Hz, 1 H), 6.98 (dd, *J*=8.4, 1.8 Hz, 1 H), 6.02 (dd, *J*=9.7, 4.4 Hz, 1 H), 4.13 (dd, *J*=8.6, 4.2 Hz, 1 H), 3.92 (d, *J*=6.6 Hz, 2 H), 3.47 (dd, *J*=14.1, 9.7 Hz, 1 H), 3.33 - 3.41 (m, 1 H), 3.26 (dd, *J*=14.1, 4.4 Hz, 1 H), 3.16 (ddd, *J*=9.8, 6.8, 6.7 Hz, 1 H), 1.83 - 1.98 (m, 1 H), 1.58 - 1.74 (m, 2 H), 1.41 - 1.57 (m, 1 H), 1.14 - 1.27 (m, 1 H), 0.48 - 0.63 (m, 2 H), 0.26 - 0.40 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-1-(phenylsulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (R,S**)-**18c.***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide, (*R**,*S***)-**16** (150 mg, 0.29 mmol), pyridine (1.8 mL),

benzenesulfonyl chloride **17c** (61.5 mg, 0.348 mmol), rt for 1h, Purification by preparative HPLC (method 1), 53 mg of the desired product (0.081 mmol, 27.8 % yield). *m/z* 657.3 $[MH]^+$, t_R = 4.03 min, Diastereomeric Ratio= 97:3 (method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.74 - 7.83 (m, 2 H), 7.66 - 7.74 (m, 1 H), 7.57 - 7.65 (m, 2 H), 7.21 (d, *J*=8.4 Hz, 1 H), 7.14 (d, *J*=l.86 Hz, 1 H), 7.09 (t, 1=75.0 Hz, 1 H), 7.00 (dd, *J*=8.4, 1.8 Hz, 1 H), 5.98 (dd, *J*=9.5, 4.6 Hz, 1 H), 4.14 (dd, *J*=8.6, 4.6 Hz, 1 H), 3.86 - 4.01 (m, 2 H), 3.37 - 3.50 (m, 2 H), 3.27 (dd, *J*=l4.l, 4.4 Hz, 1 H), 3.14 (ddd, *J*=9.8, 7.1, 6.9 Hz, 1 H), 1.45 - 1.98 (m, 4 H), 1.14 - 1.30 (m, 1 H), 0.48 - 0.63 (m, 2 H), 0.25 - 0.39 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-1-((3,4-dimethoxyphenyl)sulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**18d.***

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**16** (84 mg, 0.16 mmol), 3,4-dimethoxybenzene-1-sulfonyl chloride **17d** (38.4 mg, 0.16 mmol), pyridine (1.5 mL), rt for 3h, 20 mg of the desired product (0.028 mmol, 17 % yield). *m/z* 717.2 $[MH]^+$. t_R = 6.97 min, Diastereomeric Ratio= 99:1 (method A); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.30 (s, 2 H), 7.39 - 7.48 (m, 1 H), 7.25 - 7.35 (m, 2 H), 7.17 (dd, *J*=14.8, 8.2 Hz, 2 H), 7.03 - 7.10 (m, 1 H), 6.93 (t, *J*=75.0 Hz, 1 H), 6.10 - 6.19 (m, 1 H), 4.21 - 4.31 (m, 1 H), 4.00 (d, *J*=7.1 Hz, 2 H), 3.91 and 3.92 (2s, 2CH_3, 6 H), 3.54 - 3.65 (m, 1 H), 3.40 - 3.50 (m, 1 H), 3.22 - 3.38 (m, 2 H), 1.70 - 1.86 (m, 2 H), 1.55 - 1.71 (m, 1 H), 1.24 - 1.35 (m, 2 H), 0.53 - 0.66 (m, 2 H), 0.32 - 0.46 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-1-((3-(dimethylcarbamoyl)phenyl)sulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**18e**.*

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**16** (40 mg, 0.08 mmol), pyridine (2 mL), 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride **17e** (19 mg, 0.08 mmol), rt for 4h, 20 mg of the desired product (0.03 mmol, 35.5% yield). *m/z* 728.1 $[MH]^+$. t_R = 6.12 min, Diastereomeric Ratio= 99:1 (method A); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.60 (s, 2 H), 7.80 - 7.87 (m, 1 H), 7.68 - 7.80 (m, 3 H), 7.19 (d, *J*=7.9 Hz, 1 H), 7.14 (d, *J*=1.8 Hz, 1 H), 7.09 (t, *J*=75.0 Hz, 1 H), 6.98 (dd, *J*=8.4, 1.8

Hz, 1 H), 6.01 (dd, *J*=9.7, 4.4 Hz, 1 H), 4.18 (dd, *J*=8.6, 4.2 Hz, 1 H), 3.91 (d, *J*=7.1 Hz, 2 H), 3.47 (dd, *J*=14.3, 9.9 Hz, 1 H), 3.35 - 3.41 (m, 1 H), 3.26 (dd, *J*=14.1, 4.4 Hz, 1 H), 3.18 (dt, *J*=9.7, 6.8 Hz, 1 H), 3.02 (s, 3 H), 2.89 (s, 3 H), 1.88 - 2.02 (m, 1 H), 1.60 - 1.73 (m, 2 H), 1.47 - 1.60 (m, 1 H), 1.13 - 1.28 (m, 1 H), 0.51 - 0.60 (m, 2 H), 0.26 - 0.40 (m, 2 H).

*3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-1-((3-(dimethylcarbamoyl)phenyl)sulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxid*e (*R**,*S***)-***18e.***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide, (*R**,*S***)-**16** (50 mg, 0.1 mmol), 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride **17e** (31.1 mg, 0.13 mmol), pyridine (3 mL), rt for 4h, Purification by preparative HPLC (Method 1), 20 mg of the desired product (0.03 mmol, 30 % yield). *m/z* 728.1 $[MH]^+$. t_R = 6.20 min, Diastereomeric Ratio ≥ 99:1 (method A); $[\alpha_D]$ = +39.02 (c=0.51; $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.79 - 7.87 (m, 1 H), 7.70 - 7.76 (m, 2 H), 7.67 (d, *J*=7.5 Hz, 1 H), 7.21 (d, *J*=8.4 Hz, 1 H), 7.13 (d, *J*=1.8 Hz, 1 H), 7.10 (t, *J*=75.0 Hz, 1 H), 6.96 - 7.03 (m, 1 H), 5.94 - 6.01 (m, 1 H), 4.13 - 4.25 (m, 1 H), 3.85 - 3.97 (m, 2 H), 3.34 - 3.53 (m, 2 H), 3.23 - 3.30 (m, 1 H), 3.12 - 3.22 (m, 1 H), 3.00 (s, 3 H), 2.87 (s, 3 H), 1.86 - 2.01 (m, 1 H), 1.64 - 1.83 (m, 2 H), 1.52 - 1.63 (m, 1 H), 1.13 - 1.29 (m, 1 H), 0.56 (dd, *J*=7.9, 1.3 Hz, 2 H), 0.25 - 0.41 (m, 2 H).

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-1-((3-(dimethylcarbamoyl)-4-methoxyphenyl)sulfonyl)pyrrolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-19.**

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-pyrrolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (*S**,*S***)-**16** (40 mg, 0.08 mmol) was dissolved in pyridine (2 mL) and 5-(chlorosulfonyl)-2-methoxybenzoic acid **17f** (19 mg, 0.08 mmol) was added. The mixture was stirred at rt for 4h, to achieve completion, diluted with aqueous HCl 1N and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and concentrated under vacuum to give 50 mg of the desired product (*S**,*S***)-**18f** (0.07 mmol, 88% yield). *m/z* 731.1 $[MH]^+$. (*S**,*S***)-**18f** (20 mg, 0.03 mmol) was dissolved in DCM (2 mL). CDI (4.4 mg, 0.03 mmol) and dimethylamine 2M in THF (30 µl, 0.03 mmol) were added and the mixture stirred for 2 days at rt. The reaction was concentrated under vacuum, diluted

with EtOAc and washed with HCl aq. 1 M. The organic phase was dried over Na_2SO_4 and concentrated under vacuum to yield the desired compound that was purified by preparative HPLC (method 1) (10 mg; 0.01 mmol, 48.2 % yield). *m/z* 757.9 $[MH]^+$; t_R = 3.56min, Diastereomeric Ratio ≥ 99:1 (Method B); $[\alpha_D]$ = -55.88 (c=0.48; $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.80 (dd, *J*=8.6, 2.4 Hz, 1 H), 7.56 (d, *J*=2.7 Hz, 1 H), 7.32 (d, *J*=8.8 Hz, 1 H), 7.10 - 7.21 (m, 2 H), 7.08 (t, *J*=75.0 Hz, 1 H), 6.98 (dd, *J*=8.4, 1.8 Hz, 1 H), 5.89 - 6.11 (m, 1 H), 4.04 - 4.20 (m, 1 H), 3.87 - 3.97 (m, 5 H), 3.46 (m, 1 H), 3.08 - 3.30 (m, 3 H), 2.99 (s, 3 H), 2.75 (s, 3 H), 1.60 - 1.75 (m, 2 H), 1.43 - 1.59 (m, 2 H), 0.80 – 0.89 (m, 1 H), 0.49 - 0.62 (m, 2 H), 0.33 (d, *J*=4.4 Hz, 2 H).

Procedure for the synthesis of sulfonamides 22. *Synthesis of 3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-3-(2-methoxyethylsulfonyl)thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (S*,S**)-**22b***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S*,S***)-**21** (100 mg, 0.19 mmol) was dissolved in Py (1.5 mL, 18 mmol). 2-methoxyethanesulfonyl chloride **17b** (39 mg, 0.24 mmol) was added, and the reaction was stirred at rt for 1h to achieve completion. The reaction mixture was diluted with aqueous HCl 1N, and extracted with ethyl acetate. The organic phase was washed with aqueous HCl 1N and brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative HPLC (Method 1) to afford 67 mg of (*S*,S***)-**22b** (0. 10 mmol, 54% yield). *m/z* 657.2 $[MH]^+$. LC-MS t_R 4.57 min, Diastereomeric Ratio 98:2 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.55 (s, 2 H), 7.15 - 7.21 (m, 1 H), 7.06 - 7.13 (m, 2 H), 6.93 - 7.00 (m, 1 H), 5.97 - 6.06 (m, 1 H), 5.44 (s, 1 H), 3.85 - 3.98 (m, 3 H), 3.50 - 3.70 (m, 4 H), 3.37 - 3.50 (m, 3 H), 3.20 - 3.28 (m, 3 H), 3.01 - 3.16 (m, 2 H), 1.23 (d, *J*=5.3 Hz, 1 H), 0.51 - 0.63 (m, 2 H), 0.34 (q, *J*=4.9 Hz, 2 H).

Sulfonamides **22c-l** were prepared following the same procedure starting from the corresponding enantiomerically pure thiazolidine **21** and sulfonyl chloride **17**.

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-(phenylsulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22c***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-

carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21** (1 g, 1.75 mmol), Sulfonyl chloride **17c** (371 mg, 2.10 mmol), Py (4.5 mL, 1.75 mmol), rt, 20 min. The crude was purified by flash cromatography (579 mg, 0.86 mmol, yield 49%). *m/z* 675.2 $[MH]^+$. LC-MS t_R 4.20 min, Diastereomeric Ratio= 97:3 (Method B); $[\alpha_D]$= -37.96 (c=0.56; $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.60 (s, 2 H), 7.87 (d, *J*=7.5 Hz, 2 H), 7.75 (d, *J*=7.5 Hz, 1 H), 7.61 - 7.70 (m, 2 H), 7.18 (d, *J*=7.9 Hz, 1 H), 7.05 - 7.14 (m, 2 H), 6.96 (dd, *J*=8.4, 1.8 Hz, 1 H), 6.01 (dd, *J*=9.3, 4.9 Hz, 1 H), 5.43 (s, 1 H), 3.90 (d, *J*=7.1 Hz, 2 H), 3.75 - 3.85 (m, 1 H), 3.59 - 3.68 (m, 1 H), 3.44 (d, *J*=9.3 Hz, 1 H), 3.29 (m, 1 H), 2.91 - 3.00 (m, 1 H), 2.64 (d, *J*=11.0 Hz, 1 H), 1.18 (d, *J*=7.1 Hz, 1 H), 0.56 (dd, *J*=7.9, 1.8 Hz, 2 H), 0.26 - 0.40 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-((3,4-dimethoxyphenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22d***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21** (1.1 g, 1.92 mmol)**,** Sulfonyl chloride **17d** (546 mg, 2.31 mmol), Py (4.5 mL, 1.92 mmol), rt, 20 min. No purification needed (636 mg, 0.864 mmol, yield 45%). *m/z* 735.2 $[MH]^+$. LC-MS t_R 4.12 min, Diastereomeric Ratio=97:3 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.59 (s, 2 H), 7.45 (dd, *J*=8.4, 2.2 Hz, 1 H), 7.32 (d, *J*=1.8 Hz, 1 H), 7.15 - 7.21 (m, 2 H), 7.11 (d, *J*=1.8 Hz, 1 H), 7.08 (t, *J*=75.0 Hz, 1 H), 6.95 (dd, *J*=8.4, 1.8 Hz, 1 H), 6.01 (dd, *J*=9.3, 4.9 Hz, 1 H), 5.51 (s, 1 H), 3.77 - 3.95 (m, 9 H), 3.54 - 3.66 (m, 1 H), 3.45 (dd, *J*=14.1, 9.3 Hz, 1 H), 3.25 - 3.28 (m, 1 H), 2.87 - 3.00 (m, 1 H), 2.52 - 2.64 (m, 1 H), 1.19 - 1.28 (m, 1 H), 0.47 - 0.65 (m, 2 H), 0.27 - 0.39 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((S)-3-(3-(dimethylcarbamoyl)phenylsulfonyl)thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (S,S**)-**22e***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21** (300 mg, 0.525 mmol), sulfonyl chloride **17e** (156 mg, 0.630 mmol), Py (3 mL, 37.1 mmol), rt, 4h. Flash chromatography (DCM/IsoPrOH 98/2) (250 mg, 0.334 mmol, 64% yield). *m/z* 746.2 $[MH]^+$; $[\alpha_D]$ = -43.30 (c=0.51; $CHCl_3$), t_R = 3.66, Diastereomeric Ratio ≥ 99/1 (Method A); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm

8.58 (s, 2 H), 7.88 - 7.97 (m, 2 H), 7.68 - 7.82 (m, 2 H), 7.14 - 7.21 (m, 1 H), 7.09 - 7.13 (m, 1 H), 7.08 (t, *J*=75.0 Hz, 1 H), 6.92 - 6.99 (m, 1 H), 5.91 - 6.10 (m, 1 H), 5.54 (s, 1 H), 3.79 - 3.94 (m, 3 H), 3.60 - 3.71 (m, 1 H), 3.41 - 3.51 (m, 1 H), 3.26 - 3.32 (m, 1 H), 3.02 (s, 3 H), 2.92 - 3.00 (m, 1 H), 2.89 (s, 3 H), 2.56 - 2.70 (m, 1 H), 1.20 - 1.27 (m, 1 H), 0.53 - 0.60 (m, 2 H), 0.29 - 0.36 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-3-((3-(dimethylcarbamoyl)phenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,R**)-**22e***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*R***)-**21** (693 mg, 1.2 mmol), Py (7 mL, 1.2 mmol), 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride **17e** (780 mg, 3.36 mmol), rt, 4h. Purified by n-Hex/DCM/IprOH/MeOH 45/50/4/1 (24 mg, 0.03 mmol, 2% yield). *m/z* 746.2 $[MH]^+$; t_R = 3.57, Diastereomeric Ratio= 99:1(Method 2); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.93 - 8.00 (m, 2 H), 7.64 - 7.80 (m, 2 H), 7.22 (d, *J*=8.4 Hz, 1 H), 7.11 - 7.15 (m, 1 H), 7.10 (t, *J*=75.0 Hz, 1 H), 6.97 - 7.03 (m, 1 H), 5.89 - 6.03 (m, 1 H), 5.58 (s, 1 H), 3.93 (s, 2 H), 3.62 - 3.83 (m, 2 H), 3.41 - 3.53 (m, 1 H), 3.22 - 3.30 (m, 1 H), 2.95 - 3.07 (m, 4 H), 2.87 (s, 3 H), 2.61 - 2.73 (m, 1 H), 1.16 - 1.31 (m, 2 H), 0.48 - 0.64 (m, 2 H), 0.34 (d, *J*=4.9 Hz, 2 H).

3,5-dichloro-4-((R)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-3-((3-(dimethylcarbamoyl)phenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (R,R**)-**22e***

3,5-dichloro-4-((*R*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*R**,*R***)-**21**, (350 mg, 0.612 mmol), Py (6.55 mL, 612 mmol), 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride **17e** (414 mg, 1.67 mmol), rt, 4h. Purified by preparative HPLC (Method 1) (82 mg, 0.110 mmol, 18% yield). *m/z* 746.0 $[MH]^+$, t_R = 3.63, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.57 (s, 2 H), 7.86 - 8.00 (m, 2 H), 7.65 - 7.83 (m, 2 H), 7.18 (d, *J*=7.9 Hz, 1 H), 7.11 (d, *J*=1.3 Hz, 1 H), 7.08 (t, *J*=75.0 Hz, 1 H), 6.86 - 6.96 (m, 1 H), 6.01 (dd, *J*=9.0, 5.1 Hz, 1 H), 5.54 (s, 1 H), 3.80 - 3.98 (m, 3 H), 3.60 - 3.72 (m, 1 H), 3.46 (dd, *J*=14.1, 9.3 Hz, 1 H), 3.30 - 3.35 (m, 1 H), 2.82 - 3.06 (m, 7 H), 2.63 - 2.73 (m, 1 H), 1.15 - 1.33 (m, 1 H), 0.47 - 0.65 (m, 2 H), 0.33 (q, *J*=4.9 Hz, 2 H).

3,5-dichloro-4-((R)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-((3-

(dimethylcarbamoyl)phenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (R,S**)-**22e***

3,5-dichloro-4-((*R*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*R*,S***)-**21**, (140 mg, 0.245 mmol), Py (4.71 mL, 440 mmol), 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride **17e** (61 mg, 0.245 mmol), rt, 4h. Purified by preparative HPLC (Method 1) (35 mg, 0.047 mmol, 19% yield). *m/z* 746.0 $[MH]^+$; t_R = 3.57; 3.64, Diastereomeric Ratio= 84:16(Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.89 - 7.98 (m, 2 H), 7.63 - 7.80 (m, 2 H), 7.22 (d, *J*=7.9 Hz, 1 H), 7.06 - 7.15 (m, 2 H), 7.00 (dd, *J*=8.4, 1.3 Hz, 1 H), 5.97 (dd, *J*=9.7, 4.4 Hz, 1 H), 5.57 (s, 1 H), 3.89 - 4.02 (m, 2 H), 3.57 - 3.86 (m, 2 H), 3.47 (dd, *J*=14.1, 9.7 Hz, 1 H), 3.26 (d, *J*=4.9 Hz, 1 H), 2.80 - 3.10 (m, 7 H), 2.61 - 2.74 (m, 1 H), 1.15 - 1.31 (m, 1 H), 0.50 - 0.64 (m, 2 H), 0.34 (q, *J*=4.9 Hz, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-(pyridin-3-ylsulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22g***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*S*,S***)-**21**, (540 mg, 0.944 mmol), Py (8 mL, 1 mmol), pyridine-3-sulfonyl chloride **17g** (252 mg, 1.42 mmol), rt, 1h. Purified by Preparative HPLC (Method 1) to afford 408 mg of (*S*,S***)-***22g*** (0.603 mmol, 60% yield). *m/z* 676.2 $[MH]^+$; $[\alpha_D]$ = -43.74 (c=0.53; $CHCl_3$); t_R = 3.62, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.05 (d, *J*=2.2 Hz, 1 H), 8.91 (dd, *J*=4.6, 1.5 Hz, 1 H), 8.58 (s, 2 H), 8.31 (ddd, *J*=8.3, 1.9, 1.8 Hz, 1 H), 7.69 (dd, *J*=8.4, 4.9 Hz, 1 H), 7.18 (d, *J*=7.9 Hz, 1 H), 7.05 - 7.14 (m, 2 H), 6.95 (dd, *J*=8.2, 1.5 Hz, 1 H), 6.01 (dd, *J*=9.0, 5.1 Hz, 1 H), 5.63 (s, 1 H), 3.80 - 3.96 (m, 3 H), 3.61 - 3.72 (m, 1 H), 3.46 (dd, *J*=14.1, 9.3 Hz, 1 H), 3.28 - 3.40 (m, 2 H), 2.91 - 3.04 (m, 1 H), 2.69 (ddd, *J*=11.1, 6.4, 6.3 Hz, 1 H), 1.21 (ddd, *J*=12.1, 7.5, 4.6 Hz, 1 H), 0.49 - 0.64 (m, 2 H), 0.26 - 0.41 (m, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-((1-methyl-1H-imidazol-4-yl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22h***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*S*,S***)-**21**, (100 mg, 0.175 mmol), Py (1.87 mL, 23.1 mmol), 1-methyl-1H-imidazole-4-sulfonyl chloride **17h** (39 mg, 0.21 mmol), rt, 2 h. Purified by

Preparative HPLC (Method 1) to afford 30 mg of (*S**,*S***)-**22h** (0.04 mmol, 25% yield). *m/z* 679.2 $[MH]^+$; t_R = 3.28, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.56 (s, 2 H), 7.93 (s, 1 H), 7.87 (s, 1 H), 7.17 (d, *J*=8.4 Hz, 1 H), 7.10 (d, *J*=9.7 Hz, 2 H), 6.92 - 6.98 (m, 1 H), 5.94 - 6.11 (m, 1 H), 5.35 (s, 1 H), 3.91 (d, *J*=7.1 Hz, 2 H), 3.73 – 3.80 (m, 4 H), 3.61 - 3.71 (m, 1 H), 3.40 - 3.49 (m, 1 H), 3.23 - 3.30 (m, 1 H), 2.94 - 3.04 (m, 1 H), 2.76 - 2.88 (m, 1 H), 1.16 - 1.28 (m, 1 H), 0.56 (d, *J*=7.9 Hz, 2 H), 0.34 (d, *J*=4.4 Hz, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-((1-methyl-5-(methylcarbamoyl)-1H-pyrrol-3-yl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22i***

From 3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21**, (80 mg, 0.14 mmol), Py (1 mL, 12.4 mmol), 1-methyl-5-(methylcarbamoyl)-1H-pyrrole-3-sulfonyl chloride **17i** (40 mg, 0.168 mmol), rt, 2 h. Purified by preparative HPLC (Method 1) to afford 73 mg of (*S**,*S***)-**22i** (0.09 mmol, 66% yield). *m/z* 735.2 $[MH]^+$; t_R = 3.47, Diastereomeric Ratio ≥ 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.45 - 12.98 (bs, 1 H), 8.59 (s, 2 H), 8.27 (d, *J*=4.9 Hz, 1 H), 7.67 (d, *J*=1.8 Hz, 1 H), 7.18 (d, *J*=8.4 Hz, 1 H), 7.06 - 7.13 (m, 3 H), 6.96 (dd, *J*=8.4, 1.86 Hz, 1 H), 6.03 (dd, *J*=9.3, 4.9 Hz, 1 H), 5.22 (s, 1 H), 3.84 - 3.96 (m, 5 H), 3.58 - 3.73 (m, 2 H), 3.45 (dd, *J*=14.1, 9.3 Hz, 1 H), 3.28 - 3.30 (m, 1 H), 2.94 - 3.05 (m, 1 H), 2.75 - 2.84 (m, 1 H), 2.72 (d, *J*=4.4 Hz, 3 H), 1.22 (d, *J*=7.5 Hz, 1 H), 0.48 - 0.63 (m, 2 H), 0.33 (q, *J*=4.9 Hz, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-3-((2,5-dimethylthiophen-3-yl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**22l***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21**, (150 mg, 0.26 mmol), Py (4 mL, 49.2 mmol), 2,5-dimethylthiophene-3-sulfonyl chloride **17l** (94 mg, 0.45 mmol), rt, 18 h. Purified by Preparative HPLC (Method 2) to afford 160 mg of (*S**,*S***)-**22l** (0.22 mmol, 86% yield). *m/z* 709.02 $[MH]^+$; $[\alpha_D]$ = -50.27 (c=0.3, DCM), t_R = 4.41, Diastereomeric Ratio ≥ 95:5 (Method C); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.56 (s, 2 H), 7.19 (d, 1 H), 7.12 (d, 1 H), 6.98 (s, 1 H), 6.97 (dd, 1 H),

7.08 (t, 1 H), 6.02 (dd, 1 H), 5.40 (s, 1 H), 3.91 (d, 2 H), 3.78 (dt, 1 H), 3.62 (dt, 1 H), 3.45 (dd, 1 H), 3.30 (dd, 1 H), 3.03 (dt, 1 H), 2.80 (dt, 1 H), 2.57 (s, 3 H), 2.40 (s, 3 H), 1.11 - 1.34 (m, 1 H), 0.45 - 0.70 (m, 2 H), 0.24 - 0.45 (m, 2 H).

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-3-((3-(dimethylcarbamoyl)-4-methoxyphenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-22m**

To a solution of 3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (*S**,*S***)-**21** (60 mg, 112 mmol) in Py (1 mL, 112 mmol), Sulfonyl chloride **17f** (56 mg, 224 mmol) was added, and the reaction was stirred at rt for 1h to achieve completion. The reaction mixture was diluted with aqueous HCl 1N, and extracted with ethyl acetate. The organic phase was washed with aqueous HCl 1N and brine, dried over Na_2SO_4 and concentrated under vacuum to give 70 mg of (*S**,*S***)-**22f** No purification needed (0.093 mmol, yield 88%). *m/z* 749.1 $[MH]^+$. LC-MS t_R=3.44 min, Diastereomeric Ratio= 99:1 (Method B). 4-((*S*)-2-(((*S*)-3-((3-carboxy-4-methoxyphenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-oxide (*S**,*S***)-**22f** (70 mg, 0.093 mmol) was dissolved in DMF (1 mL). CDI (18 mg, 0.11 mmol) was added and the mixture stirred for 30 min at rt. Then dimethylamine was added (200 µl, 0.6 mmol) and the mixture stirred at rt for 2 h. The reaction was quenched with water, and the product was extracted with AcOEt. The organic layer was washed with water (2x) and NaCl saturated solution, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by preparative HPLC (Method 1) condition to give 70 mg of (*S**,*S***)-**22m** (0.09 mmol, yield 97%). *m/z* 776.2 $[MH]^+$. LC-MS t_R 3.64 min, Diastereomeric Ratio= 97:3 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.57 (s, 2 H), 7.81 - 7.97 (m, 1 H), 7.68 - 7.76 (m, 1 H), 7.32 (m, 1 H), 7.18 (d, *J*=7.9 Hz, 1 H), 7.05 - 7.13 (m, 2 H), 6.91 - 6.98 (m, 1 H), 5.92 - 6.07 (m, 1 H), 5.57 (s, 1 H), 3.75 - 4.06 (m, 6 H), 3.55 - 3.68 (m, 1 H), 3.37 - 3.49 (m, 1 H), 3.25 - 3.35 (m, 1 H), 2.99 (s, 4 H), 2.75 (s, 3 H), 2.54 - 2.65 (m, 1 H), 1.14 - 1.29 (m, 1 H), 0.56 (dd, *J*=7.9, 1.8 Hz, 2 H), 0.33 (d, *J*=5.3 Hz, 2 H).

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-3-(3-

methoxypropanoyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-24a**

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21** (150 mg, 0.262 mmol), 3-methoxypropanoic acid **23a** (14.84 µL, 0.157 mmol), DMAP (64 mg, 0.52 mmol) and EDC (50.3 mg, 0.262 mmol) were dissolved in dry DCM (6 mL) and the mixture was stirred at rt for 1h. Thus, EDC (101 mg, 0.525 mmol), 3-methoxypropanoic acid (14.84 µL, 0.157 mmol) and DMAP (32.0 mg, 0.262 mmol) were added and the mixture was stirred at rt overnight.The reaction mixture was washed with 1N HCl (1 x 30 mL) and brine (1 x 30mL). The solvent was removed in vacuo to give a residue that was purified by preparative HPLC (Method 2) to give 50 mg of (*S**,*S***)-**24a**: (0.08 mmol, 31 % yield). *m/z* 607.05 [MH] +; t_R = 3.49, Diastereomeric Ratio 77:23 (Method C) [α_D] = -46.5 (c 0.49; DCM); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.54 (s, 2 H), 7.17 (d, 1 H), 7.07 (s, 1 H), 6.94 (dd, 1 H), 7.07 (t, 1 H), 5.81 – 6.02 (m, 1 H), 5.40 (s, 0.77 H) and 5.64 (s, 0.23 H), 4.11 (s, 2 H), 3.92 (d, 2 H), 3.72 – 3.85 (m, 2 H), 3.35 – 3.53 (m, 2 H), 3.12 – 3.26 (m, 1 H), 2.83 – 3.11 (m, 2 H), 1.12 – 1.35 (m, 1 H), 0.50 – 0.62 (m, 2 H), 0.20 – 0.42 (m, 2 H);

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-3-(3-(dimethylcarbamoyl)benzoyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-24b**

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**21** (100 mg; 0.175 mmol) was dissolved in DMF (1 mL). 3-(dimethylcarbamoyl)benzoic acid **23b** (41 mg0.21 mmol), was added to the reaction solution followed by EDC (44 mg, 0.23 mmol) and DMAP (24 mg, 0.19 mmol). The reaction was stirred at rt for 4 h before to get to completion and quenched by adding 20 mL of aq HCl 1M. The aqueous layer was extracted with EtOAc and washed with HCl 1M (x3) and with aq K_2CO_3 5% (x3). The resulting organic extract was anhydrified with Na_2SO_4, filtered on a filter paper, and the solvent removed on a rotary evaporator under reduced pressure. The oil residue was purified by preparative HPLC (Method 1) to yield 45 mg of (*S**,*S***)-**24b** (0.063 mmol, yield 36%). *m/z* 710.2 [MH] +; t_R = 3.38, Diastereomeric Ratio 3:97 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.55 (s, 2 H), 7.40 - 7.65 (m, 4 H), 6.78 - 7.31 (m, 4 H), 5.85 - 6.14 (m, 1 H), 5.24 (s, 1 H), 3.69 - 4.02 (m, 4 H), 3.36 -

3.63 (m, 2 H), 3.09 - 3.21 (m, 1 H), 2.74 - 3.07 (m, 7 H), 1.12 - 1.28 (m, 1 H), 0.42 - 0.66 (m, 2 H), 0.18 - 0.39 (m, 2 H).

3,5-dichloro-4-((*S*)-2-(4-(difluoromethoxy)-3-methoxyphenyl)-2-((*S*)-3-(3-(dimethylcarbamoyl)phenylsulfonyl)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (*S,*S***)-28a.**

To a solution of 3,5-dichloro-4-((*S*)-2-(4-(difluoromethoxy)-3-methoxyphenyl)-2-((*S*)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide hydrochloride (*S**,*S***)-**27a** (350 mg, 0.658 mmol) in pyridine (6 mL) cooled at 0°C, a solution of 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride (245 mg, 0.987 mmol) in DCM (3 mL) was added drop-wise and the reaction was stirred at 0°C for 1 h. The mixture was diluted with DCM (30 mL) and washed twice with aqueous 1N HCl; the organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum. The residue was purified by preparative HPLC (Method 2) followed by flash chromatography on silica gel (DCM/MeOH = 97/3) to afford 147 mg of (*S**,*S***)-**28a** (0.208 mmol, 31.6% yield); *m/z* 705.97 $[MH]^+$, t_R = 3.28 min, Diastereomeric Ratio = 95:5 (Method C); (^{1}H NMR); $[\alpha_D]$ = - 43.1 (c=0.57, DCM); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.57 (s, 2 H), 7.94 (dt, 1 H), 7.90 (t, 1 H), 7.77 (dt, 1 H), 7.71 (t, 1 H), 7.18 (d, 1 H), 7.15 (d, 1 H), 6.97 (dd, 1 H), 7.07 (t, 1 H), 6.04 (dd, 1 H), 5.54 (s, 1 H), 3.84 (s, 3 H), 3.75 - 3.94 (m, 1 H), 3.66 (dt, 1 H), 3.48 (dd, 1 H), 3.33 (dd, 1 H), 3.02 (br. s., 3 H), 2.98 (dd, 1 H), 2.90 (br. s., 3 H), 2.66 (dt, 1 H).

3,5-dichloro-4-((*S*)-2-(3-(cyclopentyloxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-3-((3-(dimethylcarbamoyl)phenyl)sulfonyl)thiazolidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-28b**

(*S**,*S***)-**28b** was prepared following an analogous procedure as for (*S**,*S***)-**28a** from (*S**,*S***)-**27b** (195 mg, 0.33 mmol), pyridine (4 mL) 3-(dimethylcarbamoyl)benzene-1-sulfonyl chloride (149 mg, 0.599 mmol), rt, 18 h. Purified by preparative HPLC (Method 2). 94 mg of (*S**,*S***)-**28b** (0.122 mmol, 37% yield); *m/z* 760.17 $[MH]^+$, t_R = 3.98 min, Diastereomeric Ratio = 95:5 (Method C); $[\alpha_D]$ = -42.7 (c=0.50; DCM); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.57 (s, 2 H), 7.95 (dt, 1 H), 7.91 (t, 1 H), 7.77 (dt, 1 H), 7.72 (td, 1 NH), 7.16 (d, 1 H), 7.08 (d, 1 H), 6.94 (dd, 1 H), 6.99 (t, 1 H), 6.02 (dd, 1 H), 5.57 (s, 1 H), 4.82 - 5.00 (m, 1 H), 3.87 (dt, 1 H), 3.65 (dt, 1 H), 3.46 (dd, 1 H), 3.32 (dd, 1 H), 3.03 (br. s.,

3 H), 2.93 - 3.01 (m, 1 H), 2.90 (br. s., 3 H), 2.65 (dt, 1 H), 1.38 - 2.04 (m, 8 H).

3,5-dichloro-4-((*S*)-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-2-((*S*)-3-(3-(dimethylcarbamoyl)phenylsulfonyl)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (*S*,S*)-28c.**

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-((*S*)-3-(3-(dimethylcarbamoyl)phenylsulfonyl)-thiazolidine-2-carbonyloxy)ethyl)pyridine 1-oxide (*S*,S***)-**22e** (200 mg, 0.268 mmol) was dissolved in 2,2,2-trifluoroacetic acid (2 mL, 0.268 mmol) and the solution stirred overnight at rt. The reaction mixture was diluted with DCM and concentrated under vacuum to give a crude which was purified through preparative HPLC (Method 1) to give 90 mg of (*S*,S***)-**28c** (0.131 mmol, 49% yield). *m/z* 661.3 $[MH]^+$; t_R = 2.49 min, Diastereomeric Ratio = 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.05 (s, 1 H), 8.58 (s, 2 H), 7.85 - 7.97 (m, 2 H), 7.67 - 7.80 (m, 2 H), 7.11 (d, *J*=8.4 Hz, 1 H), 7.04 (t, *J*=75.0 Hz, 1 H), 6.94 (d, *J*=2.2 Hz, 1 H), 6.83 – 6.87 (m, 1 H), 5.86 - 6.02 (m, 1 H), 5.47 (s, 1 H), 3.78 - 3.92 (m, 1 H), 3.56 - 3.68 (m, 1 H), 3.37 - 3.49 (m, 1 H), 3.20 - 3.28 (m, 1 H), 2.93 - 3.09 (m, 4 H), 2.89 (s, 3 H), 2.59 - 2.69 (m 1 H).

Preparation of Compounds 35, 36a-b, 36d, 37. Compounds **35, 36a-b, 36d, 37** were synthesized following the same procedure described for the preparation of compounds (*S*,S***)-**22b** starting from corresponding intermediates **32, 33, 34** (whose preparation is described in Supporting Information).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-3-((3-(dimethylcarbamoyl)phenyl)sulfonyl)thiazolidine-4-carbonyl)oxy)ethyl)pyridine 1-oxide (*R*,S***)-***35***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-thiazolidine-4-carbonyl)oxy)ethyl)pyridine 1-oxide (*R*,S***)-**32** (75 mg, 0.14 mmol), sulfonyl chloride **17e** (42 mg, 0.17 mmol), Py (1.5 mL, 18.6 mmol), rt, 3 h. The crude was purified by Preparative HPLC (Method 1), 40 mg solid (0.056 mmol, yield 41%). *m/z* 746.1 $[MH]^+$. LC-MS t_R 6.30 min, Diastereomeric Ratio= 99:1 (Method A); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.59 (s, 2 H), 7.85 - 7.99 (m, 2 H), 7.65 - 7.79 (m, 2 H), 7.13 - 7.22 (m, 2 H), 7.09 (t, *J*=75.0 Hz, 1 H), 6.98 (dd, *J*=8.4, 1.86 Hz, 1 H), 6.00 (dd, *J*=9.3, 4.4 Hz, 1 H), 4.89 (dd, *J*=7.3, 4.6 Hz, 1 H), 4.73 (d, *J*=10.6 Hz, 1 H), 4.32 (d, *J*=10.6 Hz, 1 H), 3.92 (d, *J*=7.1 Hz, 2 H), 3.46 (dd, *J*=14.1, 9.7 Hz, 1 H), 3.23 - 3.30 (m, 1 H), 2.93 - 3.08 (m, 5 H),

2.88 (s, 3 H), 1.23 (d, *J*=7.1 Hz, 1 H), 0.49 - 0.64 (m, 2 H), 0.33 (q, *J*=4.9 Hz, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-1-((3-(dimethylcarbamoyl)phenyl)sulfonyl)piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**36a***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S**,*S***)-**33** (900 mg, 1.70 mmol), sulfonyl chloride **17e** (634 mg, 2.56 mmol), Py (7 mL, 87 mmol), 0°C, 4 h. The crude was purified by flash cromatography (DCM/IprOH 98/2), 80 mg solid (0.102 mmol, yield 6%). *m/z* 742.2 $[MH]^+$. LC-MS t_R 3.85 min, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.50 (s, 2 H), 7.57 - 7.84 (m, 4 H), 7.08 - 7.17 (m, 1 H), 7.02 - 7.08 (m, 2 H), 6.83 - 6.93 (m, 1 H), 5.77 - 5.96 (m, 1 H), 4.56 - 4.73 (m, 1 H), 3.78 - 3.99 (m, 2 H), 3.56 - 3.74 (m, 1 H), 3.33 - 3.46 (m, 2 H), 3.17 - 3.26 (m, 1 H), 2.99 (s, 3 H), 2.86 (s, 3 H), 1.99 - 2.17 (m, 1 H), 1.43 - 1.69 (m, 3 H), 1.08 - 1.31 (m, 2 H), 0.71 - 0.99 (m, 1 H), 0.43 - 0.61 (m, 2 H), 0.25 - 0.38 (m, 2 H).

4-((S)-2-(((S)-1-((3-carboxy-4-methoxyphenyl)sulfonyl)piperidine-2-carbonyl)oxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-oxide (S,S**)-**36b***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S**,*S***)-**33** (100mg, 0.376 mmol), sulfonyl chloride **17f** (94 mg, 0.38 mmol), Py (1.5 mL, 18.6 mmol), rt, 8 h. The reaction mixture was diluted with HCl 1N and extracted with AcOEt. The organic phase was washed with HCl 1N and brine, dried over Na_2SO_4 and concentrated under vacuum to give 60 mg of crude that was used without further purification. *m/z* 530.12 $[MH]^+$.

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((S)-1-(phenylsulfonyl)piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (S,S**)-**36d***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S**,*S***)-**33** (106 mg, 0.2 mmol), sulfonyl chloride **17c** (43 mg, 0.25 mmol), Py (1.5 mL, 18.6 mmol), rt, 2 h. The crude was purified by Preparative HPLC (Method 1), 19 mg solid (0.03 mmol, yield 15%). *m/z* 671.2 $[MH]^+$. LC-MS t_R 4.44 min, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.64 (s, 2 H), 7.62 - 7.70 (m, 3 H), 7.57 (d,

J=7.5 Hz, 2 H), 7.15 (m, 1 H), 7.02 - 7.10 (m, 2 H), 6.89 (m, 1 H), 5.84 - 5.91 (m, 1 H), 4.56 - 4.66 (m, 1 H), 3.87 (m, 2 H), 3.57 - 3.67 (m, 1 H), 3.36 - 3.48 (m, 1 H), 3.16 - 3.27 (m, 1 H), 2.90 - 3.08 (m, 1 H), 1.98 - 2.12 (m, 1 H), 1.41 - 1.66 (m, 3 H), 1.10 - 1.30 (m, 2 H), 0.78 - 0.98 (m, 1 H), 0.57 (m, 2 H), 0.33 (dd, *J*=4.9, 1.3 Hz, 2 H).

3,5-dichloro-4-((S)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((R)-1-((3-(dimethylcarbamoyl)phenyl)sulfonyl)piperidine-3-carbonyl)oxy)ethyl)pyridine 1-oxide (R,S**)-**37***

3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*R*)-piperidine-3-carbonyl)oxy)ethyl)pyridine 1-oxide (*R**,*S***)-**34** (248 mg, 0.47 mmol), sulfonyl chloride **17e** (138 mg, 0.56 mmol), Py (4 mL, 50 mmol), rt, 18 h. The crude was purified by Flash Cromatography on Silica Gel (DCM/MeOH = 99/1 to 98/2), 60 mg (0.146 mmol, yield 31%). *m/z* 742.1 $[MH]^+$. LC-MS t_R 3.78 min, Diastereomeric Ratio= 95:5 (Method C); $[\alpha_D]$= +2.018 (c=0.565, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.55 (s, 2 H), 7.72 - 7.84 (m, 3 H), 7.70 (t, 1 H), 7.18 (d, 1 H), 7.10 (d, 1 H), 6.96 (dd, 1 H), 7.07 (t, 1 H), 5.94 (dd, 1 H), 3.92 (d, 2 H), 3.47 - 3.59 (m, 1 H), 3.45 (dd, 1 H), 3.32 - 3.39 (m, 1 H), 3.21 (dd, 1 H), 3.02 (br. s., 3 H), 2.90 (br. s., 3 H), 2.55 - 2.69 (m, 2 H), 1.03 - 1.96 (m, 6 H), 0.47 - 0.69 (m, 2 H), 0.20 - 0.47 (m, 2 H).

Synthesis of 3,5-dichloro-4-((*S*)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(((*S*)-1-((3-(dimethylcarbamoyl)-4-methoxyphenyl)sulfonyl)piperidine-2-carbonyl)oxy)ethyl)pyridine 1-oxide (*S,*S***)-36c**

4-((*S*)-2-(((*S*)-1-((3-carboxy-4-methoxyphenyl)sulfonyl)piperidine-2-carbonyl)oxy)-2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)ethyl)-3,5-dichloropyridine 1-oxide (*S**,*S***)-**36b** (60 mg, 0.08 mmol) was dissolved in DMF (1.5 mL). CDI (26 mg, 0.16 mmol) was added and the mixture stirred for 30 min at rt. Then dimethylamine 2M in THF was added (402 µl, 0.81 mmol) and the mixture stirred at rt for 18 h. The reaction was quenched with water, and the product was extracted with AcOEt. The organic layer was washed with water (2x) and NaCl saturated solution, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by preparative HPLC (Method 1) to give 25 mg of (*S**,*S***)-**36c** (0.064 mmol, yield 40%). *m/z* 772.3 $[MH]^+$. LC-MS t_R 3.84 min, Diastereomeric Ratio= 99:1 (Method B); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.62 (s, 2 H), 7.59 -

7.77 (m, 1 H), 7.45 - 7.56 (m, 1 H), 7.18 - 7.22 (m, 1 H), 7.13 - 7.17 (m, 1 H), 7.03 - 7.09 (m, 2 H), 6.79 - 6.95 (m, 1 H), 5.83 - 5.94 (m, 1 H), 4.53 - 4.72 (m, 1 H), 3.81 - 3.96 (m, 5 H), 3.60 (d, *J*=11.9 Hz, 1 H), 3.40 (d, *J*=10.6 Hz, 1 H), 3.28 - 3.29 (m, 1 H), 3.23 (dd, *J*=14.1, 5.3 Hz, 1 H), 2.98 (s, 3 H), 2.74 (s, 3 H), 2.06 (d, *J*=12.8 Hz, 1 H), 1.43 - 1.61 (m, 3 H), 1.12 - 1.31 (m, 2 H), 0.88 (d, *J*=12.8 Hz, 1 H), 0.52 - 0.61 (m, 2 H), 0.29 - 0.36 (m, 2 H).

Biology and ADME.

Determination of inhibition of PDE4 enzymatic activity in the cell free assay. See ref. 23.

***In vitro* determination of anti-inflammatory activity in the PBMCs assay.** See ref. 23.

Human lung S9 stability. See ref. 23.

Plasma protein binding. See ref. 23.

Caco-2 membrane permeability assay. See ref 23.

Plasma stability. See ref. 23.

Human hepatocyte stability. Test compounds were incubated, in duplicate, at a concentration of 1 µM with hepatocytes (1 million cells/mL) in Leibovitz L-15 Medium, at 37°C. At 0, 10, 20, 30, 60 and 90 minutes, 50 µL aliquots of the incubates weretaken, added with 80 µL of ice-cold acetonitrile and 20 µL of 1 µM warfarin in acetonitrile (injection check), and samples centrifuged. The supernatant were analysed for unchanged compound by LC-MS/MS. 7-Ethoxycoumarin (1 µM) and 7-hydroxycoumarin (30 µM), positive controls, respectively, of phase I and phase II activities of hepatocytes, as well as control incubations with test compounds incubated in the absence of hepatocytes (medium only) for 0 and 90 min, were included. Control samples were processed as test compounds samples.

Human microsomes stability. Test compounds were incubated, in duplicate, at the concentration of 1 µM with liver microsomes (0.8 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, 5, 10, 20, 30 and 60 min) 50 µL aliquots of the incubates were taken, added with 80 µL of ice-cold acetonitrile and 20 µL of 1 µM warfarin in acetonitrile (injection check) to stop the reaction and samples centrifuged. The supernatant were analysed by LC-MS/MS for unchanged compounds. Test compounds were incubated with liver microsomes in

Dulbecco's buffer in the absence of NADPH for 0 and 60 minutes, as control. Midazolam at the concentration of 1 μM, was incubated with microsomes as positive control for phase I activity of microsomes. Control samples were processed as test compounds samples.

***In vitro* metabolite profile.** Commercialy available cryopreserved hepatocytes of pooled mixed sex humans were used. Test compounds were incubated, in duplicate, at the concentration of 10 μM with human liver hepatocytes (1 mL / 1 million cells) in Leibovitz L-15 medium, at 37°C. At t =0, 60 minutes samples aliquots of the incubates were taken. The sample workup included evaporation of protein-free supernatant under nitrogen and reconstitution of the residue in mobile phase for HPLC analysis. Control incubations were performed with test compounds incubated in the absence of hepatocytes (medium only) for 60 min. Control samples were processed as test compounds samples. Samples were analyzed by LC-MS/MS.

PDEs Enzyme Assays. All the PDE isoenzymes were prepared as human recombinant proteins from insect Sf9 cells, , with the exception of PDE6, purified from bovine retina.[25] The radiometric assay method is a modification of the two-step method of Thompson and Appleman.[26] Compounds (*S*,S***)-**18e** and (*S*,S***)-**22e** were tested against PDEs at five concentrations (1% final DMSO concentration) in duplicate with a starting concentration of 30 μM and a 1:10 serial dilution. All the PDEs enzymatic assays were performed at Eurofins (Taiwan).

***In Vivo* Pharmacology.**

ED_{50} determination of inhibition of ovalbumin-induced lung eosinophilia in rats.

Adult male Brown-Norway rats (Charles River Laboratories Italy, Calco, Italy) were sensitized by intraperitoneal injection of a suspension containing ovalbumin (OVA; 1 mg/rat) and $Al(OH)_3$ (100 mg/rat) in 1 mL of saline for 3 consecutive days. Then, 2 to 3 weeks later, the animals were exposed to an aerosol of OVA solution (1% in saline) by a nose-only apparatus system for 30 minutes to trigger an influx of inflammatory cells into the airways. Vehicle-control treated animals were exposed to an aerosol of saline using the same general conditions. For the determination of inhibitory potency, test compounds were administered intratracheally 2 hours before and 6 hours after the antigen (OVA) aerosol. For the intratracheal administration of test compounds or vehicle, animals were anesthetized

with isoflurane (4% in oxygen), and a laryngoscope was moved forward into the mouth to visualize the trachea and guide the insertion of a fine-tipped dry powder insufflator (PennCentury, Philadelphia, PA) directly into the trachea and located 1–2 mm above the bifurcation. Dry powder formulations were prepared by blending coarse respiratory grade lactose and the micronized test compound to achieve 10 mg/kg body weight total particulate powder. Dry powders were blown into the airways during the spontaneous phase inspiration in an air volume of 3 mL. The difference in weight of the needle before and after the administration was used to calculate the dose administered. Control animals received 10 mg/kg of lactose. At 24 hours after exposure either to OVA or saline aerosol, animals were sacrificed by an overdose of anesthetic. Bronchoalveolar lavage (BAL) fluid was obtained by gently washing the lungs with 3 aliquots (4 mL each) of solution A (10× Hanks' balanced salt solution, 100 mL; EDTA 100 mM, 100 mL; HEPES 1 mM, 10 mL; distilled water, 790 mL). Routine recovery of BAL fluids did not significantly differ among animals with ~80% of instilled volume recovered (9.5–10.5 mL). The resulting BAL fluid was centrifuged at 800*g* for 10 minutes at 4°C, and the supernatants were removed and discharged. The pellets were resuspended in a volume of 1.5 mL, and total and differential cell counts were performed within 2 hours using an automated cell counter (Sysmex, Dasit, Cornaredo, Italy). A dose response curve was constructed for the inhibition of Ova-induced lung eosinophilia at 2 h pre-dosing, and half-maximal effect doses (ED_{50}) was estimated from the fitted curve.

All the experimental procedures and conditions were reviewed and approved by the local ethics committees and were performed in full compliance with the international European ethics standards (86/609- EEC), the Italian legislative decree 116/1992, the French National Committee (décret 87/848) for the care and use of laboratory animals, and the Animals (Scientific Procedures) Act of 1986.

Pharmacokinetics of (*S**,*S***)-**18e** and (*S**,*S***)-**22e in rats.** (*S**,*S***)-**18e** and (*S**,*S***)-**22e** were administered to Sprague-Dawley rats (250–400 g; Charles River Laboratories Italy) by the intratracheal route as micronized powder as described previously. At the time of sacrifice, blood samples were collected in heparinized tubes. The samples were immediately separated by centrifugation at 1200g for 15 minutes. Lung samples were collected at 0.25, 1, 2, 4, 8, 16, and 24 hours after the administration of the test compounds (with $n = 3$ for each sampling time). Animals were sacrificed by overdose of

anesthetic (4% isoflurane in 100% O_2). At the time of sacrifice, the lungs were excised and washed with saline 3 times. A portion of lung tissue was homogenized into polypropylene tubes in 3 volumes of a acetonitrile/saline solution (50%:50%; v/v) using an Ultra-Turrax homogenizer (IKA-Werke GmbH, Staufen, Germany) at 3000 rpm for 30 seconds at room temperature. All biologic samples were then transferred into polypropylene tubes and put in the freezer at −80°C until analysis by electrospray liquid chromatography–tandem mass spectrometry using an Alliance Waters high-pressure liquid chromatography (Waters Corporation, Milford, MA) coupled to a ABI-Sciex API2000 (AB Sciex, Framingham, MA) operated in positive ion mode.

Molecular modeling. PDE4B protein structures in complex with ligands were prepared using the default settings of the Protein Preparation Wizard in Maestro.[27] Only the water molecules that are coordinated to the metal ions or are relevant for binding in the solvent-exposed region were retained for docking. Three-dimensional structures for all ligands were generated with LigPrep,[28] using Epik[29] for the determination of ligand ionization and tautomeric states. Preliminary binding poses of compound *(S)*-**14a** and (*S**,*S***)-**18b** in the PDE4B catalytic site were obtained with the Glide[30] standard precision (SP) mode giving the possibility to generate up to five distinct poses, which were then optimized applying the Glide XP refinement method. A visual analysis of the docked poses allowed the selection of the most relevant binding mode.

Expression, Purification, and Crystallization. The DNA sequence encoding the human PDE4B cat - UCR2 (241-659) sequence was cloned into pFastbac1 (Life Technologies) using BamH1 and EcoR1 restriction sites. Recombinant bacmid DNA was produced according to the Bac-to-Bac baculovirus expression method. The resulting recombinant baculovirus was used to infect 1 L of suspension cultures of Sf-9 cells. Following 72 h of incubation at 27 °C the supernatant was harvested, pelleted and stored at -80 °C prior to purification. The pellets were resuspended in lysis buffer and mechanically broken using a continuous cell disrupter (Constant Systems) at a pressure of 20 kpsi. All purifications were subsequently carried out eluting the protein with a buffer (50 mM Tris HCl pH 8.0, 300 mM NaCl, 350 mM imidazole, 1 mM DL-dithiothreitol) directly onto a G-25 desalt column pre-equilibrated in desalt buffer (50 mM Tris HCl pH 8.0, 50 mM NaCl, 1 mM DL-dithiothreitol). The elution was loaded onto

a Q-sepharose HP column (GE Healthcare) and eluted with a gradient of Q Buffer B (50 mM Tris HCl pH 8.0, 500 mM NaCl, 10 mM imidazole, 1 mM DL-dithiothreitol). Fractions containing PDE4B cat – UCR2 were subsequently purified by size exclusion chromatography (Superdex S100; 10 mM HEPES, 100 mM NaCl, 1 mM DL-dithiothreitol). Crystals of human PDE4B cat - UCR domain (10 mg/mL) were grown at 20 °C by hanging drop vapour diffusion. Drops comprised 2 µl protein solution mixed with a reservoir buffer consisting of 100 mM sodium acetate pH 4.5, 14% PEG400 and 50 mM calcium acetate. Crystals, up to 100 µm in largest dimension, grew from precipitate after 1-2 weeks. Crystals were transferred to a cryoprotectant solution (100 mM sodium acetate pH 4.5, 14% PEG400, 50 mM calcium acetate and 20% glycerol) and then flash-frozen prior to X-ray data collection.

Structure Determination. Data were collected at the DIAMOND synchrotron on a Pilatus P2M detector on beamline I04.1 with a wavelength of 0.92 Å. Crystals of the PDE4B/(*S**,*S***)-**18e** complex diffracted at 1.65 Å and belonged to the $P2_12_12_1$ space group, with cell parameters a = 55.27 Å, b = 55.73 Å, c = 225.56 Å. Phasing was performed with PHASER[31] and refinement was done with REFMAC.[32] The final model obtained an R_{work} of 18.4% and an R_{free} of 21.1%.

ASSOCIATED CONTENT

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website:

Synthesis and characterization of not commercially available acids **13c** and **13d** and preparation of intermediates **15**, **16**, **20**, **21**, **25**, **26**, **27**, **29**, **30**, **31**, **32**, **33** and **34**.

Summary of crystallographic data of the hPDE4Bcat-UCR in complex with the inhibitor (*S**,*S***)-**18e** and refinement statistics.

Molecular formula strings and biological data

Accession Codes. PDB code of compound (*S**,*S***)-**18e** 5OHJ (see Figure 5)

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Notes

The authors declare no competing financial interest.

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Abbreviations Used

ACN, acetonitrile; AcOH, acetic acid; ADME, absorption, distribution, metabolism, and excretion; AMP, adenosine monophosphate; BOC , tert-butoxycarbonyl; Boc_2O, di- tert-butyl dicarbonate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; Compd, compound; COPD, chronic obstructive pulmonary disease; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide); ESI, electrospray ionization; Et_2O, diethylether; EtOAc, ethyl acetate; EtOH, ethyl alcohol; FBS, fetal bovine serum; FCS, fetal calf serum; FEV1, forced expiratory volume; HPLC, high-performance liquid chromatography; iPr_2NH, diisopropylamine; $iPrO_2$, diisopropyl ether; iPrOH, isopropyl alcohol; LARBS, low-affinity rolipram binding site; LC-MS , liquid chromatography–mass spectrometry; LPS, lipopolysaccharides; *m*CPBA, meta-chloroperoxybenzoic acid; MeOH, methyl alcohol; MRT, mean residence time; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NEAA, nonesssential amino acids; NMR, nuclear magnetic resonance; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffer saline; PDE4, phosphodiesterase-4; Pgp, P-glycoprotein; PPB, plasma protein binding; RPMI, Roswell Park Memorial Institute; tR, retention time; TEA, triethylamine; THF, tetrahydrofuran; TLC, thin layer

chromatography; TNF-α, tumor necrosis factor-alpha; TWC, total white cells; UCR2, upstream conserved region 2; XRD, X-ray diffraction.

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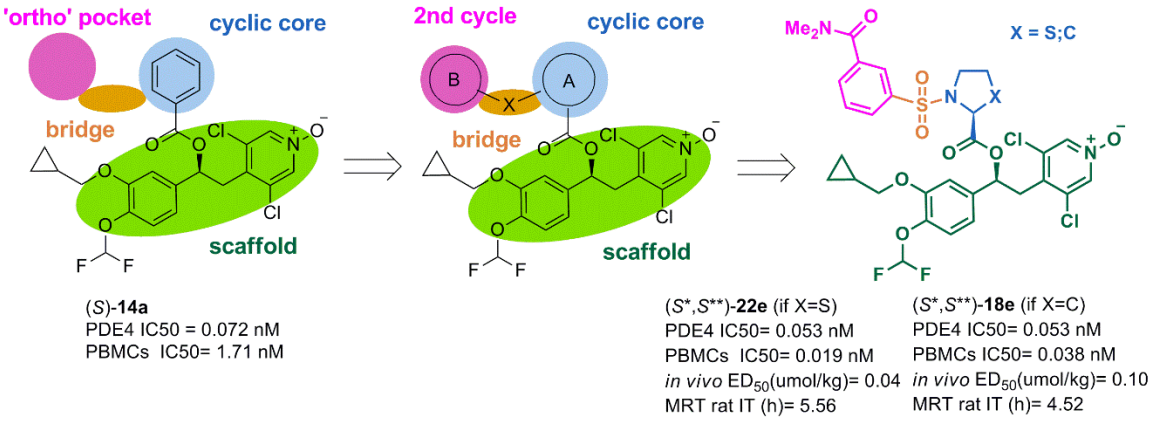
Table of Contents graphic



(*S*)-**14a**
PDE4 IC50 = 0.072 nM
PBMCs IC50= 1.71 nM

(*S**,*S***)-**22e** (if X=S)
PDE4 IC50= 0.053 nM
PBMCs IC50= 0.019 nM
in vivo ED_{50}(umol/kg)= 0.04
MRT rat IT (h)= 5.56

(*S**,*S***)-**18e** (if X=C)
PDE4 IC50= 0.053 nM
PBMCs IC50= 0.038 nM
in vivo ED_{50}(umol/kg)= 0.10
MRT rat IT (h)= 4.52