Discovery of (*S*)-*N*-{2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl}acetamide (Apremilast), a Potent and Orally Active Phosphodiesterase 4 and Tumor Necrosis Factor-α Inhibitor

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Abstract: In this communication, we report the discovery of **1S** (apremilast), a novel potent and orally active phosphodiesterase 4 (PDE4) and tumor necrosis factor-α inhibitor. The optimization of previously reported 3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(3,4-dimethoxyphenyl)propionic acid PDE4 inhibitors led to this series of sulfone analogues. Evaluation of the structure−activity relationship of substitutions on the phthalimide group led to the discovery of an acetylamino analogue **1S**, which is currently in clinical trials.

PDE4[a] is one of 11 known members of the PDE enzyme family.[1] It is a cyclic adenosine monophosphate (cAMP) specific enzyme, which hydrolyzes cAMP to AMP. Thus, inhibition of PDE4 leads to an increase of cAMP concentration, which influences multiple intracellular signaling pathways.[2] Since PDE4 is localized to specific subcellular sites in different parts of body and cells,[3] regulating the localized cAMP gradients in cells provides opportunities to affect various therapeutic areas, such as inflammation,[4] respiratory,[5,6] and central nervous system diseases.[7] Therefore, PDE4 has long been considered to be an important therapeutic target. Among various activities of PDE4 inhibitors, anti-inflammatory activity probably is the most widely studied and reported area. One of the driving forces for the anti-inflammatory activity of PDE4 inhibitors is the inhibition of production of TNF-α, a key cytokine in the inflammatory cascade. Elevated levels of TNF-α have been associated with a number of inflammatory diseases such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease. In the past decade, the success of anti-TNF-α biologics has valuated the importance of the blockade of TNF-α production in the treatment of patients with various inflammatory diseases.[8,9] Monocytes and macrophages are key producers of TNF-α, and PDE4 is a major PDE enzyme present in these cells. Therefore, production of TNF-α can be suppressed by inhibition of PDE4 in stimulated human peripheral blood mononuclear cells (hPBMC) and in human whole blood (WB).[10] This activity provides us with an important tool to examine the anti-inflammatory activity of our PDE4 inhibitors in human cells. We have previously reported on the development of a novel series of PDE4 and TNF-α inhibitors derived from 3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(3,4-dimethoxyphenyl)propionic acid derivatives.[11] Herein, we report the discovery of **1S** (apremilast), a novel orally active PDE4 and TNF-α inhibitor (Figure 1). **1S** is currently in clinical trials for the treatment of psoriasis.[12]

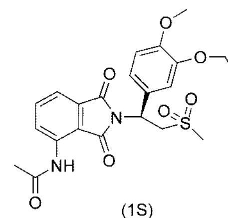

Figure 1. Structure of **1S**.

The racemates and enantiomers of phthalimide β-amino-β-aryl acid **4**, ester **5**, amide **6**, and nitrile **7** analogues were prepared as previously described.[11] The β-aminosulfone **3** was prepared by treatment of in situ generated imine of 3-ethoxy-4-methoxybenzaldehyde by lithium hexamethyldisilazide, with lithium dimethylsulfone and boron trifluoride etherate in a 41% yield. Resolution of the β-aminosulfone **3** was achieved by treatment of **3** with *N*-acetyl-L-leucine to give the *S*-isomer **3S** with an ee of 98.4%. The *R*-isomer **3R** could be obtained in a similar manner using *N*-acetyl-D-leucine with a 97.6% ee. Treatment of the corresponding phthalic anhydrides with **3** provided **9**−**11**. Coupling of 3-nitrophthalic anhydride with **3**, followed by hydrogen reduction gave **12**. When hydrogenation was performed in the presence of formaldehyde, the dimethylamino analogue **13** was obtained. Catalytic hydrogenation of 3-nitrophthalic acid followed by acetylation with acetic anhydride afforded 3-*N*-acetylaminophthalic anhydride. Compound **1S** was prepared by condensation of 3-*N*-acetylaminophthalic anhydride with β-aminosulfone **3S** in acetic acid in a 75% yield (Scheme 1). The enantiomeric purity of **1S** could be further enhanced by recrystallization from ethanol to give a sample with a 99.5% ee.[13]

Three assays were used to assess the in vitro potency of our PDE4 inhibitors. PDE4 inhibitory activity was measured with PDE4 enzyme isolated from U937 cells as previously reported.[14] TNF-α inhibitory activity was measured in lipopolysaccharide (LPS) stimulated hPBMC or WB.[11] The hPBMC and WB cells are human cells, and thus, these cellular assays may have greater clinical relevance than inhibition of purified PDE4. Furthermore, the difference between hPBMC and WB TNF-α inhibitory activity may serve as an indirect indicator of the degree of protein binding, and the WB data are a more realistic view of the clinical situation.

In a previous report, we described a novel series of 3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(3,4-dimethoxyphenyl)propionic acid and derivatives with TNF-α inhibitory activity.[11] Later, we reported that inhibition of PDE4 was the mechanism of action for TNF-α inhibition by this class of compounds.[14] In the effort to optimize the potency, isosteric replacement of the acid moiety was explored. Replacement of the acid moiety of **4a** with a methyl ester **5a** resulted in a 6-fold increase in PDE4

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[a] Abbreviations: PDE4, phosphodiesterase 4; TNF-α, tumor necrosis factor-α; SAR, structure−activity relationship; cAMP, cyclic adenosine monophosphate; hPBMC, human peripheral blood mononuclear cells; WB, human whole blood; LPS, lipopolysaccharide; CYP450, cytochrome P450.

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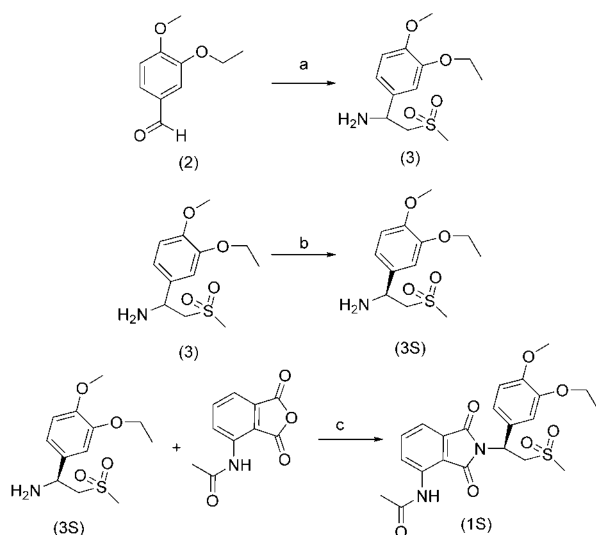
Scheme 1[a]



[a] Reagents and conditions: (a) $LiN(SiMe_3)_2$, then Me_2SO_2/*n*-BuLi/BF_3Et_2O, −78 °C; (b) *N*-Ac-L-leucine, MeOH; (c) HOAc, reflux.

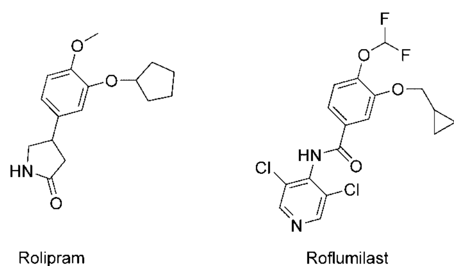


Figure 2. Structures of rolipram and roflumilast.

Table 1. TNF-α and PDE4 Inhibition (IC_{50} in μM) by 3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-(3-alkoxy-4-methoxyphenyl)-propionic Acid and Derivatives

compd	R	Y	TNF-α[a]	WB[b]	PDE4
4a	Et	COOH	35	ND[c]	1.3
4b	cyclopentyl	COOH	36	ND[c]	0.34
5a	Et	COOMe	0.7	4.0	0.22
5b	cyclopentyl	COOMe	1.7	ND[c]	1.6
6a	Et	$CONH_2$	2.7	10.5	1.9
6b	cyclopentyl	$CONH_2$	2.5	ND[c]	1.1
7a	Et	CN	0.24	0.49	0.04
7b	cyclopentyl	CN	1.6	ND[c]	0.34
8a	Et	SO_2Me	1.3	2.5	0.22
8b	cyclopentyl	SO_2Me	1.5	ND[c]	0.26

[a] TNF-α inhibition in LPS-stimulated hPBMC. [b] TNF-α inhibition in LPS-stimulated WB. [c] ND: not determined.

Table 2. TNF-α and PDE4 Inhibition (IC_{50} in μM) by Substituted 3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-(3-ethoxy-4-methoxyphenyl)-propionic Acid and Derivatives

compd	stereo	Y	Z	TNF-α[a]	WB[b]	PDE4
1	rac	SO_2Me	NHAc	0.19	0.44	0.082
1S	*S*	SO_2Me	NHAc	0.077	0.11	0.074
1R	*R*	SO_2Me	NHAc	0.37	ND[c]	0.61
9	rac	SO_2Me	OH	1.47	ND[c]	0.82
10	rac	SO_2Me	OMe	0.34	ND[c]	0.69
11	rac	SO_2Me	Me	0.27	2.4	0.068
12	rac	SO_2Me	NH_2	0.37	2.2	0.12
13	rac	SO_2Me	NMe_2	0.22	1.4	0.12
14	*R*	$CONH_2$	NHAc	3.0	ND[c]	0.71
15	rac	CN	NHAc	0.12	1.6	0.13

[a] TNF-α inhibition in LPS-stimulated hPBMC. [b] TNF-α inhibition in LPS-stimulated WB. [c] ND: not determined.

inhibitory activity and a 50-fold increase in TNF-α inhibitory activity. The PDE4 inhibitory activity of the amide analogue **6a** showed no improvement, but **6a** is 13-fold more potent in the inhibition of TNF-α in hPBMC compared to **4a**. Nitrile analogue **7a** was a potent compound, which resulted in a near 32-fold increase in PDE4 inhibitory activity and 145-fold increase in TNF-α inhibitory activity compared to **4a**. The sulfone moiety is also a good isosteric replacement of the acid moiety. Sulfone **8a** is 6-fold more potent in the inhibition of PDE4 and 27-fold more potent in the inhibition of TNF-α than **4a**. Interestingly, the ethoxy analogues **7a** and **8a** have similar potency against TNF-α in hPBMC and WB assays.

The 3,4-dialkoxyphenyl moiety is one of many known pharmacophores in PDE4 inhibitors.[15] Generally, a methoxy group at the 4-position is the optimal group, and a bulky group, such as cyclopentoxy, at the 3-position leads to more potent analogues in a majority of reported series of PDE4 inhibitors, which contain the 3,4-dialkoxyphenyl moiety. This feature can be exemplified by rolipram, a prototypical PDE4 inhibitor as shown in Figure 2. Therefore, to improve the potency, we also investigated the effect of the 3-alkoxy group. Surprisingly, the inhibitory activities of TNF-α and PDE4 of ethoxy analogues appeared to be better or no worse than those of cyclopentoxy analogues (**4**−**8a** vs **4**−**8b**) (Table 1), since literature precedence suggests that larger alkoxy moieties are preferred. Nevertheless, the 3-ethoxy-4-methoxyphenyl group was chosen as the standard group to further optimize the potency.

The effect of substitutions on the phthalimide group was explored to further improve the potency. Below we describe the effect of substitutions at the 4-position of the phthalimide ring in the sulfone series (Table 2). The details of SAR will be reported in future publications. Hydroxyl and methoxy substitutions of the 4-hydrogen in **8a** resulted in less potent PDE4 inhibitors **9** and **10**, respectively. Substitutions of 4-hydrogen with methyl **11**, amino **12**, or dimethylamino **13** groups led to slightly improved PDE4 inhibitory activity and TNF-α inhibitory activity in hPBMC, compared to those of the parent compound **8a**. However, their TNF-α inhibitory activities decreased 6- to 9-fold in WB versus the results in hPBMC. Introduction of a 4-*N*-acetylamino group led to the sulfone analogue **1**, which was 3-fold more potent than **8a** in the PDE4 assay and, more importantly, 6-fold more potent in the TNF-α assays. Interestingly, the 4-*N*-acetylamino group in analogues of amide **14** and nitrile **15** did not provide significant improvement on TNF-α inhibitory activity compared to their parent compounds **6a** and **7a**, respectively.

A single enantiomer is preferred in drug development. Resolution of the racemate of aminosulfone **3** followed by phthalimide formation yielded the *S*-isomer **1S** that was found to be the more active enantiomer, which was >5-fold more potent than the *R*-isomer **1R**. Some of the activity of the **1R** isomer may be due to residual **1S**, since **1R** contained 1.2% of **1S** by chiral HPLC.[13b] The PDE4 IC_{50} of **1S** from the PDE4

Table 3. Pharmacokinetics of **1S** in Female Rat

female rat	dose (mg/kg)	C_{max} (ng/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)	*F* (%)	Cl ((L/h)/kg)	V_{ss} (L/kg)
IV	5	6600	11 000	3		0.5	2
oral	10	1100	14 000	5	64		

enzymatic assay, and its TNF-α IC_{50} from hPBMC and WB cell based assays are very similar. Even though the TNF-α (in hPBMC) and PDE4 inhibitory activity of **1S** is significantly weaker than that of roflumilast (IC_{50} of 0.02 and 0.3 nM, respectively), the TNF-α IC_{50} in WB is only 2-fold different between **1S** and roflumilast (IC_{50} = 50 nM).[16] **1S** was further profiled for DMPK properties and tested in two in vivo models.

Compound profiling of **1S** revealed that it was stable in the presence of human microsomes ($t_{1/2} > 60$ min). It demonstrated IC_{50} values of >10 μM against cytochrome P450 (CYP450) enzymes, including 1A2, 2C9, 2C19, 2D6, and 3A4. **1S** was 90% protein bound in human plasma. Oral and intravenous administration of **1S** in female rats showed that it had good pharmacokinetics with low clearance, a moderate volume of distribution, and a 64% oral bioavailability (Table 3).

Two models of inflammation were used to characterize the in vivo efficacy of **1S**. The first model, a LPS-induced TNF-α inhibition model in rats, examined the TNF-α inhibitory ability of **1S** in vivo. A significant suppression of TNF-α production was observed at oral dose levels as low as 0.01 mg/kg, and the ED_{50} was determined to be 0.03 mg/kg. The second model, a LPS-induced neutrophilia model in rats,[17] measured the inhibition of neutrophils in bronchoalveolar lavage fluid, a common symptom observed in chronic obstructive pulmonary disease patients.[18] In this model, **1S** exhibited an ED_{50} of 0.3 mg/kg po in the inhibition of neutrophilia. In a similar model, roflumilast was reported to have an ED_{50} of 0.9 mg/kg.[19] The in vitro potency, in vivo oral efficacy, and excellent bioavailability supported advancement of **1S** to clinical development.

In summary, we optimized the structures of a series of 3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(3,4-dialkoxyphenyl)propionic acid analogues to enhance PDE4 and TNF-α inhibitory activity. We discovered that the sulfone moiety is a good isostere for the carboxylic acid moiety in this series. Substitutions at the 4-position of the phthalimide group did not have significant impact on PDE4 inhibitory activity in the enzymatic assay. However, addition of the *N*-acetylamino group led to the discovery of **1S**, which had enhanced potency in cell based assays. **1S** demonstrated good rat pharmacokinetics and oral efficacy in two rat inflammation models. **1S** was advanced into development, and two phase II clinical trials in psoriasis were reported with significant improvement in treated patients.[20] Further details of inhibition of PDE4 subtypes, other proinflammatory mediators, and adverse effects will be reported in future publications.

Supporting Information Available: Elemental analytical data of **1**, **1S**, **1R**, and **9**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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