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Highly potent PDE4 inhibitors with therapeutic potential

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Abstract—Based on the hypothesis that the dose-limiting side effects of PDE4 inhibitors could be mediated via the central nervous system (CNS), design and synthesis of a hydrophilic analogue is considered to be one approach to improving the side-effect profile of Ariflo™ **1**. Water-soluble piperidine derivatives were found to possess therapeutic potential.

Phosphodiesterase 4 (PDE4)[1–3] is a cyclic adenosine monophosphate (cAMP)-specific PDE that is expressed in inflammatory cells such as eosinophils. Inhibition of PDE4 results in elevation of the cAMP level in these cells, which in turn downregulates the inflammatory response.[4] The potential of PDE4 inhibitors as anti-inflammatory agents for the treatment of asthma and other inflammatory disorders has therefore received considerable attention.[5] However, no selective PDE4 inhibitor has reached the market yet because the early compounds (such as rolipram) caused dose-limiting side effects like nausea and emesis, which severely restricted their therapeutic utility.

The improved therapeutic index of Ariflo™ **1**[6] (Fig. 1) has been attributed to increased selectivity for the ability to inhibit PDE4 catalytic activity (LPDE4) versus the ability to compete for high-affinity[^{3}H]rolipram-binding site (HPDE4).[7,8] A second strategy is related to the design of PDE4 subtype-selective inhibitors.[9–13]

The dose-limiting side effects of PDE4 inhibitors are thought to be mediated via the central nervous system (CNS).[14,15] Accordingly, the negatively charged physical property of Ariflo **1** at a physiological pH might decrease the occurrence of side effects because of limited penetration into the CNS. Molecular design of a highly hydrophilic PDE4 inhibitor was thought to be another possible approach because compounds that show good penetration into the CNS have a relatively high lipophilicity.[16] As shown in Figure 1, focus was placed on the design and synthesis of a less lipophilic and/or zwitter-ionic piperidine derivative, which is structurally related to Ariflo **1** and expected to show the more limited penetration into the CNS.[17] A structure–activity relationship (SAR) study was started with the expectation that this approach would provide compounds with an improved therapeutic index.

As outlined in Scheme 1, test compounds were prepared from the benzyl cyanide **16**[6] by the following two synthetic pathways. Dialkylation of **16** with *cis*-2-butenyl-1,4-dichloride **17** afforded the cyclopentene derivative **18** in 73% yield. Oxidative cleavage of the cyclopentene moiety of **18** gave a dialdehyde **19**, which was used for the next reaction without isolation. Reductive amination of the dialdehyde **19** with an appropriate amino ester in the presence of sodium triacetoxyborohydride afforded **20a** and **20b** at moderate yields.[18] Deprotection of the benzylester **20a–b** was accomplished by catalytic

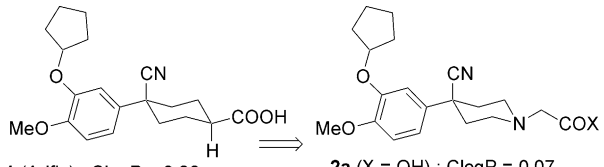

Figure 1. Molecular design of hydrophilic molecules.

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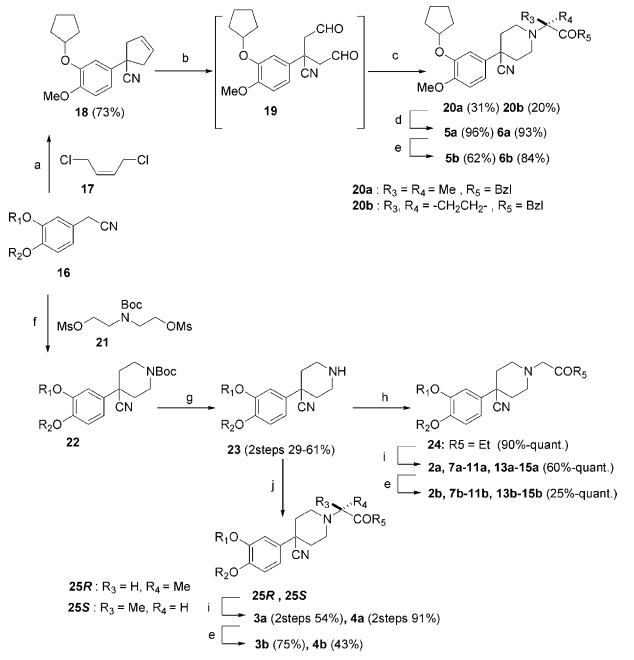


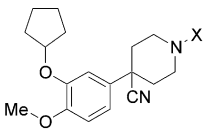
Scheme 1. (a) **17**, LiHMDS, THF, −78 °C; (b) O_3, CH_2Cl_2, then PPh_3, −78 °C; (c) $NaBH(OAc)_3$, AcOH, DCE, $NH_2CR_3R_4COR_5$; (d) H_2, 10% Pd/C, MeOH; (e) EDC, HOBt, DMF, $NH_2OC(CH_3)_2(OCH_3)$, then 2 N HClaq, MeOH; (f) **21**, LiHMDS, THF, −78 °C; (g) TFA, $C_6H_5SCH_3$, CH_2Cl_2, then 4 N HCl/EtOAc; (h) K_2CO_3, $BrCH_2COOEt$, DMF; (i) 2 N NaOHaq, EtOH; (j) methyl-*S*-(−)-lactate or methyl-*R*-(+)-lactate, Tf_2O, CH_2Cl_2, 2,6-lutidine, Et_3N.

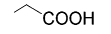
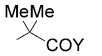
hydrogenation to give **5a** and **6a**, which were converted to the corresponding hydroxamic acid analogues **5b** and **6b**, respectively, by condensation with (1-methoxy-1-methyethyl)oxyamine followed by acidic deprotection. Compounds **2a**, **7a–11a**, and **13a–15a** were also prepared from **16** as follows. Dialkylation of **16** with dimesylate **21** gave the piperidine derivative **22**, acidic deprotection of which produced an amine derivative **23**. *N*-Alkylation of **23** with ethyl 2-bromoacetate afforded **24**, alkaline hydrolysis of which gave the carboxylic acid analogues **2a**, **7a–11a**, and **13a–15a**. Condensation of these carboxylic acid analogues with (1-methoxy-1-methyethyl)oxyamine, followed by acidic deprotection, produced the hydroxamic acid analogues **2b**, **7b–11b**, and **13b–15b**, respectively.

The series of piperidine derivatives listed in the Tables 1–3 were synthesized and biologically evaluated for the ability to inhibit PDE4 prepared from U937 cells[19] (derived from human monocytes). The results of the assays were expressed as IC_{50} values, that is the test compound concentration that caused 50% inhibition. Test compounds were also evaluated for the ability to inhibit lipopolysaccharide (LPS)-induced production of tumor necrosis factor-α (TNF-α) in rats.[20] The results were expressed as ID_{50} values, that is the dose that resulted in 50% inhibition relative to the effect of the vehicle.

In Table 1, the biological activities of these piperidine derivatives are summarized. Compound **2a** was designed to reduce the hydrophobicity of Ariflo **1** and to remove the configurational isomerism; it exhibited potent LPDE4 activity, with an oral dose of 3 mg/kg

Table 1. Activity profile of piperidine derivatives

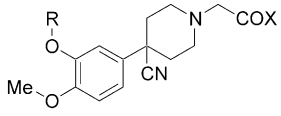


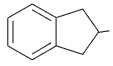
Compd	X	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α[b] ID_{50} (mg/kg, po)
2a		66 ± 39	(74%)[c]
2b		0.080 ± 0.050	0.04
3a (Y = OH)		> 300	NT[e]
3b (Y = NHOH)		0.91 ± 1.9	(26%)[d]
4a (Y = OH)		> 300	NT[e]
4b (Y = NHOH)		5.9 ± 7.9	(49%)[d]
5a (Y = OH)		> 1000	NT[e]
5b (Y = NHOH)		34 ± 11	NT[e]
6a (Y = OH)		> 1000	NT[e]
6b (Y = NHOH)		4.6 ± 1.4	2.0

[a] Inhibition of PDE4 prepared from U937 cells (a cell line derived from human monocytes). IC_{50} represent a mean of $n = 2$.
[b] ID_{50} for inhibition of LPS-induced TNF-α production in rats ($n = 7$) 0.5 h after oral dosing of a test compound.
[c] Inhibition % at 3 mg/kg, po.
[d] Inhibition % at 1 mg/kg, po.
[e] Not tested.

causing 74% inhibition of LPS-induced TNF-α production in rats. Based on reported information,[11] the corresponding hydroxamic acid analogue **2b** was extremely potent in the in vitro LPDE4 assay and showed an ID_{50} value of 0.04 mg/kg, orally in the in vivo TNF-α production assay. To improve the predicted rapid metabolic hydrolysis of **2b**, the design and synthesis of α-substituted and α,α-disubstituted analogues **3b–6b** was carried out as illustrated in Table 1. Compounds

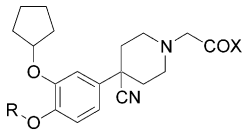
Table 2. Activity profile of piperidine derivatives



Compd	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
7a (X = OH)	Me-	> 100	NT[d]
7b (X = NHOH)		4.7 ± 1.1	0.23
8a (X = OH)	Et-	> 100	(0%)[b]
8b (X = NHOH)		0.52 ± 0.12	0.04
9a (X = OH)		> 100	(12%)[b]
9b (X = NHOH)		0.29 ± 0.03	0.03
10a (X = OH)	*i*-Propyl—	> 100	NT[d]
10b (X = NHOH)		1.0 ± 0.8	0.09
11a (X = OH)	*c*-Butyl	> 100	NT[d]
11b (X = NHOH)		0.18 ± 0.02	0.03
12 (X = OH)		67 ± 19	(29%)[c]

[a] See corresponding footnotes from Table 1.
[b] Inhibition % at 1 mg/kg, po.
[c] Inhibition % at 3 mg/kg, po.
[d] Not tested.

Table 3. Activity profile of piperidine derivatives



Compd	R	LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
13a (X = OH)	Et-	2500 ± 980	NT[b]
13b (X = NHOH)		6.2 ± 1.1	0.7
14a (X = OH)	CHF_2-	65 ± 11	1.1
14b (X = NHOH)		0.051 ± 0.007	0.02
15a (X = OH)	*i*-Propyl—	> 1000	NT[b]
15b (X = NHOH)		> 30	NT[b]

[a] See corresponding footnotes from Table 1.
[b] Not tested.

3a–**6a** were also synthesized and evaluated for comparison with the corresponding hydroxamic acid analogues. All of the corresponding carboxylic acid analogues **3a**–**6a** exhibited much less potency in the LPDE4 assay than their corresponding hydroxamic acid analogues **3b**–**6b,** respectively. Compounds **3a**–**4a** and **5a**–**6a** exhibited less than 50% inhibition at 0.3 and 1 μM, respectively. The above chemical modification was found to cause marked reduction of the activity of the new chemical lead **2b**. More details will be discussed in the subsequent full report.

As shown in Table 2, further attempts at optimization of the cyclopentyl moiety of **2a**–**b** were made. Replacement of the cyclopentyl moiety of **2b** with *n*-alkyl chains such as methyl and ethyl moieties resulted in **7b** and **8b**, respectively. Compound **7b** showed reduced potency in both in vitro and in vivo tests, while **8b** showed slightly reduced in vitro potency and retained its in vivo potency. Replacement of the cyclopentyl moiety of **2b** with a branched alkyl moiety, such as an isopropyl moiety, gave **10b**, which showed nearly 10-fold reduction of in vitro potency and 2-fold reduction of in vivo potency. Replacement of the cyclopentyl moiety of **2b** with a cyclopropylmethyl moiety afforded **9b**, which also had slightly reduced in vitro potency and retained its in vivo potency. Ring contraction of the cyclopentyl moiety of **2b** produced a cyclobutyloxy analogue **11b**, which had slightly less in vitro activity and retained its in vivo potency. Interestingly, indan-2-yloxy analogue **12**[21] demonstrated equipotent LPDE4 activity compared with **2a**, while it exhibited less in vivo potency than **2a**. The corresponding carboxylic acids **7a**–**11a** demonstrated markedly reduced LPDE4 inhibitory activity compared with **2a**.

As shown in Table 3, further optimization of the methoxy moiety of **2a**–**b** was carried out. Replacement of the methoxy moiety of **2b** with an ethyloxy moiety gave **13b**, which had nearly 100-fold less LPDE4 inhibitory activity and nearly 10-fold less in vivo activity. Replacement of the methoxy moiety of **2b** with an isopropyloxy moiety resulted in **15b**, also with markedly reduced LPDE4 inhibitory activity. Interestingly, replacement of the methoxy moiety of **2b** with a difluoromethoxy[22] moiety produced **14b**, which had slightly increased LPDE4 activity and demonstrated 2-fold greater in vivo potency. The corresponding carboxylic acid derivatives **13a** and **15a** showed much lower potency both in vitro and in vivo, although **14a** had nearly the same potency as **2a** in both the LPDE4 assay and the LPS-induced TNF-α production assay. As described above, chemical modification of the methoxy moiety of **2a**–**b** was found to be much more limited than that of the cyclopentyl moiety, while the difluoromethoxy moiety was identified as another optimized moiety that could be replaced by methoxy. Among the several possible changes in the physical properties of a molecule that occur when a hydrogen atom is replaced by a fluorine atom, an increase of at least three factors can be expected, which are steric bulkiness, lipophilicity, and metabolic stability. More details will be discussed using the Log D values and the molal volume calculated by QMPRPLUS™,[23] in the subsequent full report.

Further biological evaluation of compounds **2b**, **8b**, **9b** and **14a**, which were selected based on the in vitro LPDE4 assay and in vivo TNF-α production, was carried out as shown in Table 4. These compounds were evaluated for the inhibition of slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction[24,25] for their beneficial effect and gastric acid emptying in rats[26] for their side effect. These compounds were also evaluated for inhibition of LPS-induced TNF-

Table 4. Biological profile of **1**, **2b**, **8b**, **9b** and **14a**

Compd	SRS-A-mediated bronchoconstriction[a] ID_{50} (mg/kg, po)	Inhibition of TNF-α production[b] ID_{50} (mg/kg, po)	Inhibition of gastric emptying[c] ID_{50} (mg/kg, po)	Inhibition of TNF-α production[d] IC_{50} (μM) in HWB
1 (Ariflo)	4.5	1.7	5.7	18
2b	0.3	0.04	0.3	0.0089
8b	0.04	0.04	0.7	0.0050
9b	0.3	0.03	0.3	0.0021
14a	4.2[e]	1.1	4.7	0.84

[a] Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs ($n = 3$–6); OVA challenge 0.15 mg/kg 1 h after oral dosing of a test compound.
[b] See corresponding footnotes from Table 1.
[c] Inhibition of gastric emptying in rats ($n = 5$).
[d] Inhibition of LPS-induced TNF-α production in human whole blood. IC_{50} represent a mean of $n = 3$.
[e] Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs ($n = 3$–6); OVA challenge 0.5 mg/kg 1 h after oral dosing of a test compound.

α production in human whole blood (HWB)[27] to estimate their clinical potential. The effect of these compounds on SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs was not always consistent with their potency for the inhibition of LPS-induced TNF-α production in rats, probably because of differences in this cross-species comparison. For example, **2b** and **9b** were much less potent in guinea pigs than expected from their effects on TNF-α production, while **8b** was equipotent in each assay. To assess one of the safety problems, inhibition of gastric emptying by **2b**, **8b**, **9b** and **14a** was evaluated in rat. The ID_{50} values at which these compounds affected gastric emptying were found to be higher than the values for TNF-α production, which is one of their beneficial effects.

Several compounds (**2b**, **8b**, **9b**, and **14a**) demonstrated more potency than Ariflo **1** against SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs and LPS-induced TNF-α production in rats. Based on their much more potent effect on LPS-induced TNF-α production in HWB compared with Ariflo **1**, these compounds could show improved therapeutic potential in clinical applications.

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