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New orally active PDE4 inhibitors with therapeutic potential

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Abstract—Structural optimization of pyrazolopyridine derivative **2**, which is one of the newly discovered chemical leads for PDE4 inhibitors from our in-house library, was carried out successfully. The process of discovery of new orally active PDE4 inhibitors, which are expected to possess therapeutic potential, is presented and their structure–activity relationships are discussed.
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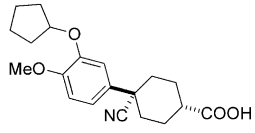
Phosphodiesterase 4 (PDE4) is an enzyme that is responsible for the inactivation of cyclic adenosine monophosphate (cAMP).[1,2] Inhibition of this enzyme results in elevation of cAMP expression in inflammatory cells such as eosinophils. The anti-inflammatory effects of PDE4 inhibitors have been well documented both in vitro and in vivo in a variety of animal models,[3] but no inhibitor has yet been used clinically because of dose-limiting side effects such as nausea and vomiting that restrict their therapeutic potential.[4]

Two strategies have already been reported for the production of efficient PDE4 inhibitors with less side effects. Ariflo™ **1**[5] (Fig. 1) and the other second-generation PDE4 inhibitors were reported to be more LPDE4-selective[6] than (*R*)-rolipram and/or to be PDE4 subtype-selective.[7,8]

In previous papers, we have described the design and synthesis of bicyclo[3·3·0]octane derivatives[9] and piperidine derivatives[10] as interesting new classes of PDE4 inhibitors with therapeutic potential. As we reported previously, investigation of the structure–activity relationship (SAR) profile of PDE4 inhibitors based on the structural features of Ariflo has revealed that modification of Ariflo can give a series of PDE4 inhibitors with an improved therapeutic index relative to the classical inhibitor, rolipram. To achieve the molecular design of efficient new inhibitors with improved therapeutic potential, discovery and biological evaluation of a new chemical lead with a completely different structure was considered to provide us with another approach to the chemical modification of rolipram.

The pyrazolopyridines have been reported to be active compounds for PDEs, adenosine and benzodiazepine receptors.[11–13] We identified compound **2** as a lead compound by high throughput screening (HTS) of our in-house library. As a result of structural optimization of **2**, an orally active PDE4 inhibitor, compound **12** (Fig. 2) was discoverd. The results of our SAR study are also reported.

Synthesis of the test compounds listed in Tables is outlined in Scheme 1.[12] Michael addition of **16** to an acceptor **21** followed by the removal of an ethoxy moiety provided **17**, after which ring closure was accomplished with phosphorus oxychloride under reflux to afford chloride **18**. Alkaline hydrolysis of **18** gave carboxylic acid **19**. Heating **19** with thionyl chloride followed by treatment with aqueous ammonia resulted in the amide **20**. Replacement of the chloro moiety of **20** with appropriate anilines gave us the test compounds **2**–**15**.



1 (Ariflo)

Figure 1. Structure of Ariflo **1**.

Keywords: PDE4; Inhibitor; Pyrazolopyrioline; Orally active.
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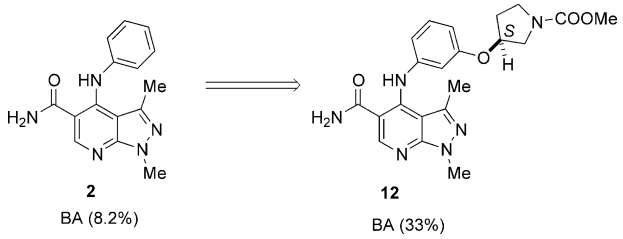


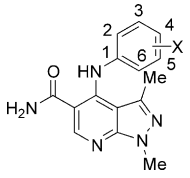
Figure 2. Discovery of a new chemical lead **2** and identification of an orally bioavailable PDE4 inhibitor **12**.

A series of pyrazolopyridine derivatives were synthesized and evaluated for the ability to inhibit PDE4 prepared from U937 cells[14] (derived from human monocytes). The results of the assays were expressed as IC_{50} values, i.e., the test compound concentration that achieved 50% inhibition relative to the vehicle. Test compounds were also evaluated for the ability to inhibit lipopolysaccharide (LPS)-induced production of tumor necrosis factor-α (TNF-α) in rats.[15] The results were expressed as ID_{50} values, that is, the dose that resulted in 50% inhibition relative to the vehicle.

During the course of screening of PDE4 inhibitors, compound **2** was found to demonstrate moderate potency in both the in vitro and in vivo assays. As shown in Table 1, design and synthesis of *mono*-substituted aniline derivatives **3**–**10** was carried out. Introduction of a *ortho*-methoxy group into the aniline moiety of **2** afforded **3**, which was slightly less potent in vitro assay. Introduction of a *meta*-methoxy group into the phenyl moiety of **2** gave **4**, which showed 10-fold greater potency in vitro assay. Interestingly, *para*-methoxy derivative **5** did not show LPDE4 inhibitory activity up to a concentration of 0.1 μM. Thus, *meta*-methoxy derivative **4** exhibited the most potent inhibitory activity in both the in vitro and in vivo assays among the three isomers **3**–**5**. Further chemical modification of the *meta*-methoxy moiety of **4** was subsequently carried out. Replacement of the *meta*-methoxy group of **4** with *meta*-cyclopentyloxy, *meta*-cyclohexyloxy, and *meta*-cyclobutyloxy groups resulted in **6**, **7**, and **8**, respectively, which all showed a marked decline of LPDE4 inhibitory activity. Removal of the methyl portion of the methoxy moiety of **4** afforded *meta*-hydroxy derivative **9**, which was nearly 19-fold less potent in its LPDE4 inhibitory activity, while **9** showed 40% inhibition of TNF-α production in rats at an oral dose of 10 mg/kg. Replacement of the methoxy group of **4** with a nitrile group gave **10**, which showed a nearyl 17-fold decline of LPDE4 inhibitory activity and also failed to inhibit TNF-α production in rats at an oral dose of 3 mg/kg.

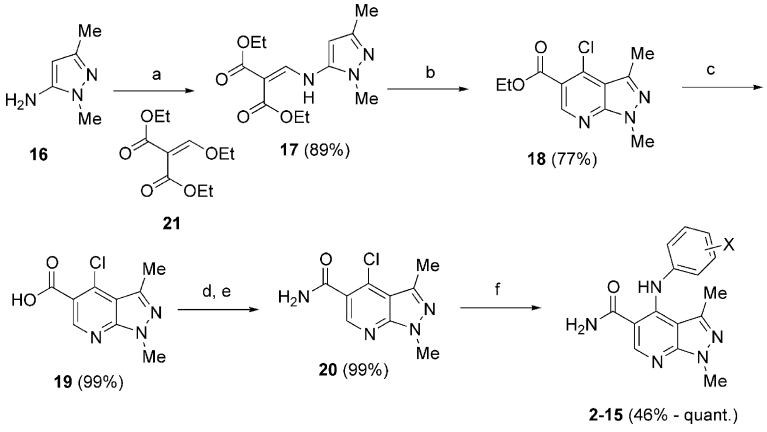
Compounds **2** and **4** demonstrated very poor bioavailability (BA) despite relatively good oral activity (the BA of **2** and **4** was 8.2 and 1.7%, respectively). Design and

Table 1. Activity profile of pyrazolopyridine derivatives **2**–**10**



Compd	X	Inhibition of LPDE4[a]	Inhibition of TNF-α[b]
		IC_{50} (μM)	ID_{50} (mg/kg, po)
2	H	0.036±0.015	(48%)[c]
3	2-OMe	0.076±0.037	(68%)[c]
4	3-OMe	0.0050±0.0007	2.4
5	4-OMe	>0.1	NT[e]
6	3-*c*Pentyloxy	>0.1	(39%)[c]
7	3-*c*Hexloxy	>0.1	NT[e]
8	3-*c*Butyloxy	>0.1	NT[e]
9	3-OH	0.097±0.083	(40%)[c]
10	3-CN	0.085±0.005	(2%)[d]

[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 10 mg/kg, po.
[d] Inhibition% at 3 mg/kg, po.
[e] Not tested.



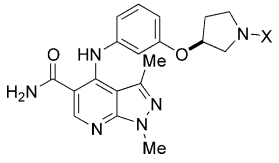
Scheme 1. (a) **21**, 120 °C; (b) $POCl_3$, 120 °C; (c) KOHaq, dioxane; (d) $SOCl_2$, 80 °C; (e) NH_3aq, THF; (f) Aniline derivatives, dioxane, reflux.

synthesis of PDE4 inhibitors with a better oral BA was attempted, and compound **12** was discovered to show both good potency and a good BA (33%). Thus, further chemical modification of the pyrolidine moiety of **12** was carried out as illustrated in Table 2. Removal of the methoxycarbonyl moiety of **12** afforded **11**, which showed complete loss of LPDE4 inhibitory activity at 0.3 μM. Therefore, the *N*-methoxycarbonyl moiety of **12** seems to be essential for its potent inhibitory activity. Surprisingly, the *R*-enantiomer of **12** exhibited less than 50% inhibition (~20%) at 0.1 μM. Replacement of the methoxycarbonyl moiety of **12** with a *tert*-butoxycarbonyl moiety gave **13**, which also showed loss of activity at 0.3 μM. The *N*-acetyl pyrolidinyloxy derivative **14** exhibited 41% inhibition in LPDE4 assay at a concentration of 0.3 μM. LPDE4 inhibitory activity was restored in the case of *N*-sulfonylmethyl derivative **15**, which had an IC_{50} value of 0.25 μM. As a result, compounds with good potency and good BA such as **12** were obtained by very limited chemical modification.

Further biological evaluation of compounds **4** and **12**, which were selected based on their oral activity against LPS-induced TNF-α production assay, was carried out as shown in Table 3. These compounds were evaluated for their ability to inhibit slow reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction.[16,17] The results were expressed as ID_{50} values, that is, the dose that resulted in 50% inhibition relative to the vehicle. These compounds were also evaluated for the ability to inhibit TNF-α production in human whole blood (HWB)[18] to estimate their clinical potential. The results of the assays were expressed as IC_{50} values, that is, the test compound concentration that resulted in 50% inhibition relative to the vehicle. The potency of these compounds for inhibiting in SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs was not always consistent with their potency in inhibiting LPS-induced TNF-α production in rats, probably because of differences in pharmacokinetics due to cross-species comparison. Compounds **4** and **12** demonstrated 50% inhibition of SRS-A-induced bronchoconstriction at ID_{50} values of 6.8 mg/kg, po and 45% inhibition at 10 mg/kg, po, respectively, while their ID_{50} values for TNF-α production were 2.4 and 1.0 mg/kg, respectively. With respect to inhibition of gastric emptying in rats,[19] the ID_{50} values of **4** and **12** were higher than that of Ariflo **1**, while their IC_{50} values for LPS-induced TNF-α production in HWB were lower than that of Ariflo **1**. Based on the above-mentioned biological data, **4** and **12** were estimated to be likely to have improved therapeutic potential and an improved side effect profile. Both of the compounds were also evaluated in the in vivo ferret emesis model. According to our evaluation, they did not cause emesis up to the oral dose of 10 mg/kg.

Design and synthesis of efficient new PDE4 inhibitors with improved therapeutic potential was accomplished by the structural optimization of chemical lead **2**, which was found in our in-house library. Among the compounds tested, **4** and **12** could have improved therapeutic potential with an improved side effect profile based on biological data obtained using both cross-species comparison and same-species comparison. Full details will be reported in due course.

Table 2. Activity profile of pyrazolopyridine derivatives **11**–**15**



Compd	X	Inhibition of LPDE4[a]	Inhibition of TNF-α[a]
		IC_{50} (μM)	ID_{50} (mg/kg, po)
11	H	>0.3	NT[a]
12	COOMe	0.010±0.005	1.0
13	COO*t*Bu	>0.3	NT[a]
14	COMe	>0.3	NT[a]
15	SO_2Me	0.25±0.01	NT[a]

[a] See corresponding footnotes from Table 1.

Table 3. Activity profile of pyrazolopyridine derivatives **4**, **12** and Ariflo **1**

Compd	Inhibition of bronchoconstriction[a]	Inhibition of TNF-α production[b]	Inhibition of gastric emptying[c]	Inhibition of TNF-α production in HWB[d]	Ferret emesis[e] (vomiting/tested)		
	ID_{50} (mg/kg, po)	ID_{50} (mg/kg, po)	ID_{50} (mg/kg, po)	IC_{50} (μM)	3	10 (mg/kg, po)	30
1(Ariflo)	4.5	1.7	5.7	18	NT[g]	NT[g]	NT[g]
4	6.8	2.4	23%[f]	3.0	0/2	0/2	1/2
12	45%[f]	1.0	15	4.8	0/2	0/3	2/2

[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] Vomiting test in fasted ferrets.
[f] Inhibition% at 10 mg/kg, po.
[g] Not tested.

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