Bioorganic & Medicinal Chemistry Letters 24 (2014) 2689–2692

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of 2,3-disubstituted pyridines as potent, non-emetic PDE4 inhibitors

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ARTICLE INFO

Article history:
Received 18 March 2014
Revised 10 April 2014
Accepted 12 April 2014
Available online 20 April 2014

Keywords:
Phosphodiesterase 4
PDE4 inhibitors
2,3-Disubstituted pyridine
Structure activity relationship
Anti-inflammatory agent

ABSTRACT

A series of 2,3-disubstituted pyridines were synthesized as potential non-emetic PDE4 inhibitors. To decrease brain exposure and minimize emesis, we modified the lipophilic moiety of a series of emetic PDE4 inhibitors and found that introduction of a hydroxy group into the pyridine moiety of the side chain led to non-emetic compounds with preserved PDE4 inhibitory activity. Following optimization at the phenoxy group, we identified compound **1** as a potent non-emetic PDE4 inhibitor. Compound **1** showed significant efficacy in an animal model of asthma without inducing emesis.

Type 4 cAMP phosphodiesterase (PDE4) specifically hydrolyzes cAMP, a pivotal second messenger, and its inhibition is known to effectively increase intracellular cAMP levels and to regulate various cellular functions.[1] PDE4 is reported to be expressed in key effector cells involved in asthma, particularly airway smooth muscle cells as well as in inflammatory cells, including eosinophils, neutrophils, T-lymphocytes, and macrophages. It has been reported that PDE4 inhibitors significantly increase intracellular cAMP in airway smooth muscle cells, thereby providing a bronchodilatory effect.[2–4] In addition to the first generation PDE4 inhibitor rolipram, a number of second generation PDE4 inhibitors (Fig. 1) have been reported with roflumilast (Daxas™, Daliresp™) recently approved for severe chronic obstructive pulmonary disease (COPD).[5–7] Despite significant progress in this area, PDE4 inhibitors are often associated with side effects such as nausea, emesis, and vasculopathy, all of which limit the therapeutic use of these agents.[8] This highlights the need for novel pharmacophores that would allow the design of safer PDE4 inhibitors. Here, we describe the discovery of 2,3-disubstituted pyridines as a new class of potent non-emetic PDE4 inhibitors and the evaluation of identified compound **1** (Fig. 1).

In our search for safer PDE4 inhibitors, we identified compound **2** (Fig. 2) as a potent, orally available candidate.[9] However, further in vivo evaluation of this compound revealed emesis as side effect in ferrets. To overcome this problem, we investigated ways to improve compound **2** safety profile without affecting its PDE4 inhibitory activity. Based on the hypothesis that compound **2** safety concerns may be attributed to its high lipophilicity, which leads to high brain exposure, we considered structural modifications that would allow reduction of compound **2** lipophilicity.

Our strategy for generation of lead non-emetic PDE4 inhibitors is shown in Figure 2. Basically, we hypothesized that inhibition of PDE4 in the central nerve system (CNS) causes emesis and that reduced brain exposure by increasing hydrophilicity leads to decrease the potency to induce emesis in ferrets.

Structure activity relationships (SARs) of the prepared 2,3-disubstituted pyridines are summarized in Table 1. First, we changed the S-linker of the phenylthio moiety of compound **2** to an O-linker (**3**), NH-linker (**4**), carbonyl linker (**5**), or a methylene linker (**6**). Replacement of the S-linker by an O-linker or a methylene linker had no effect on PDE4 inhibitory activity. On the other hand, PDE4 inhibition decreased following use of an NH-linker or a carbonyl linker. Second, we introduced a hydroxyl group at the 4-pyridyl group. This is because it has been reported that metabolism of roflumilast to form an N-oxide (Fig. 1) results in low brain penetration and therefore reduced emetic effect.[7] To avoid decrease in

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http://dx.doi.org/10.1016/j.bmcl.2014.04.052
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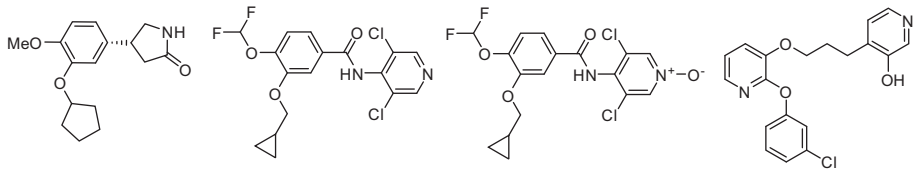


Figure 1. Chemical structures of selected PDE4 inhibitors.

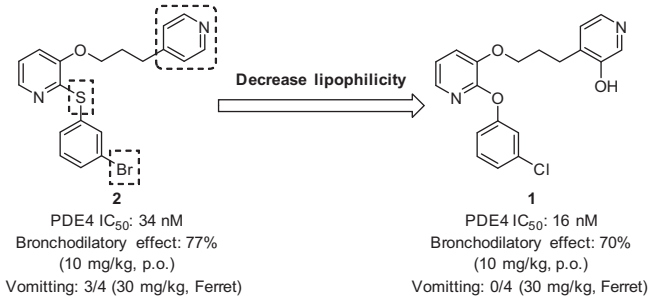


Figure 2. Strategy for reducing compound **2** lipophilicity.

the pyridyl group N-coordinating ability for binding to the metal site of PDE4 enzyme, we introduced a hydroxyl group at the 3-position of the pyridine ring, allowing the formation of pyridone from 2-hydroxy pyridine. Evaluation of the synthesized compounds **7**, **8** and **9** revealed a maintained PDE4 inhibitory activity in vitro, although the bronchodilating effect of compounds **7** and **9**, with the S-linker and methylene linker, respectively decreased. Compound **8**, on the other hand, showed bronchodilating effect with no vomiting in ferrets even at 300 mg/kg, po.

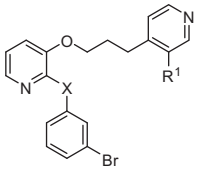
Next, we considered various substituents at the 3-position of the phenyl ring (Table 2). Replacement of the bromo group in compound **8** with a chloro (**1**) or a fluoro (**10**) group resulted in maintained PDE4 inhibitory activity. Among these compounds, oral administration of compound **1** at 4 h before ovalbumin (OVA) challenge produced bronchodilating effect. Based on this long-lasting bronchodilating effect, we selected compound **1** as a lead compound and considered various substituents at the 2- and 4-positions of the phenoxy ring. Introduction of a chloro group at the 2- (**11**) or 4-position (**12**) decreased PDE4 inhibitory activity. In addition, introduction of two chloro groups at the 3- and 5-positions diminished PDE4 inhibitory activity (**13**).

The results of further in vitro evaluation of compound **1** are summarized in Table 3. Compound **1** inhibited both human and guinea pig PDE4 without inhibiting PDE3. In addition, compound **1** inhibited both antigen-induced immediate asthmatic response (IAR) and late asthmatic response (LAR) in guinea pigs. Based on these encouraging results, we selected compound **1** as a potent non-emetic PDE4 inhibitor drug candidate.

Finally, we evaluated compound **1** potential for inducing vomiting in ferrets. Oral administration of compound **1**, given at up to 300 mg/kg, did not cause vomiting (Table 4). On the other hand, both rolipram and compound **2** dose-dependently caused vomiting in ferrets. These results indicate that modification of the lipophilic moiety of compound **2** expanded its therapeutic margin.

To clarify the mechanism by which compound **1** avoids emesis, we conducted a pharmacokinetic study of this compound in guinea pigs. The results of this study revealed that compound **1** is immediately metabolized to its sulfate **14** and glucuronide **15** (Fig. 3). Investigation of compound **1** distribution after oral administration of ^{14}C-labelled compound **1**[10] showed a lower concentration in the brain than in the plasma, lung or trachea (Table 5). In addition, the unchanged compound **1** was detected in a much lower concentration than its metabolites **14** and **15** in the plasma (Table 5). These findings suggest that distribution of the unchanged compound **1** into the brain is limited. This distribution profile is believed to contribute to significant reduction of emetic side effect. On the other hand, the concentrations of compound **1** in the lung and trachea were higher than its concentration in the plasma. These results indicate that compound **1** is reformed by deconjugation of its glucuronide (metabolite **15**) with β-glucuronidase in the lung and

Table 1
SAR of the prepared 2,3-disubstituted pyridines



Compound	X	R^1	PDE4 inhibition[9] (%, 100 nM)	Bronchodilatory effect[9] (%, 10 mg/kg, po)	Cell infiltration inhibition[9] (%, 10 mg/kg, po)	Vomit[a] (30 mg/kg, po)
2	S	H	80	77	50	4/4
3	O	H	80	49	23	NT
4	NH	H	51	NT	NT	NT
5	CO	H	27	NT	NT	NT
6	CH_2	H	89	63	43	NT
7	S	OH	66	8	33	NT
8	O	OH	77	70	50	0/4
9	CH_2	OH	87	13	44	NT

NT: Not tested
[a] Male Marshall ferrets were used in this experiment. The number of vomits for each animal was recorded for 2 h following oral administration of test compound. The number of responding animals was also recorded.