Bioorganic & Medicinal Chemistry Letters 19 (2009) 3174–3176

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of selective PDE4B inhibitors

Kenji Naganuma, Akifumi Omura, Naomi Maekawara, Masahiro Saitoh, Naoto Ohkawa, Takashi Kubota, Hiromitsu Nagumo, Toshiyuki Kodama, Masayoshi Takemura, Yuji Ohtsuka, Junji Nakamura, Ryuichi Tsujita, Koh Kawasaki, Hirotsugu Yokoi, Masashi Kawanishi *

Pharmaceuticals Research Center, Asahi Kasei Pharma Corporation, 632-1 Mifuku, Izunokuni-shi, Shizuoka 410-2321, Japan

ARTICLE INFO

Article history:
Received 15 March 2009
Revised 22 April 2009
Accepted 24 April 2009
Available online 3 May 2009

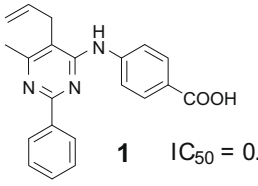
Keywords:
PDE4B
Neutrophilia
2-Arylpyrimidine

ABSTRACT

In this study the first PDE4B selective inhibitor is described. Optimization of lead 2-arylpyrimidine derivatives afforded a series of potent PDE4B inhibitors with >100-fold selectivity over the PDE4D isozyme. With a good pharmacokinetic profile, a selected compound exhibited potent anti-inflammatory effects in vivo and showed less emesis compared with Cilomilast.

Phosphodiesterase 4 (PDE4) is a hydrolytic enzyme responsible for the degradation of the second messenger cAMP in many cell types, and has been proposed as an attractive target in various diseases including asthma and chronic obstructive pulmonary disease (COPD).[1,2] There are a number of PDE4 inhibitors including roflumilast[3] and Cilomilast[4] which have been applied in clinical trials, however, extensive use of these compounds is limited because of dose-limiting adverse effects such as nausea and emesis.[2] The PDE4 family consists of four isoforms; PDE4A, 4B, 4C, and 4D. Knockout studies have revealed that PDE4B ablation suppresses TNF-α production[5], and PDE4D may be responsible for the occurrence of nausea and emesis,[1a,6] heart failure and the risk of arrhythmias.[7] In this regard, a PDE4B inhibitor selective over PDE4D is expected to be a useful anti-inflammatory agent and a chemical probe is needed to investigate the role of PDE4B in detail. However, high structural similarity between PDE4B and PDE4D has made it difficult to develop a highly selective inhibitor which to date has not been possible.[8] In this letter, we describe the discovery of the first PDE4B selective inhibitor with novel structural features which has potential as an anti-inflammatory drug.

By screening our in-house compound library, a pyrimidine derivative **1** was identified with potent inhibitory activity against PDE4B. **1** has an IC_{50} of sub-micromolar range and a potential 10-fold selectivity over PDE4D. We focused on investigations on the structure–activity relationships to optimize this lead compound.



1 IC_{50} = 0.19μM

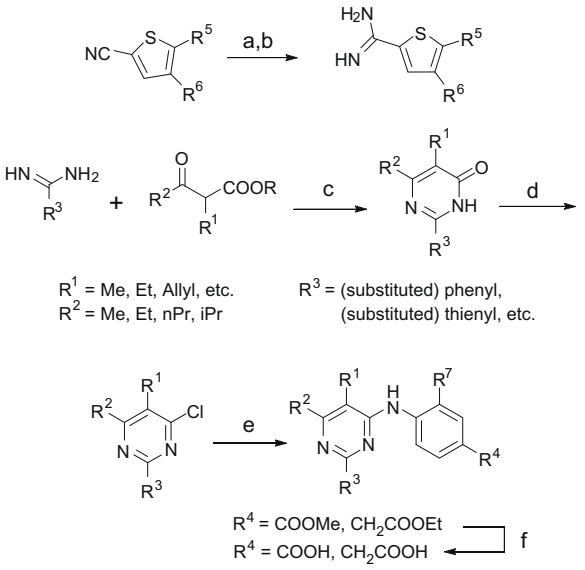
The synthesis of the pyrimidine derivative is outlined in Scheme 1.[9] The amidines used in this study were commercially available or were prepared from nitriles via methyl imidates. Condensation of amidines with β-ketoesters under basic conditions gave 4-oxopyrimidines, followed by chlorination with phosphorus oxychloride. The resulting 4-chloropyrimidines were substituted with anilines in the presence of acid to afford 4-aminopyrimidines. Carboxylic acids were prepared by saponification of corresponding esters.

Firstly, the structure–activity relationships (SAR) of the alkyl groups at 5- and 6-positions on the pyrimidine ring were investigated (Table 1).[10] The compounds bearing allyl ethyl, cyano, or formyl groups at 5-position were equipotent for PDE4B inhibition and 4B/4D selectivity (**1**, **3**, **8** and **10**). Bulkier groups at this position reduced the potency (**4**–**7**, **13** and **14**). Compound **12** which had an ethyl group on 6-position showed enhanced potency but less selectivity compared with methyl (**1**) analog. Thus it seemed that the steric effect was significant in these positions.

SAR exploration of the phenyl group at 2-position of the pyrimidine ring revealed substitution to 2- or 3-thienyl group (**15** and **16** in Table 2, respectively) which could be suitable alternatives without a loss of potency or selectivity. The introduction of a 2-methyl-

* Corresponding author. Fax: +81 558 76 5755.
E-mail address: kawanishi.mb@om.asahi-kasei.co.jp (M. Kawanishi).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.121

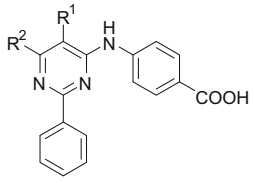


Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, rt; (b) NH_4Cl, EtOH, 90 °C; (c) NaH, MeOH, 55 °C; (d) $POCl_3$, 100 °C; (e) aniline, HClaq, EtOH, 55 °C; (f) NaOHaq, MeOH, rt.

thiazol-4-yl (**17**), 2-pyridyl (**18**), or pyradinyl (**19**) group resulted in a decrease in potency.

During the course of the SAR work to enhance PDE4B selectivity over the 4D isozyme, we found a suitable combination of substituents at 2-position and the carboxylic acid moiety at the phenyl amino group, R^3 and R^4, respectively (Table 3). A substituted phenyl or thienyl group at R^3 did not improve selectivity when R^4 was a carboxy group (**20**–**24**), similar results were observed when R^3 was substituted with phenyl and R^4 with a carboxymethyl group (**25**–**28**). However, compounds **29** and **30**, bearing a substituted thienyl group at R^3 and a carboxymethyl group at R^4 showed significant selectivity greater than 25-fold.

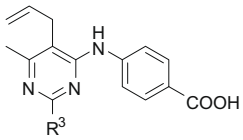
Table 1
Inhibitory activity of pyrimidine derivatives **1**–**14**



Compound	R^1	R^2	PDE4B IC_{50} (nM)	PDE4D IC_{50} (nM)	Ratio 4D/4B IC_{50}
Cilomilast			37	27	0.7
1	$-CH_2CH{=}CH_2$	Me	190	1900	9.7
2	Me	Me	430	3400	7.9
3	Et	Me	140	2100	15
4	*n*-Pr	Me	1300	7600	5.6
5	*i*-Pr	Me	>1000	n.d.	n.d.
6	*n*-Bu	Me	>1000	n.d.	n.d.
7	Bn	Me	>1000	n.d.	n.d.
8	–CN	Me	120	1500	12
9	$-CH_2NH_2$	Me	>1000	n.d.	n.d.
10	–CHO	Me	300	1500	5.2
11	$-CH_2OH$	Me	540	8000	15
12	$-CH_2CH{=}CH_2$	Et	34	82	2.4
13	$-CH_2CH{=}CH_2$	i-Pr	>1000	n.d.	n.d.
14	$-CH_2CH{=}CH_2$	n-Pr	690	2400	3.4

n.d. = Not determined.

Table 2
Inhibitory activity of pyrimidine derivatives **15**–**19**



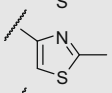
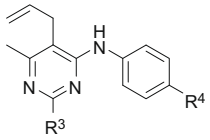
Compound	R^3	PDE4B IC_{50} (nM)	PDE4D IC_{50} (nM)	Ratio 4D/4B IC_{50}
1	Ph	190	1900	9.7
15		120	1300	11
16		68	990	15
17		2800	12,000	4.2
18		3700	15,000	4.1
19		860	3000	3.5

Table 3
Inhibitory activity of pyrimidine derivatives **20**–**30**



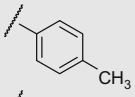
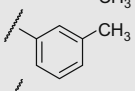
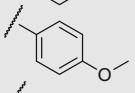
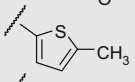
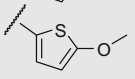
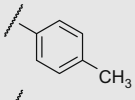
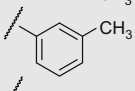
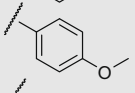
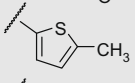
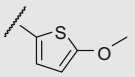
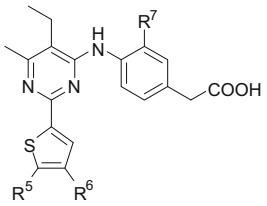
Compound	R^3	R^4	PDE4B IC_{50} (nM)	PDE4D IC_{50} (nM)	Ratio 4D/4B IC_{50}
1	Ph	–COOH	190	1900	9.7
20		–COOH	220	2800	13
21		–COOH	210	1500	6.9
22		–COOH	220	2000	8.9
23		–COOH	150	1300	8.6
24		–COOH	78	760	9.8
25	Ph	$-CH_2COOH$	1800	>10,000	n.d.
26		$-CH_2COOH$	940	11,000	12
27		$-CH_2COOH$	1200	9900	8.0
28		$-CH_2COOH$	600	4800	8.0
29		$-CH_2COOH$	320	8800	28
30		$-CH_2COOH$	190	5300	29

Table 4
Compounds with improved inhibitory activity and selectivity



Compound	R^5	R^6	R^7	PDE4B IC_{50} (nM)	PDE4D IC_{50} (nM)	Ratio 4D/4B IC_{50}
31	SCH_3	H	H	34	1500	43
32	Br	H	H	19	1600	87
33	Cl	H	H	15	1700	114
34	CH_3	Br	H	6.8	2900	420
35	F	H	F	15	3100	205

Table 5
Pharmacokinetic data[a] for compound **33** and **35**

Compound	Mice		Ferret	
	AUC[b] (μg h/mL)	C_{max} (μg/mL)	AUC[b] (μg h/mL)	C_{max} (μg/mL)
33	52.3	8.7	33.5	4.5
35	21.9	10.9	340.9	14.8

[a] Dosed at 2.0 mg/kg po as suspension in 0.5% methylcellulose.
[b] AUC of 0–∞.

Table 6
Biological evaluation of compound **33**

Compound	Inhibition of TNF-production		Neutrophilia inhibition[c]	Vomiting test[d]
	In vitro mice PBMC[a] IC_{50} (M)	*In vivo* mice[b] ID_{50} (mg/kg, po)	*In vivo*, ferret inhibition% @ dose (po)	Dose in ferret (mg/kg, po), Emetic responders (vomiting /tested)
Cilomilast	0.20	2.2	35% @ 10 mg/kg	0.3, 0/6 1, 1/6 10, 2/3
33	0.50	14	44% @ 12.5 mg/kg 46% @ 25 mg/kg	100, 0/4 200, 2/2

[a] Inhibitory concentration on LPS-induced TNF-α production from mouse peripheral blood mononuclear cells (PBMC).
[b] Inhibitory dose on LPS-induced (ip) TNF-α production in mice.
[c] The percentage inhibition for a change in the number of neutrophils in lung lavage after LPS inhalation in ferrets.
[d] Occurrence of vomiting episode after administration for 6 h.

We then focused on optimization of 2-thienyl derivatives. After extensive investigations to introduce a wide variety of functional groups on the thiophene moiety and a combination with substituents on the other position, a series of compounds with highly improved potency and selectivity were found. Compounds **33**–**35** in Table 4 showed excellent potency on 4B and >100-fold selectivity over 4D.[11]

These compounds generally showed good pharmacokinetic properties. Examples are compounds **33** and **35** in Table 5. Bioavailability of **33** and **35** in mice was 85% and 73%, respectively (data not shown). Compounds **33** and **35** were not inhibitors (IC_{50} > 10 μM) of CYP1A2, CYP3A4, CYP2C9, and CYP2D6 (data not shown).

Compound **33** was subjected to further biological evaluation. Table 6 shows a summary of the results compared with Cilomilast. Compound **33** had the same potency as Cilomilast in inhibiting TNF-α production in mouse peripheral blood mononuclear cells (PBMC) in vitro, reflecting the same degree of activity against PDE4B, or had an additional effect due to the PDE4D inhibiting activity of Cilomilast. Oral activity against LPS-induced TNF-α production in mice was evaluated, and it was found that compound **33** showed potential activity in this assay. We further evaluated the inhibitory activity against neutrophil accumulation in lung induced by LPS, and the emetic adverse effect in ferrets. Cilomilast showed an effect on neutrophilia at a dose of 10 mg/kg, and vomiting was observed at a dose of 1 mg/kg, indicating a rather narrow therapeutic window. Compound **33** had the same potency on neutrophilia, and a significantly lower emetic effect with no vomiting at a dose of 100 mg/kg. The greater safety index of **33** is possibly due to PDE4B selectivity over PDE4D. To the best of our knowledge, this is the first disclosure of PDE4B inhibitors which show both strong potency and high selectivity (>100-fold) against PDE4D. A further exploration of these PDE4B inhibitors and their application in COPD and other therapeutic areas is now ongoing.

In summary, a novel class of potent, selective, and orally available PDE4B inhibitors has been developed. Biological evaluation of the compound identified as **33** had anti-inflammatory therapeutic potential and a wider safety margin regarding emesis.

Acknowledgments

The authors thank the analytical groups in the Research Center, Asahi Kasei Pharma Corporation for analytical and spectroscopic data. The authors thank Naoko Yamaguchi for biological evaluation.

References and notes

1. (a) Zhang, K. Y. J.; Ibrahim, P. N.; Gillette, S.; Bollag, G. *Exp. Opin. Ther. Targets* **2005**, *9*, 1283; (b) Burnouf, C.; Pruniaux, M.-P. *Curr. Pharm. Design* **2002**, *8*, 1255; (c) Kodimuthali, A.; Jabaris, S. S. L.; Pal, M. *J. Med. Chem.* **2008**, *51*, 5471.
2. Houslay, M. D.; Schafer, P.; Zhang, K. Y. *Drug Discovery Today* **2005**, *10*, 1503.
3. Timmer, W.; Leclerc, L.; Birraux, G.; Neuhauser, M.; Hatzelmann, A.; Bethke, T.; Wurst, W. *J. Clin. Pharmacol.* **2002**, *42*, 297.
4. Christensen, S. B.; Guider, A.; Forster, C. J.; Gleason, J. G.; Bender, P. E.; Karpinski, J. M.; DeWolf, W. E., Jr.; Barnette, M. S.; Underwood, D. C.; Griswold, D. E.; Cieslinski, L. B.; Burman, M.; Bochnowicz, S.; Osborn, R. R.; Manning, C. D.; Grous, M.; Hillegas, L. M.; Bartus, J. O.; Ryan, M. D.; Eggleston, D. S.; Haltiwanger, R. C.; Torphy, T. J. *J. Med. Chem.* **1998**, *41*, 821.
5. Jin, S.-L. C.; Conti, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7628.
6. Robichaud, A.; Stamatiou, P. B.; Jin, S.-L. C.; Lachance, N.; MacDonald, D.; Laliberte, F.; Liu, S.; Huang, Z.; Conti, M.; Chan, C. C. *J. Clin. Invest.* **2002**, *110*, 1045.
7. Lehnart, S. E.; Wehrens, X. H. T.; Reiken, S.; Warrier, S.; Belevych, A. E.; Harvey, R. D.; Richter, W.; Jin, S.-L. C.; Conti, M.; Marks, A. R. *Cell* **2005**, *123*, 25.
8. Srivani, P.; Usharani, D.; Jemmis, E. D.; Sastry, G. N. *Curr. Pharm. Des.* **2008**, *14*, 3854.
9. Alternatively, compounds **8**–**11** having functionalized substituent at 5-position of pyrimidine ring were synthesized via 1,6-dihydro-4-methyl-6-oxo-2-phenyl-5-pyrimidine-carbonitrile described in Ellingboe, J.; Alessi, T.; Millen, J.; Sredy, J.; King, A.; Prusiewicz, C.; Guzzo, F.; VanEngen, D.; Bagli, J. *J. Med. Chem.* **1990**, *33*, 2892.
10. Recombinant human PDE4B1 (catalytic domain, a.a. 324–736) and human PED4D3 (catalytic domain, a.a. 244–673) were used. Inhibitory activity of the compounds were assayed using 3 μM cAMP with 0.83 μCi [^{3}H]-cAMP for 10 min. The reaction was stopped by adding 25 μL of trichloroacetic acid to the mixture and the solution was charged on a neutral alumina column equilibrated with 0.1 M of 2-[[tris(hydroxylmethyl)methyl]-amino]-1-ethanesulfonic acid (TES) buffer (pH 8.0). After washing the column with a sufficient amount of 0.1 M TES buffer solution, the column was eluted with 2 N NaOH, and the radioactivity of [^{3}H]-5′AMP product was measured.
11. Compound **33** showed no inhibition (IC_{50} > 2 μM) toward PDE 1–3, 5 and 6 (data not shown).