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Identification of the 5,5-dioxo-7,8-dihydro-6*H*-thiopyrano[3,2-*d*] pyrimidine derivatives as highly selective PDE4B inhibitors

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ABSTRACT

A PDE4B subtype selective inhibitor is expected to have a wider therapeutic window than non-selective PDE4 inhibitors. In this Letter, two series of 7,8-dihydro-6*H*-thiopyrano[3,2-*d*]pyrimidine derivatives and 5,5-dioxo-7,8-dihydro-6*H*-thiopyrano[3,2-*d*]pyrimidine derivatives were evaluated for their PDE4B subtype selectivity using human PDE4B2 and PDE4D2 full length enzymes. To improve their PDE4B selectivity over PDE4D, we optimized the substituents on the pyrimidine ring and the side chain phenyl ring, resulting in several derivatives with more than 100-fold selectivity for PDE4B. Consequently, we identified 2-(3-chloro-4-methoxy-phenyl)-5,5-dioxo-7,8-dihydro-6*H*-thiopyrano[3,2-*d*]pyrimidine derivative **54** as a highly selective PDE4B inhibitor, which had potent hPDE4B inhibitory activity with an IC_{50} value of 3.0 nM and 433-fold PDE4B selectivity over PDE4D.

Phosphodiesterase 4 (PDE4) is expressed in inflammatory and immune cells and mediates a hydrolysis of cyclic adenosine monophosphate (cAMP).[1] Since an up-regulation of cAMP contributes to a suppression of inflammatory responses, PDE4 has been established as an attractive target for the treatment of inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and so on.[2] Since the 1980s, PDE4 inhibitors with a wide variety of chemotypes have been developed as anti-inflammatory agents, and Roflumilast[3], a second generation PDE4 inhibitor, has been approved for the treatment of severe COPD. Indeed, Roflumilast and the other PDE4 inhibitors demonstrated moderate efficacy in COPD in the clinical studies, however, the maximum dose was limited by the mechanism-associated side effects such as nausea, emesis, and diarrhea.

PDE4 isozymes are coded by four distinct genes to give four isoforms (PDE4A-D), and 22 splice variants of these four isoforms are known.[4] In the knockout studies, it is implied that PDE4D inhibition promotes undesired adverse effects[5] and PDE4B inhibition evokes a desired anti-inflammatory effect.[6] Consequently, a PDE4B selective inhibitor is expected to have a wider therapeutic window than non-selective PDE4 inhibitors, and several attempts to obtain a selective PDE4B inhibitor have been reported.[7] However, to our knowledge, there are no compounds advancing into clinical study as a selective PDE4B inhibitor.

Our challenge to acquire PDE4B selective inhibitors started with a series of fused bicyclic sulfone derivatives that we previously reported as novel PDE4 inhibitors.[8] We initially evaluated the selectivity for PDE4B over PDE4D of our representative compounds (Fig. 1). Compound **1** exhibited good in vitro PDE4B inhibitory activity (IC_{50} = 25 nM) and high selectivity (112 folds) over PDE4D for recombinant human PDE4B (152–564) and PDE4D (78–508). However, when full length human PDE4B2 and PDE4D2 were used for the assay, the PDE4B inhibitory activity of **1** decreased (IC_{50} = 150 nM) and the PDE4B selectivity dropped to 3.7 folds. Similarly, sulfide **2** and amide **3** showed only moderate PDE4B inhibition and low selectivity for the full length enzymes.

It is known that PDE4 enzymes contain unique regulatory domains, called upstream conserved regions 1 and 2 (UCR1 and UCR2), and the UCR2 or C-terminal regulatory domain can interact with the catalytic domain of PDE4 enzymes to regulate enzyme

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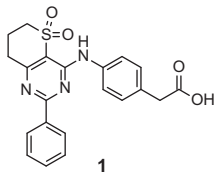
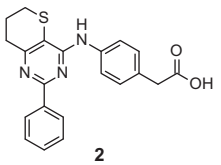


1

hPDE4B (152-564) IC_{50} = 25 nM
hPDE4D (78-508) IC_{50} = 2800 nM
Selectivity (D/B) = 112

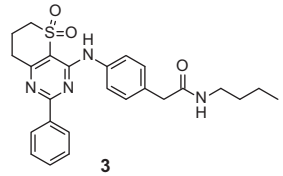
hPDE4B2 (full) IC_{50} = 150 nM
hPDE4D2 (full) IC_{50} = 550 nM
Selectivity (D/B) = 3.7



2

hPDE4B (152-564) IC_{50} = 29 nM
hPDE4D (78-508) IC_{50} = 1300 nM
Selectivity (D/B) = 45

hPDE4B2 (full) IC_{50} = 210 nM
hPDE4D2 (full) IC_{50} = 1300 nM
Selectivity (D/B) = 6.2



3

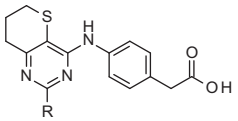
hPDE4B (152-564) IC_{50} = 22 nM
hPDE4D (78-508) IC_{50} = 260 nM
Selectivity (D/B) = 12

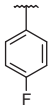
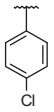
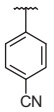
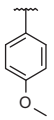
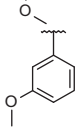
hPDE4B2 (full) IC_{50} = 70 nM
hPDE4D2 (full) IC_{50} = 180 nM
Selectivity (D/B) = 2.6

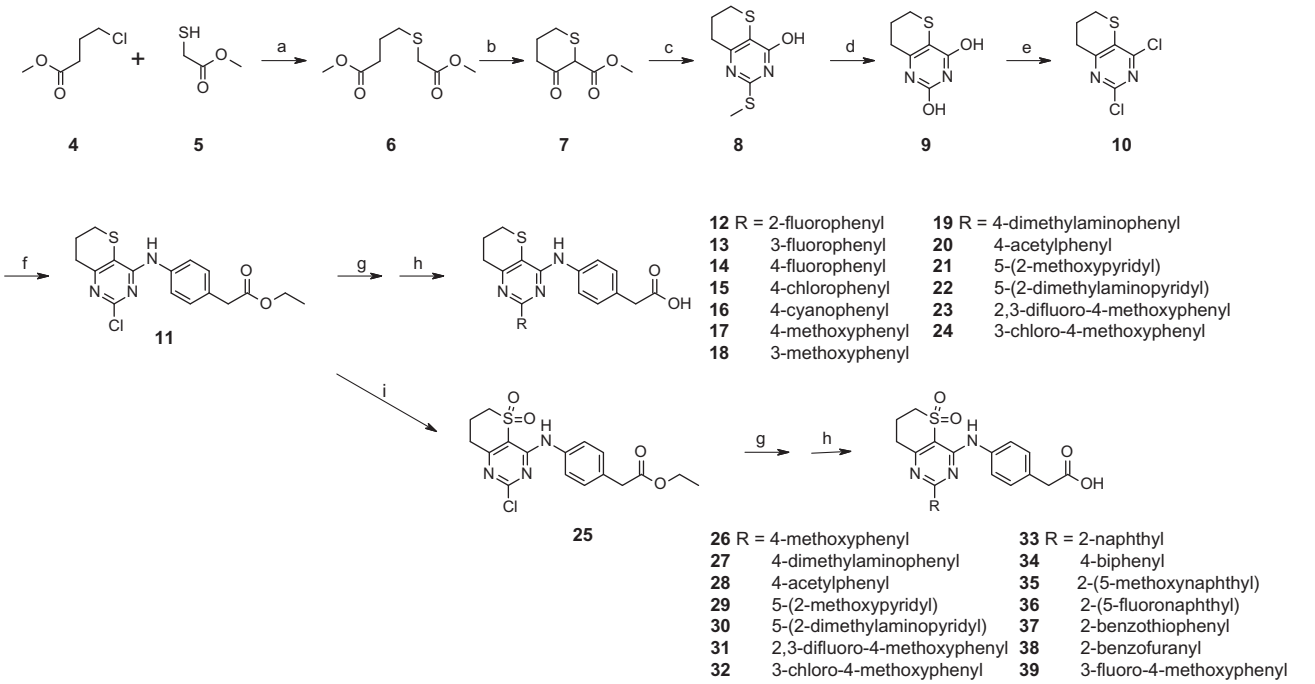
Figure 1. In vitro profiles of compounds **1**–**3**.

activity.[9] Since the access of PDE4 inhibitors to the catalytic pocket would be controlled by these helix-capped conformations, the inhibitory activity and selectivity with full length enzymes were different from those with truncated enzymes. We considered that the in vitro result with full length enzymes would reflect an

Table 1
In vitro inhibitory activity and selectivity of compounds **12**–**18**



No.	R	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)
12		880	3400	3.9
13		57	680	12
14		120	1000	8.3
15		120	420	3.5
16		30	290	9.7
17		11	280	25
18		120	1800	15



Scheme 1. Reagents and conditions: (a) NaOMe, NaI, MeOH, 65 °C; (b) NaOMe, toluene, 110 °C (98% from **5**); (c) 2-methylisothiourea sulfate, KOH, MeOH; (d) AcOH, H_2O, 110 °C (30% from **7**); (e) $POCl_3$, *N*,*N*-dimethylaniline, 90 °C (82%); (f) ethyl 2-(4-aminophenyl)acetate, DIPEA, EtOH, reflux (93%); (g) arylboronic acid or arylboronic acid pinacol ester, $PdCl_2dppf{\cdot}CH_2Cl_2$, Na_2CO_3, DME, H_2O, 130 °C, microwave; (h) 1 N NaOH, MeOH, THF, 60 °C; (i) mCPBA, CH_2Cl_2 (79%).

in vivo response better than that with truncated enzymes. Therefore, we used full length human PDE4B2 and PDE4D2 enzymes for the in vitro assay to explore PDE4B subtype selective inhibitors in this study.

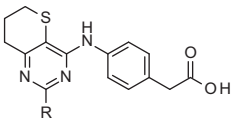
The synthetic study started with sulfide **2** as the lead compound, because the PDE4B selectivity of **2** was superior to that of **3**, even though the cell-based activity of **2** was lower than that of **3**. Additionally, sulfone compounds which were supposed to be more stable for metabolism than sulfide compounds were also synthesized.

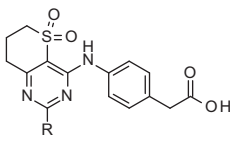
The target compounds (**12**–**24**, **26**–**39**) were synthesized according to the procedure outlined in Scheme 1. The initial reaction of methyl 4-chlorobutanoate (**4**) with methyl thioglycolate (**5**) gave diester **6**, and a successive Dieckmann condensation reaction provided ketoester **7**. The pyrimidine ring was constructed by a cyclization reaction of ketoester **7** with *S*-methylisothiourea and the resultant **8** was treated with acetic acid to provide 2,4-dihydroxypyrimidine **9**. After conversion of the hydroxyl groups to chlorine atoms using phosphoryl chloride, ethyl 2-(4-aminophenyl)acetate was introduced selectively to the 4-position of the pyrimidine ring to afford **11**. Introduction of substituents at the 2-position of **11** were performed by the Suzuki–Miyaura coupling reaction with commercially available or prepared boron reagents, and the hydrolysis of the ester gave the target sulfide ring derivatives (**12**–**24**). On the other hand, sulfide **11** was oxidized with mCPBA to furnish sulfone intermediate **25**, which was converted to the sulfone derivatives (**26**–**39**) by the same procedure used to obtain the aforementioned sulfide derivatives.

Initially, modification of the phenyl group at the 2-position of the pyrimidine ring was conducted to assess the influence of substituents on selectivity (Table 1). In the case of fluorine substituted derivatives (**12**–**14**), the 3-substitution (**13**) was favorable for both PDE4B inhibition and selectivity. Replacement of the 4-fluorine atom with a chlorine atom lowered the selectivity (**15**). On the other hand, **16** with the 4-cyano group showed an increased inhibitory activity for PDE4B with 9.7-fold selectivity. Subsequently, the 4-methoxy substitution exhibited further improvement of PDE4B inhibition with 25-fold selectivity (**17**). However, the 3-methoxy substitution (**18**) weakened the PDE4B inhibitory activity compared to the 4-methoxy substitution.

Considering the result that 4-methoxy derivative **17** was the most potent inhibitor for PDE4B with the highest PDE4B selectivity over PDE4D among these derivatives (**12**–**18**), we assumed that a proton acceptor at the 4-position would be favorable for selective inhibition of PDE4B. Therefore, we next explored derivatives having proton acceptors at the 4-position. Table 2 summarizes the results with the sulfide derivatives (**17**, **19**–**24**) and the corresponding sulfone derivatives (**26**–**32**).

Table 2
In vitro inhibitory activity and selectivity of compounds **17**, **19**–**24**, and **26**–**32**





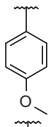
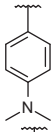
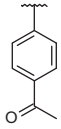
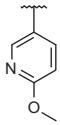
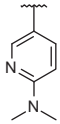
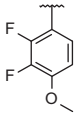
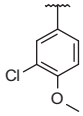
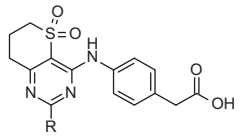
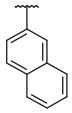
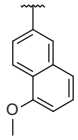
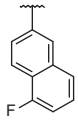
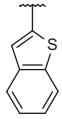
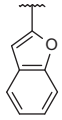
R	No.	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)	No.	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)
	**17**	11	280	25	**26**	44	1500	34
	**19**	38	790	21	**27**	38	2100	55
	**20**	34	1000	29	**28**	10	860	86
	**21**	140	7400	53	**29**	250	3800	15
	**22**	140	1600	11	**30**	56	1200	21
	**23**	140	2200	16	**31**	46	3400	74
	**24**	71	430	6.1	**32**	13	1300	100

Table 3
In vitro inhibitory activity and selectivity of compounds **33**–**38**

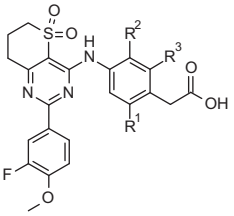


No.	R	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)
33		19	520	27
34		38	790	21
35		30	290	9.7
36		14	440	31
37		8.3	400	48
38		20	1100	55

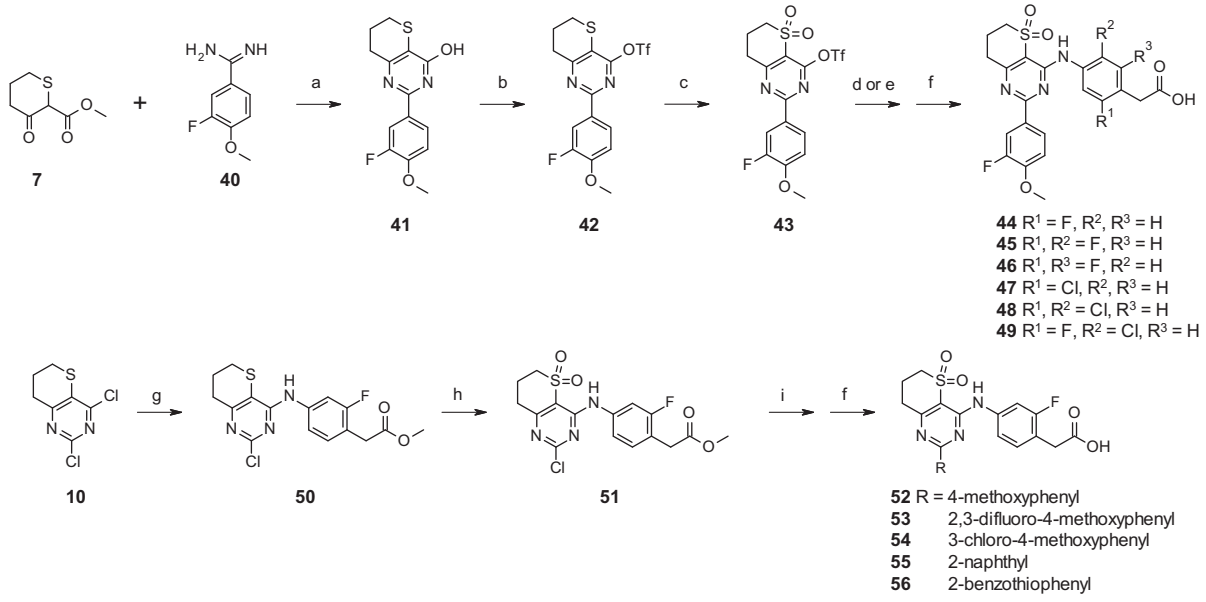
Dimethylamino and acetyl derivatives (**19**, **20**) showed good PDE4B selectivity equal to that of **17**, although their PDE4B inhibitory activity was slightly decreased. Exchanging the benzene ring of **17** with a pyridine ring gave 5-(2-methoxy)pyridyl derivative **21** which exhibited higher selectivity (53 folds) than **17**, whereas 5-(2-dimethylamino)pyridyl derivative **22** exhibited lower selectivity (11 folds) than the corresponding benzene derivative **19**. Multiple substitution of the 4-methoxyphenyl moiety of **17** with halogen atoms did not cause improvement of both PDE4B inhibitory activity and selectivity (**23**, **24**).

On the other hand, the sulfone derivative **26** which corresponds to an oxidized compound of **17** had higher selectivity (34 folds) than **17**, although the PDE4B inhibitory activity was decreased. Moreover, the dimethylamino derivative **27** showed 55-fold selectivity and the acetyl derivative **28** displayed 86-fold selectivity

Table 4
In vitro inhibitory activity and selectivity of compounds **39**, **44**–**49**

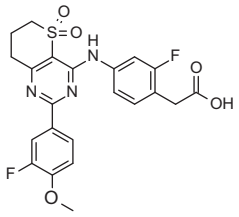


No.	R^1	R^2	R^3	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)
39	H	H	H	10	1000	100
44	F	H	H	4.6	620	135
45	F	F	H	16	1300	81
46	F	H	F	5.2	580	112
47	Cl	H	H	64	1300	20
48	Cl	Cl	H	49	600	12
49	F	Cl	H	9.9	560	57



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH (81%); (b) Tf_2O, triethylamine, DMAP, CH_2Cl_2 (34%); (c) mCPBA, CH_2Cl_2 (79%); (d) substituted (4-aminophenyl)acetate, DMF, 60 °C; (e) substituted (4-aminophenyl)acetate, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, dioxane, 100 °C; (f) 1 N NaOH, MeOH; (g) methyl (4-amino-2-fluorophenyl)acetate, $Pd_2(dba)_3$, xantphos, Na_2CO_3, dioxane, 70 °C (74%); (h) mCPBA, CH_2Cl_2 (98%); (i) arylboronic acid or arylboronic acid pinacol ester, $PdCl_2dppf{\cdot}CH_2Cl_2$, Na_2CO_3, DME, H_2O, 130 °C, microwave.

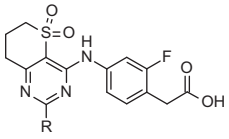
Table 5
Structure and profile of **44**

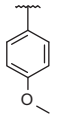
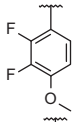
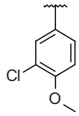
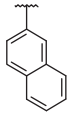
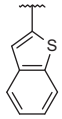


hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)	mTNF-α IC_{50} (nM)	Log*D* (pH 7.4)	PAMPA Pe*(10^{-6} cm/sec) (pH 7.4)	Mouse PK (10 mg/kg, p.o.)		
						AUC (μg h/mL)	C_{max} (μg/mL)	$T_{1/2}$(h)
4.6	620	135	18	0.8	14.3	2.77	1.70	2.0

* Permeability coefficient in parallel artificial membrane permeability assay.

Table 6
In vitro inhibitory activity and selectivity of compounds **52**–**56**



No.	R	hPDE4B2 IC_{50} (nM)	hPDE4D2 IC_{50} (nM)	Selectivity (D/B)	mTNF-α IC_{50} (nM)
52		7.5	1300	173	96
53		8.2	1400	171	131
54		3.0	1300	433	11
55		5.9	810	137	44
56		3.8	600	158	11

with strong PDE4B inhibitory activity (IC_{50} = 10 nM). However, the pyridine derivatives (**29**, **30**) failed to improve the selectivity (15 and 21 folds) compared to the corresponding benzene compounds (**26**, **27**). Introduction of the halogen atoms to the 4-methoxyphenyl moiety of **26** had positive effects on the selectivity for the sulfone derivatives (**31**, **32**). Compounds **31** and **32** showed excellent PDE4B selectivity (74 and 100 folds) retaining potent PDE4B inhibitory activity in contrast to the decrease of potency and selectivity for the corresponding sulfide derivatives (**23**, **24**). These results have indicated that the sulfone scaffold is appropriate for selective PDE4B inhibition.

We further searched for a suitable substituent for the 2-position of the pyrimidine core (Table 3). Naphthyl derivative **33** had good PDE4B inhibitory activity with an IC_{50} value of 19 nM and moderate selectivity (27 folds), while biphenyl derivative **34** showed decreased inhibitory activity with 21-fold selectivity. From the previous results that the introduction of a methoxy group and a fluorine atom improved selectivity, a methoxy group or a fluorine atom was introduced to the naphthyl group (**35**, **36**). Unexpectedly, **35** resulted in reduced activity and selectivity, and **36** showed similar PDE4B inhibitory activity and selectivity to those of **33**. On the other hand, the benzothiophene and benzofuran groups (**37**, **38**) were preferable to the naphthyl group for PDE4B inhibition and selectivity.

We next focused on substituents for the phenyl group of the side chain. The derivatives (**44**–**49**, **52**–**56**) were synthesized as shown in Scheme 2. Intermediate **7** was condensed with 3-fluoro-4-methoxybenzamidine **40** to form pyrimidine **41**. The hydroxyl group of **41** was converted to the corresponding triflate (**42**), and the sulfur atom was oxidized by mCPBA to give sulfone **43**. The side chains were introduced to **43** by a substitution reaction or palladium catalyzed cross-coupling reaction with substituted 4-aminophenylacetate. Hydrolysis of the terminal ester provided the target compounds (**44**–**49**). On the other hand, **52**–**56** were synthesized from intermediate **10**. Methyl 4-amino-2-fluorophenylacetate was selectively introduced at the 4-position of pyrimidine under a cross-coupling condition and subsequent oxidation of the sulfur atom gave 2-chloropyrimidine intermediate **51**. Several aromatic rings were introduced at the 2-position by the Suzuki–Miyaura coupling reaction and deprotection of the ester group furnished the target compounds (**52**–**56**).

Table 4 summarizes the results of the derivatives with fluorine and chlorine atoms on the phenyl group of the side chain. 2-Fluoro derivative **44** and 2,6-difluoro derivative **46** showed higher PDE4B inhibitory activity (IC_{50} = 4.6 and 5.2 nM) and selectivity (135 and 112 folds) than unsubstituted derivative **39**, whereas 2,5-disubstituted derivative **45** failed to improve the selectivity. 2-Chloro derivative **47** and 2,5-dichloro derivative **48** revealed to have only low PDE4B selectivity (20 and 12 folds) with moderate PDE4B inhibitory activity. Replacement of the 2-chlorine atom of **48** with a fluorine atom showed a recovery of the selectivity to 57 folds (**49**), but this selectivity was lower than that of the other fluoro derivatives (**44**–**46**). From these results, the mono fluorine substituted side chain was considered to be appropriate for expressing high PDE4B selectivity.

The in vitro profile and pharmacokinetic property of **44** are shown in Table 5. Compound **44** showed high inhibitory activity against lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) production in mouse splenocytes with an IC_{50} value of 18 nM, and exhibited good exposure by oral administration at a dose of 10 mg/kg in mice.

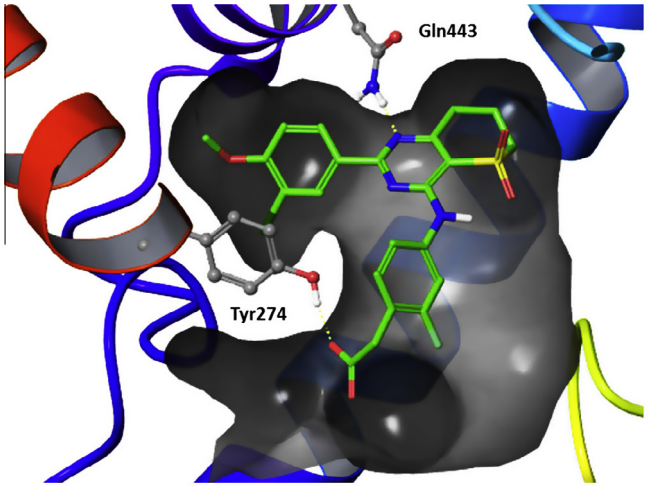


Figure 2. Putative binding model of **54** to UCR2-PDE4B (PDB accession code: 3G45). The compound model of **54** was generated according to our reported structure (PDB accession code: 3W5E).

Since **44** showed a potential for an orally available agent in Table 5, we next tried to replace the 3-fluoro-4-methoxyphenyl group of **44** with other aromatic rings which were found to have potent PDE4B inhibitory activity and high selectivity in Tables 1–3, and the results are summarized in Table 6. The 4-methoxy substituted derivatives (**52**–**54**) exhibited high PDE4B inhibitory activity with single nano molar IC_{50} and had an excellent PDE4B selectivity of more than 170 folds. Especially, 3-chloro-4-methoxyphenyl derivative **54** showed 433-fold selectivity, which was the highest selectivity in all of the derivatives. Bicyclic derivatives (**55**, **56**) also showed single nano molar potency of PDE4B inhibition. However, **55** and **56** had lower selectivity (137 and 158 folds) than the 4-methoxyphenyl derivatives (**52**–**54**).

In addition, we evaluated inhibitory activity of each compound against LPS-induced TNF-α production in mouse splenocytes. In this assay, the inhibitory activity correlated to the PDE4B2 inhibitory activity of each compound. For example, **54** which exhibited potent PDE4B2 inhibitory activity with an IC_{50} value of 3.0 nM also showed high inhibitory activity against TNF-α production (IC_{50} = 11 nM). On the other hand, **53** which had the lowest PDE4B2 inhibitory activity (IC_{50} = 8.2 nM) in Table 6 exhibited the lowest inhibitory activity against TNF-α production (IC_{50} = 131 nM). Of course, the PDE4D2 inhibitory activity also would affect the inhibition of TNF-α production. However, the PDE4D2 inhibitory activity of these compounds was supposed to be low enough to ignore. From these results, although the difference in inhibitory activity against TNF-α production seemed to be somewhat drastic compared to that of PDE4B2 inhibition, we concluded that the PDE4B inhibitory activity was a significant factor for the inhibition of TNF-α production and the selective PDE4B inhibitor could be a potent anti-inflammatory agent.

In order to rationalize the high PDE4B selectivity of **54**, we analyzed a binding model of **54** with PDE4B. Figure 2 shows a binding model of **54** based on the crystal structure of amide compound with truncated PDE4B (152–528)[8] superimposed on the reported crystal structure of PDE4B including UCR2.[9] In this model, Tyr274 from the UCR2 helix extends into the active site and is positioned between the substituent on 2-position of the pyrimidine ring and the carboxylic acid moiety. The Tyr274 residue, which corresponds to Phe196 in PDE4D, is considered to be the one of the key factors to make the subtype selectivity. The hydroxyl group of Tyr274 seems to interact with carboxylic acid moiety of **54**, which would contribute to enhancement of the PDE4B inhibitory activity. Whereas, the lack of this interaction with Phe196 of PDE4D might cause the weakness of PDE4D inhibitory activity. These hypotheses were supported by the result that amide derivatives of **39** showed lower PDE4B inhibitory activity than **39**.[10] The 3-chloro-4-methoxyphenyl moiety of **54**, which is proximal to UCR2, may also contribute to the stabilization of the capping conformation of UCR2.

In summary, in the course of the exploration of novel PDE4B selective inhibitors, it was revealed that in vitro PDE4B and PDE4D inhibitory activities should be evaluated in the assay with full length enzymes. Modification of the phenyl ring moiety at the 2-position of the pyrimidine ring in 7,8-dihydro-6*H*-thiopyrano[3,2-*d*]pyrimidine derivative **2** was effective in improving the PDE4B selectivity over PDE4D, which gave 4-methoxyphenyl derivative **17** with 25-fold selectivity. Successive optimization of the 2-position substituent, oxidation of the sulfur atom, and introduction of the fluorine atom to the phenyl ring of the side chain culminated in the identification of potent and highly selective PDE4B inhibitor **54** with an IC_{50} value of 3.0 nM for hPDE4B2, and 433-fold selectivity over hPDE4D2. Further investigation of this series of potent and selective PDE4B inhibitors including an in vivo model study and toxicity study has been reported in another publication.[11]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.076.

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10. The amide compound **A** exhibited lower PDE4B inhibitory activity compare to the corresponding carboxylic acid compound **39**, which made the PDE4B selectivity low (Figure 3). The change of the hydrogen bonding interaction with Tyr274 and the lipophilicity of amide alkyl group might reduce the PDE4B

inhibitory activity of **A**. On the other hand, in the case of interaction with PDE4D, a hydrophobic interaction of amide alkyl group with Phe196 was considered to cause the gain of the PDE4D inhibitory activity.

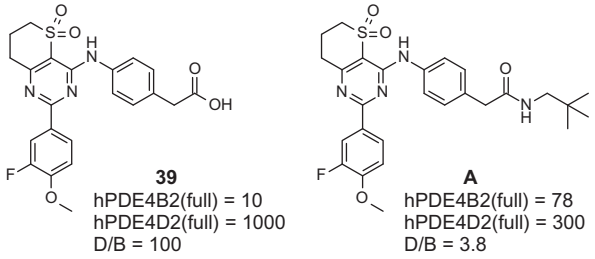

Figure 3. Comparison of the *in vitro* activity and selectivity between the carboxylic acid **39** and the amide **A**

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