Bioorganic & Medicinal Chemistry Letters 22 (2012) 2594–2597

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of oxazole-based PDE4 inhibitors with picomolar potency

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ARTICLE INFO

Article history:
Received 4 December 2011
Revised 26 January 2012
Accepted 30 January 2012
Available online 14 February 2012

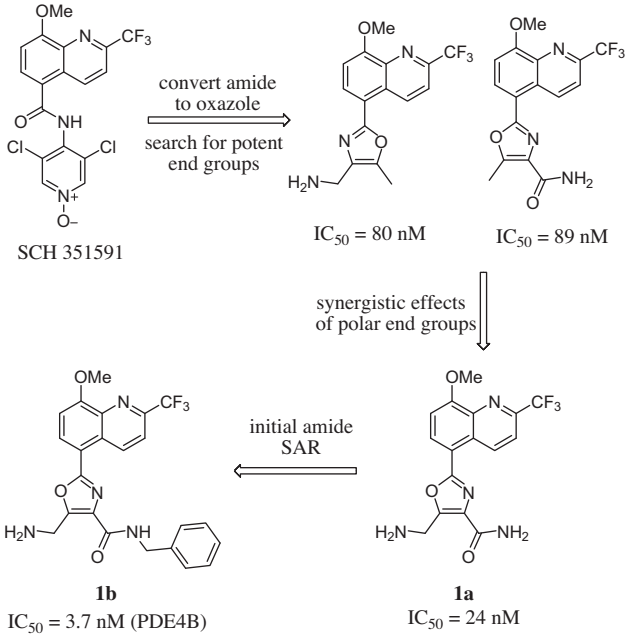
Keywords:
PDE4 inhibitor
Phosphodiesterase 4
Oxazole
Picomolar
Inflammation

ABSTRACT

Optimization of oxazole-based PDE4 inhibitors has led to the discovery of a series of quinolyl oxazoles, with 4-benzylcarboxamide and 5-α-aminoethyl groups which exhibit picomolar potency against PDE4. Selectivity profiles and in vivo biological activity are also reported.

Phosphodiesterase 4 (PDE4) is expressed predominantly in inflammatory and immune cells and is one of the phosphodiesterase (PDEs) isozymes that are responsible for the breakdown of the signaling molecule cAMP.[1] Inhibition of PDE4 effectively increases the intracellular cAMP level, which in turn provides critical negative regulation of various cellular functions in these cells. The anti-inflammatory effects of PDE4 inhibitors have been demonstrated in various animal models of airway diseases as well as in other biological disorders. Roflumilast, a selective PDE4 inhibitor, has recently been approved by the FDA for severe COPD,[2] while several PDE4 inhibitors have advanced to various stages of clinical or preclinical development for COPD, asthma, allergic rhinitis, Crohn's disease, psoriasis, atopic dermatitis, and depression.[3–10] Despite significant progress in this area, PDE4 inhibitors are often associated with dose-limiting side-effects such as nausea, emesis, and vasculopathy, which limit their therapeutic potential.[11–13] This further highlights the need to discover novel pharmacophores for designing PDE4 inhibitors with an improved therapeutic index.

We have previously reported the discovery of a potent class of quinolyl oxazole-based PDE4 inhibitors by re-designing the core structure of SCH 351591.[14,15] A novel PDE4 inhibitory pharmacophore revealed in the new core structure consists of an oxazole motif bearing the 4-carboxamide and 5-aminomethyl groups (Scheme 1). Weak inhibition of PDE10 and PDE11 has also been



Scheme 1. Design and discovery of oxazole-based PDE4 inhibitors.

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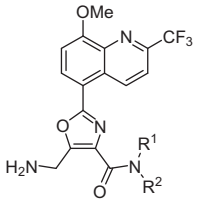
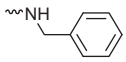
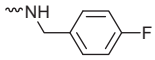
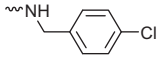
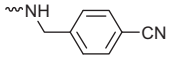
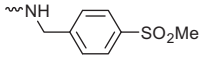
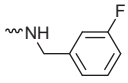
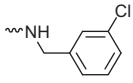
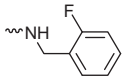
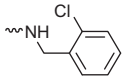
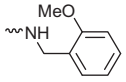
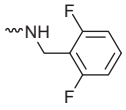
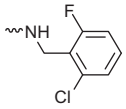
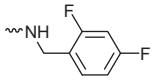
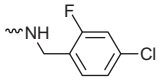
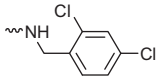
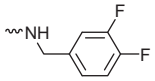
doi:10.1016/j.bmcl.2012.01.115

Table 1
SAR of 4-benzylcarboxamide group[a]



Compd	–N R^1R^2	IC_{50} (nM)		
		PDE4B	PDE10	PDE11
1b		3.7	1200	4800
1c		4.1	1300	2700
1d		3.4	2100	2700
1e		26	ND	ND
1f		37	ND	ND
1g		1.8	810	3900
1h		1.5	770	2100
1i		0.9	500	3600
1j		1.2	610	2300
1k		0.5	320	870
1l		0.46	240	2500
1m		0.1	410	1300
1n		0.6	1000	2200
1o		2.7	2200	2700
1p		4.3	1300	1100
1q		2.6	1300	2200

ND = not determined.
[a] Values of IC_{50} are the means of at least two experiments.

observed for compounds derived from this scaffold. Among the potent carboxamides identified from our initial screening, the *N*-benzylcarboxamide (**1b**) was found to exhibit good selectivity for PDE4 over PDE10 and PDE11. Herein, we report on our optimization of this series of compounds which led to the discovery of highly selective PDE4 inhibitors with picomolar potency.

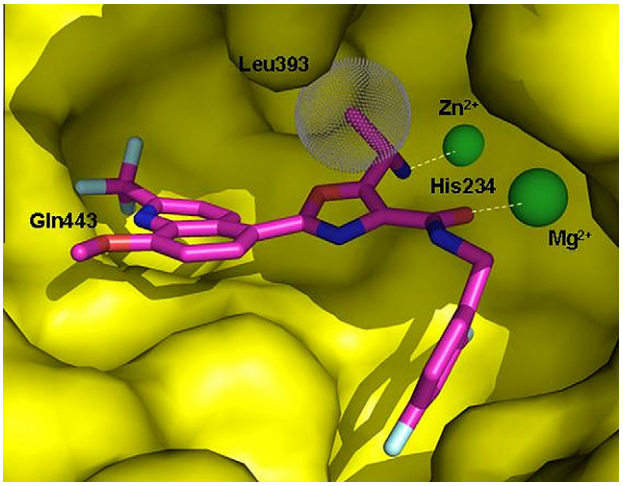
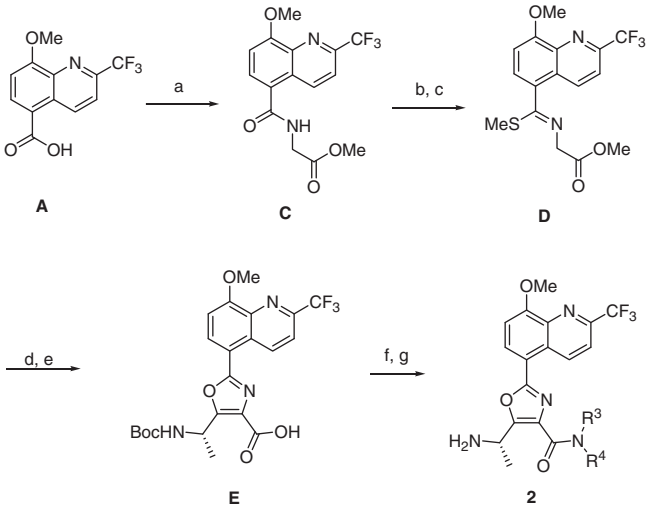


Figure 1. Modeling of **2n** binding to PDE4 with (*S*)-methyl group being highlighted.



Scheme 2. Reagents and conditions: (a) $SOCl_2$, toluene, reflux, then glycine-OEt·HCl, TEA, 100%; (b) Lawesson's reagent, THF, 70 °C, 1 h, 87%; (c) $Me_3O{\cdot}BF_4$, CH_2Cl_2, 0 °C, 3 h, 95%; (d) Boc-L-Ala-F, KHMDS, THF, −78 to −50 °C, 1 h, 50–70%; (e) LiOH, H_2O/THF, 100%; (f) HNR^3R^4, HATU, DMF/CH_2Cl_2, rt; (g) 4 N HCl, dioxane, rt 100%.

A series of oxazoles with substituted *N*-benzylcarboxamides as the 4-carboxamide moiety was first prepared according to the previously reported route.[15] These compounds were screened in vitro against PDE4B, PDE10 and PDE11.[16] Subtype B of PDE4 was chosen because it is expressed predominantly in inflammatory cells, and our goal is to develop PDE4 inhibitors as anti-inflammatory agents. The IC_{50} data is summarized in Table 1.

We first examined the effects of substitution on the phenyl ring of the *N*-benzyl group. *Para*-substitution with small halogens (F and Cl in compounds **1c** and **1d**, respectively) shows little effect on potency, while larger groups such as –CN, and $–SO_2Me$ (compounds **1e**–**1f**) result in 5- to 10-fold decrease in PDE4 potency. In contrast, *meta*- and especially *ortho*-substitution with –F, –Cl, and –OMe (compounds **1g**–**1k**) improve the IC_{50} for PDE4. The importance of the *ortho*-F substitution was further confirmed with the disubstituted benzylamide analogs, in which the most potent compounds (compounds **1l**–**1n**) are those with two *ortho*-substitutions (–F or –Cl), and a small *para*-substitution (–F or –H). This

series of analogs are generally very selective for PDE4 over PDE11. Weak inhibition of PDE10 is present in this series of compounds. Substitution on the benzyl group appears to have parallel effects on PDE4 and PDE10, but these effects are much more profound on PDE4 than on PDE10. Therefore, the most potent PDE4 compounds (compounds **1m**–**1o**) are also the most selective compounds. However, subtype selectivity between PDE4B and PDE4D has generally not been found in the series. For example, the IC_{50} of compound **1n** is 0.6 and 0.7 nM for PDE4B and PDE4D, respectively.

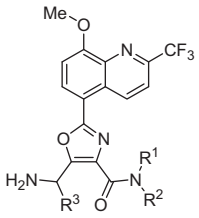
With the PDE4 inhibitory potency attaining the sub-nanomolar level through the optimization of the benzylamide group, computer modeling studies indicated that optimization of the 5-aminomethyl moiety may provide another opportunity for further improvement of the PDE4 potency. Docking studies revealed that the $-NH_2$ of the 5-aminomethyl group chelates with Zn^{2+} and makes a hydrogen bond with the His-234 side chain, which are ocated at the bottom of the active site. A small hydrophobic pocket exists between the methylene group of the 5-aminomethyl moiety and the enzyme. Substitution on the methylene group with small groups such as an (*S*)-methyl or an (*S*)-ethyl should occupy this hydrophobic space and may further increase PDE4 inhibitory potency (Fig. 1).

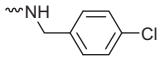
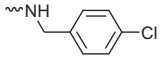
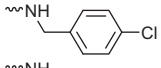
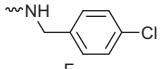
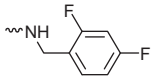
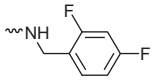
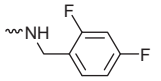
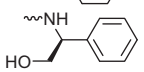
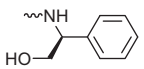
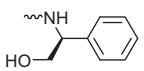
To explore the effects of α-substitution on PDE4 inhibition, a novel synthesis of the oxazole core has been developed (Scheme 2), where the oxazole ring formation step involves the reaction of a thioimidate intermediate (**D**)[17] with an *N*-Boc amino acid fluoride[18] in the presence of 2 equiv of KHMDS at −78 to −50 °C. The α-substituents can be introduced directly from the side-chains of chiral α-amino acids, and no racemization was observed. A series of compounds with different α-substituents has been prepared and their IC_{50} data for PDE4 are tabulated in Table 2.

For each of the three different benzylamide moieties used for comparison in Table 2, the (*S*)-methyl analogs (compounds **2d**, **2n**, and **2r**) are consistently 10-fold more potent than the corresponding unsubstituted analogs (compounds **1d**, **1n**, and **1r**) and also more potent than the (*S*)-ethyl (**3d**) and (*S*)-isopropyl (**5n**) analogs. The hydrophilic (*S*)-hydroxymethyl group (**4d**) decreases the PDE4 inhibitory potency by more than three-fold. As predicted from modeling studies, the (*S*)-isomer of the α-substitution is preferred and is 80-fold more potent than the corresponding (*R*)-isomer (comparing compounds **2r** and **6r**).

With the (*S*)-methyl group clearly positioned as the optimal α-substituent, a series of analogs were synthesized to explore the potential synergistic effects of combining the (*S*)-methyl

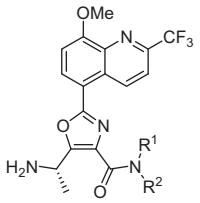
Table 2
SAR of α-substitution on the 5-aminomethyl group

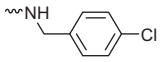
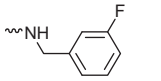
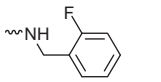
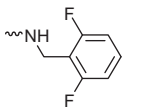
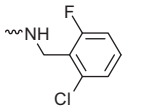
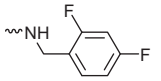
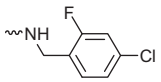
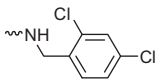
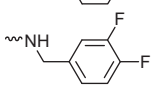
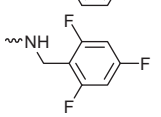


Compd	$-R^3$	$-N\ R^1R^2$	PDE4B IC_{50} (nM)
1d	H		3.4
2d	(*S*)-Me		0.35
3d	(*S*)-Et		0.81
4d	(*S*)-CH_2OH		12.7
1n	H		0.6
2n	(*S*)-Me		0.06
5n	(*S*)-*i*Pr		0.36
1r	H		1.6
2r	(*S*)-Me		0.11
6r	(*R*)-Me		8.4

[a]Values of IC_{50} are the means of at least two experiments.

Table 3
SAR of 4-benzylcarboxamide group[a]



Compd	$-N\ R^3R^4$	IC_{50} (nM)		
		PDE4B	PDE10	PDE11
2d		0.35	650	1100
2g		0.3	160	1400
2i		0.3	80	1100
2l		0.05	125	1400
2m		0.03	120	1600
2n		0.06	950	2300
2o		0.7	700	1100
2p		0.7	1700	1800
2q		0.6	1400	2000
2s		0.04	500	3400

[a] Values of IC_{50} are the means of at least two experiments.

substitution with the *ortho*-F benzylamide on both PDE4 inhibitory potency and selectivity (Table 3).

After introducing the (*S*)-methyl group to the 5-aminomethyl moiety, all compounds in the benzylamide series in Table 3 exhibit PDE4 inhibitory potency at the subnanomolar level. Compounds **2l, 2m**, **2n**, and **2s**, containing the *ortho*-F benzylamide moiety, exhibit IC_{50} values in the range of 30–60 pM and represent the most potent PDE4 inhibitors reported to date. In comparing the corresponding compounds from Tables 1 and 3, the (*S*)-methyl group has a much greater effect on PDE4 than on PDE10 and PDE11 potency, which results in further improvement of the selectivity for PDE4 over PDE10 and PDE11. Compound **2n** is the most potent PDE4 inhibitor with the highest relative selectivity for PDE4/PDE10 (15800-fold). This compound was chosen for profiling in our in vivo studies.

In the cell-based assay, compound **2n** inhibits TNFα release with an IC_{50} of 0.6 nM. In a rat PK study, compound **2n** produces a C_{max} = 960 nM with 24% bioavailability at a 10 mg/kg oral dose. This plasma level is quite sufficient for in vivo activity, and in the in vivo rat LPS(lipopolysaccharide)-induced pulmonary inflammation model,[19] compound **2n** is highly efficacious with an ED_{50} = 0.1 mg/kg PO. In addition, compound **2n** exhibits no P450 liver enzyme issues with IC_{50} for 3A4, 2D6, 2C9, and 2C19 > 8 μM. Further development of this and related compounds will be reported in the future.

In summary, a novel class of PDE4 inhibitors based on the quinolyl oxazole scaffold has been discovered. Optimization of both the 4-carboxamide and the 5-aminomethyl groups has led to the discovery of the most potent PDE4 inhibitors reported to date (IC_{50} = 30–60 pM), which are also highly selective and orally active. The oxazole motif, bearing the 4-carboxamide and 5-α-aminoethyl groups, is a highly potent and unprecedented pharmacophore for phosphodiesterase inhibition. It may also provide a new working model for the rational design of inhibitors for other PDE enzymes.

Acknowledgment

We thank Dr. Jesse Wong, Dr. Mark Liang, and Mr. Yan Jin for scale-up of intermediates.

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