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Discovery of a highly potent series of oxazole-based phosphodiesterase 4 inhibitors

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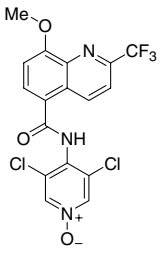
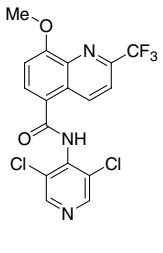
Abstract—Substituted quinolyl oxazoles were discovered as a novel and highly potent series of phosphodiesterase 4 (PDE4) inhibitors. Structure–activity relationship studies revealed that the oxazole core, with 4-carboxamide and 5-aminomethyl groups, is a novel PDE4 inhibitory pharmacophore. Selectivity profiles and in vivo biological activity are also reported.

The phosphodiesterases (PDEs) comprise a diverse family of enzymes that are responsible for the hydrolysis of cAMP and cGMP, and are key components for the cyclic nucleotide signaling systems.[1] Phosphodiesterase 4 (PDE4), one of the cAMP-specific PDE isozymes, is expressed predominantly in inflammatory and immune cells. Inhibition of PDE4 effectively increases the intracellular cAMP level, which in turn provides critical negative regulation of various cellular functions in these cells. Therefore, the development of PDE4 inhibitors as anti-inflammatory drugs has attracted extensive research efforts for more than a decade.[2–4] The anti-inflammatory effects of PDE4 inhibitors have been demonstrated in various animal models of airway diseases as well as in other biological disorders. Several PDE4 inhibitors have advanced to various stages of clinical or preclinical development, and have shown promising efficacy for COPD, asthma, allergic rhinitis, Crohn's disease, psoriasis, atopic dermatitis, and depression.[5–10] 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 which exhibit an improved therapeutic index.



SCH 351591
IC_{50} = 60 nM



SCH 365351
IC_{50} = 20 nM

We have previously reported the synthesis and pharmacological studies of SCH 351591, a potent and orally active PDE4 inhibitor.[14–16] However, in a multiple rising

Keywords: Phosphodiesterase 4; PDE4 inhibitor; Oxazole; Quinoline; Anti-inflammatory; COPD.
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dose study, this compound was found to cause vasculopathy in monkeys.[17] Another potential issue with SCH 351591 was its metabolic conversion to a more potent pyridine derivative SCH 365351. The extent of this metabolic conversion varied with species. Therefore, in order to identify PDE4 inhibitors with an improved therapeutic index, we began our search for a superior surrogate of the dichloropyridine *N*-oxide moiety of SCH 351591. Herein, we report our research efforts which have led to the discovery of a highly potent series of PDE4 inhibitors with a novel pharmacophore (Fig. 1) .

The design of a new inhibitor scaffold was facilitated by the published crystal structures of PDE4.[18–20] Modeling studies on SCH 351591 and related compounds suggested that the quinoline moiety binds to the adenosine recognition site, while the amide portion serves as a linker to anchor a group containing a polar atom which provides favorable interactions with the metal ion binding site of PDE4. The quest for a replacement of the dichloropyridine *N*-oxide moiety thus became a search for novel combinations of *linkers* and *polar end groups* as PDE4 pharmacophores.

Five-membered ring heterocycles were first explored as possible linkers to replace the amide portion. The oxazole moiety was found to be a highly versatile linker and became the core of our current series of PDE4 inhibitors. Compounds derived from this core were screened in vitro against PDE4B,[21] the subtype of PDE4 expressed predominantly in inflammatory cells, since our goal is to develop PDE4 inhibitors as anti-inflammatory agents.

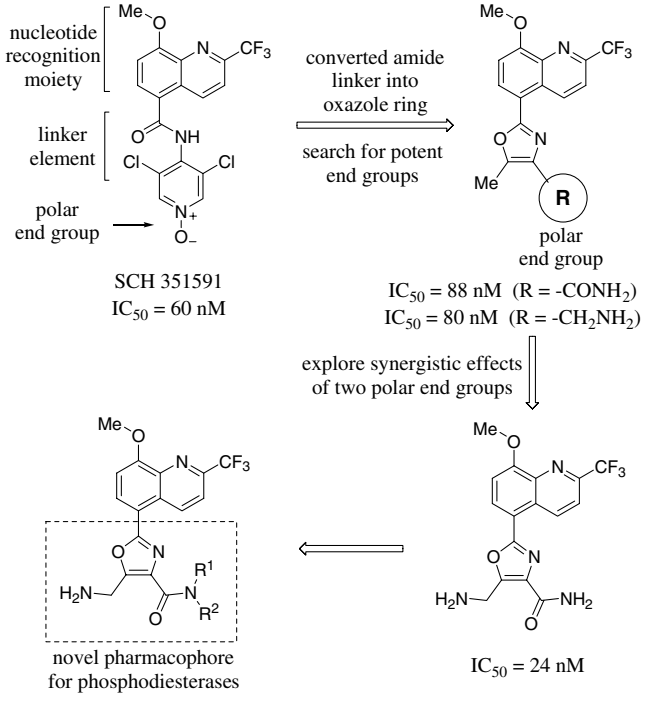


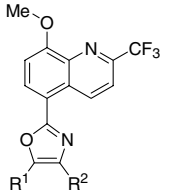
Figure 1. Design and discovery of oxazole-based PDE4 inhibitors.

Initially, only the 4-position of the oxazole was used to anchor a polar group (compounds **4**–**8**, Table 1) in order to explore potential favorable interactions with the highly polar region of the PDE4 active site, where the metal ions Zn^{2+} and Mg^{2+}, conserved water molecules, and polar residues (His, Asp, Glu) are located. It was interesting that very different polar groups such as an aminomethyl moiety (compound **7**) and a carboxamide moiety (compound **8**) both exhibited good PDE4 inhibitory activity. Since the oxazole ring can assume either one of two rotamer conformations, which are inter-converted by rotating 180° relative to the quinoline moiety, we postulated that the two different polar groups in compounds **7** and **8** may bind to different residues or metal ions of the active site. Therefore, attaching two polar groups, one on the 4-position and the other on the 5-position of the oxazole ring, might provide multiple synergistic interactions with the enzyme. Indeed, the first such designed compound (**10**, IC_{50} = 24 nM) was found to be a more potent inhibitor of PDE4. Interestingly, it is also 3-fold more potent than its regioisomer **11**. Compound **10** thus became the prototype structure for further optimization efforts.

Computer modeling studies of compound **10** revealed a unique motif providing multiple interactions with the hydrolysis site (metal ion binding site) of the enzyme, which is not seen among other reported PDE4 inhibitors. While the quinoline moiety is expected to bind to the adenyl site, as with SCH 351591, the oxazole ring serves as a linker for (**1**) positioning the primary amino group to simultaneously coordinate with both the Zn^{2+} and His-234, and (**2**) anchoring the amide oxygen to coordinate with the Mg^{2+} in the catalytic site (Fig. 2).

Indeed, the requirement of a primary amino group (5-aminomethyl) for high potency was immediately established as we explored the effect of substitution on the amino group of compound **10**. The results of representa-

Table 1. SAR of polar end group[a]



Compound	R^1	R^2	PDE4B IC_{50} (nM)
1	H	Me	350
2	H	Et	670
3	Me	$-CH_2Cl$	470
4	Me	$-CH_2OH$	470
5	Me	$-CO_2H$	390
6	Me	–CHO	310
7	Me	$-CH_2NH_2$	80
8	Me	$-CONH_2$	88
9	$-CH_2NH_2$	$-CO_2H$	53
10	$-CH_2NH_2$	$-CONH_2$	24
11	$-CONH_2$	$-CH_2NH_2$	76

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

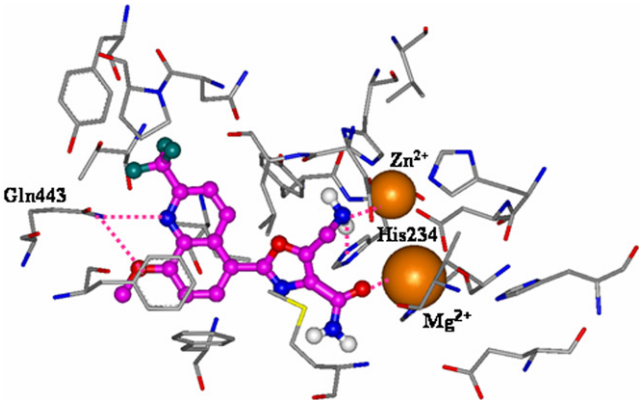


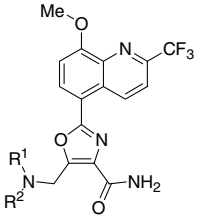
Figure 2. Modeling of compound **10** binding to the active site of PDE4.

tive substitutions are shown in Table 2. Even a small substituent such as methyl (compound **12**) is sufficient to abolish most of the PDE4 inhibitory activity of compound **10**. All other substituents (compounds **13**–**17**) essentially lead to inactive compounds.

In contrast, the 4-carboxamide group was found to tolerate a large variety of substituents when we explored the SAR of the amide group. Results of representative structures of 4-carboxamides are listed in Table 3.

4-Carboxamide analogs derived from small-size amines or simple cyclic amines (compounds **18**–**22**) give similar or slightly decreased PDE4 inhibitory activity, in comparison to the primary amide (–$CONH_2$). However, as the size and length of the carboxamide group increase,

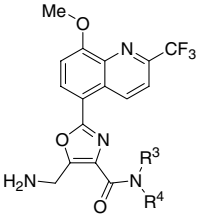
Table 2. SAR of 5-aminomethyl group[a]

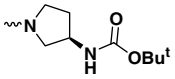
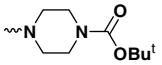
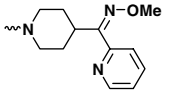
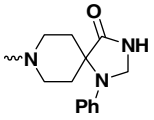
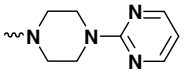
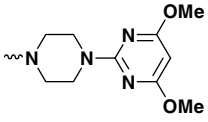
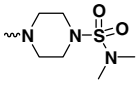
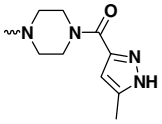
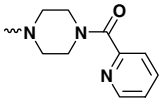


Compound	–N R^1R^2	PDE4B IC_{50} (nM) or % inhibition at 1 μM
10	–NH_2	24
12	–NHMe	580
13	–NMe_2	40%
14	–NEt_2	21%
15		31%
16		27%
17		39%

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

Table 3. SAR of 4-carboxamide group[a]



Compound	–N R^3R^4	IC_{50} (nM)		
		PDE4B	PDE10	PDE11
10	–NH_2	24	2800	7600
18	–NHMe	110	ND	ND
19	–NEt_2	62	ND	ND
20		31	ND	ND
21		17	ND	ND
22		49	ND	ND
23		1.4	130	820
24		4.3	1100	600
25		2.0	600	500
26		1.6	120	1500
27		1.0	20	130
28		19	430	2000
29		2.2	760	970
30		1.2	1700	3300
31		0.5	1500	1000
32		1.0	1900	2100
	SCH 351591	60	>10,000	>10,000
	SCH 365151	20	>10,000	7300

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

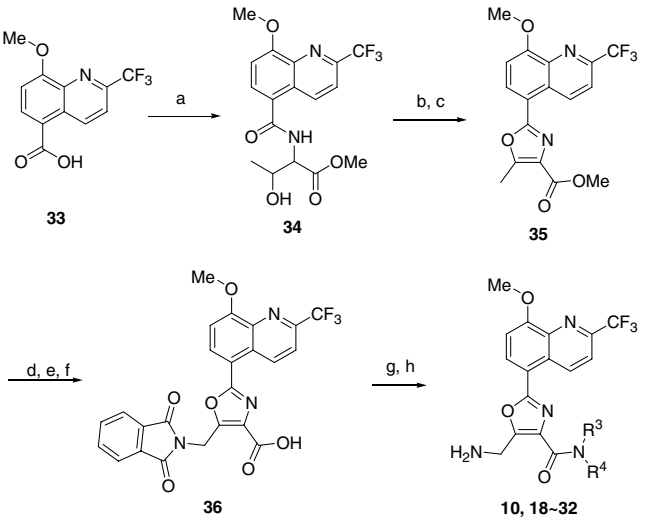
many compounds (**23**–**32**) were found to be much more potent than **10**. Modeling studies suggest that these carboxamide groups provide additional favorable interactions with the enzyme by extending into a side-pocket or the entrance area of the active site. Examples (compounds **23**–**32**) of highly potent carboxamide groups have shown a wide range of structural diversity, including both hydrophilic and hydrophobic groups. This tolerability of structural modification in the 4-carboxamide region is advantageous as we further optimize the selectivity, pharmacokinetics, and in vivo efficacy profile of this series of PDE4 inhibitors.

One unique feature of the oxazole-based PDE4 inhibitor template is the proposed multiple interactions of both its $-CH_2NH_2$ and amide functions with the metal ion site of PDE4. Since this metal ion site is conserved among the PDE superfamily, an inhibitor motif with strong interactions with this site, on one hand, could serve as a general template for designing inhibitors of other members of the PDE superfamily; on the other hand, it may pose a challenge to achieving high selectivity for PDE4.

In the current series, the quinoline moiety is the built-in element for PDE4 recognition. Selectivity screening[21] has shown that these quinolyl oxazoles are inactive toward PDE1-3 and PDE5-9. However, significant inhibitory activity for PDE10 and PDE11 has been detected in this series of compounds as shown in Table 3. For example, compound **27** is a potent inhibitor of PDE10 (IC_{50} = 20 nM) and PDE11 (IC_{50} = 130 nM), although it is still 20-fold (over PDE10) and 130-fold (over PDE11) more selective for PDE4. By exploring the SAR of the 4-carboxamide moiety, over 1000-fold selectivity has been achieved for PDE4 over PDE10 and PDE11 (compounds **30**–**32**, Table 3, all are acyl or sulfonyl piperazine analogs). However, subtype selectivity between PDE4B and PDE4D has generally not been found (within 2-fold of difference, data not shown).

A typical synthesis of these oxazole-based PDE4 inhibitors is illustrated in Scheme 1. The oxazole core is synthesized from quinolyl acid **33**[15,23] and L-threonine methyl ester. The amide ester, **34**, cyclizes to an oxazoline, which is then oxidized with $BrCCl_3$/DBU into oxazole **35**.[22] The 8-step synthesis is conducted without chromatographic purification until the last step.

In order to obtain a preliminary in vivo profile of this new class of PDE4 inhibitors, several selected compounds were studied in the rat LPS (lipopolysaccharide)-induced pulmonary inflammation model[24] (Table 4). Compounds **23**, **26**, and **32** which exhibit both high PDE4 potency and good plasma level were found to be highly efficacious in this model. Compounds **25** and **28** with lower plasma level in turn exhibit lower efficacy. In addition, compounds **24** and **28,** with their high plasma level compensating for their lower PDE4 potency, were also found to be efficacious in this model although at a higher dose level.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, toluene, reflux, then L-threonine–OMe·HCl, TEA, 100%; (b) $SOCl_2$, CH_2Cl_2, −15 °C to rt, 24 h, 97%; (c) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 3 h, 80%; (d) NBS, CCl_4, reflux, 1 h, 96%; (e) potassium phthalimide, DMF, rt, 24 h, 100%; (f) BCl_3, CH_2Cl_2, −78 °C, 2 h, 87%; (g) HNR^3R^4, HATU, DMF/CH_2Cl_2, rt; (h) H_2NNH_2, MeOH, rt.

Table 4. C_{max} and in vivo efficacy of selected compounds in rats

Compound	C_{max}, po (μM)[a]	ED_{50} or % inhibition (dose)[b]
23	0.61	0.2 mg/kg
24	1.4	56% (3 mg/kg)
25	0.17	2 mg/kg
26	0.61	45% (1 mg/kg)
28	9.5	64% (3 mg/kg)
29	0.23	2 mg/kg
32	0.73	0.6 mg/kg

[a] C_{max} data were obtained at the 10 mg/kg po dose.
[b] ED50 values were determined from dose–response curves of PMN inhibition. Percent inhibitions were obtained only at fixed doses. Compounds were administered orally to rats 5 h prior to LPS challenge.

Because of its exceptionally high plasma level in rats, compound **28** was chosen for further studies in Cynomolgus monkeys, wherein no emetic effect was observed at 30 mg/kg po (AUC = 130 μM h, and C_{max} = 13 μM).

In summary, a novel and highly potent PDE4 inhibitor scaffold based on a quinolyl oxazole has been discovered. Compounds derived from this scaffold are selective and orally active. The oxazole motif, bearing the 4-carboxamide and 5-aminomethyl groups for multiple interactions with the metal ion binding site of PDE4, may also provide a new working model for rational design of inhibitors of other PDE enzymes.

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