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Discovery and structure–activity study of a novel benzoxaborole anti-inflammatory agent (AN2728) for the potential topical treatment of psoriasis and atopic dermatitis

Tsutomu Akama*, Stephen J. Baker, Yong-Kang Zhang, Vincent Hernandez, Huchen Zhou, Virginia Sanders, Yvonne Freund, Richard Kimura, Kirk R. Maples, Jacob J. Plattner

Anacor Pharmaceuticals, Inc., 1020 E. Meadow Circle, Palo Alto, CA 94303, USA

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ABSTRACT

A series of phenoxy benzoxaboroles were synthesized and screened for their inhibitory activity against PDE4 and cytokine release. 5-(4-Cyanophenoxy)-2,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2728) showed potent activity both *in vitro* and *in vivo*. This compound is now in clinical development for the topical treatment of psoriasis and being pursued for the topical treatment of atopic dermatitis.

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Psoriasis is a chronic skin disorder caused by inflammatory cell infiltration into the dermis and epidermis, and is accompanied by keratinocyte hyperproliferation.¹ Once triggered, a strong T-cell response is mounted, and a cascade of cytokine and chemokine production is induced. Down-regulation of certain cytokines and chemokines is considered to be a good approach to treatment, and indeed, the biologics targeting TNF- α demonstrate the effectiveness of this approach.^{2–4} However, biologics have intrinsic challenges, such as limited administration route, side effects, quality control and production cost. Small molecule approaches to treat psoriasis include systemic or topical steroids, cyclosporine, psoralen plus UVA (PUVA), retinoids, methotrexate, and vitamin D₃ analogs.^{2,3} Atopic dermatitis is an allergic skin disorder, which is typically treated with topical steroids, antihistamines, and calcineurin inhibitors.⁵ However, there is still a need for new treatment with improved safety profile. Recently phosphodiesterase 4 (PDE4) inhibitors have been in development for such skin diseases. CC-10004 is in development as an oral treatment for psoriasis and atopic dermatitis.⁵ AWD-12-281 was, until recently, in development for the topical treatment of atopic dermatitis.^{5,6} In addition, roflumilast is under Phase 1 development for both diseases.⁷

We previously reported several classes of boron-containing compounds as potential therapeutic agents, including AN0128 as

an antibacterial/anti-inflammatory agent⁸ and AN2690 as an anti-fungal agent.^{9,10} As our boron-containing compound library grew and was screened in various assay systems, a series of 5-phenoxybenzoxaborole derivatives was found to exhibit inhibitory activity against the release of cytokines, such as TNF- α and IFN- γ , from peripheral blood mononuclear cells (PBMCs) stimulated by lipopolysaccharide (LPS) or phytohemagglutinin (PHA). We also discovered that this class of compounds inhibited the PDE4 enzyme as part of its mechanism of action. Herein we describe the discovery of a novel series of benzoxaborole derivatives including AN2728 (compound **2**, Fig. 1) as an optimized developmental candidate and we report the structure–activity relationships (SAR) in terms of PDE4 enzyme inhibition and cytokine release inhibition.

5-Phenoxybenzoxaborole derivatives were synthesized as shown in Schemes 1 and 2. The diaryl ether scaffold was made by a nucleophilic aromatic substitution. The formyl group of compound **2** was protected as an acetal to avoid self condensation of **2**. The formyl group of **4** was reduced to the alcohol and protected as a THP ether. The boron atom was then introduced by halogen–metal exchange with *n*-BuLi in the presence of borate, which is known as the *in situ* quench protocol.¹¹ Upon deprotection of the alcohol by HCl, the resulting hydroxymethyl group spontaneously cyclized to afford the desired oxaboroles. The carboxy derivative (**5e**) was obtained by the base hydrolysis of the corresponding cyano compound (**5b**). Amide derivatives (**5f** and **5g**) were synthesized from **5e** using regular EDC/HOBt conditions. Some compounds of the

* Corresponding author. Tel.: +1 650 543 7527; fax: +1 650 543 7660.
E-mail address: takama@anacor.com (T. Akama).

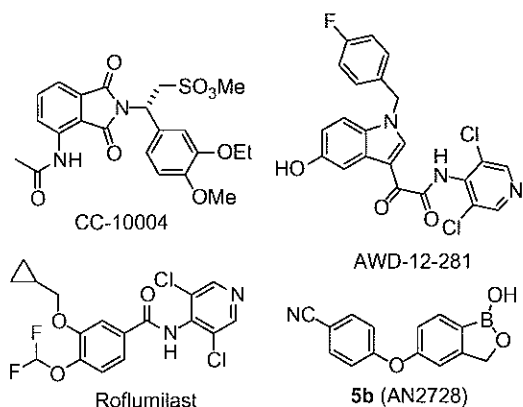
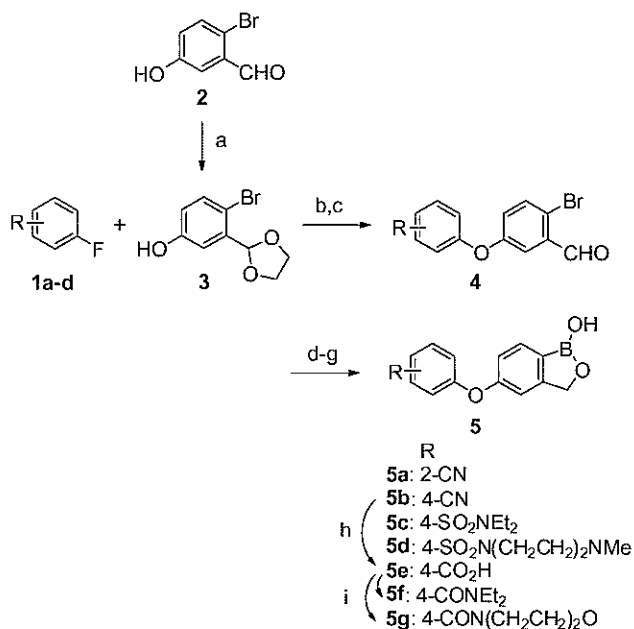


Figure 1. PDE4 inhibitors aiming at skin inflammatory diseases.

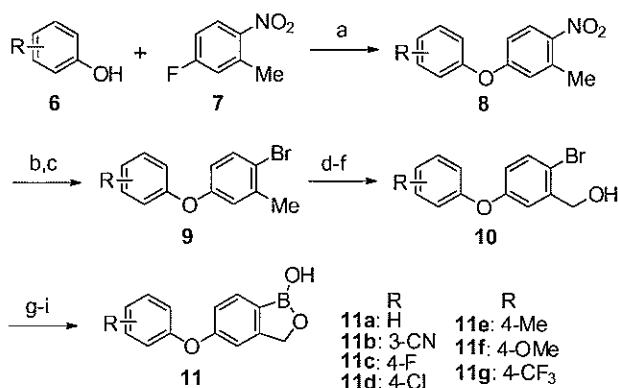
series (**11a–g**) were synthesized from phenols (**6**) and 5-fluoro-2-nitrotoluene (**7**) as shown in Scheme 2.

Regioisomers of 4-cyanophenoxy derivatives (**17a–c**) were synthesized as shown in Scheme 3 in similar ways to make compounds **5**.

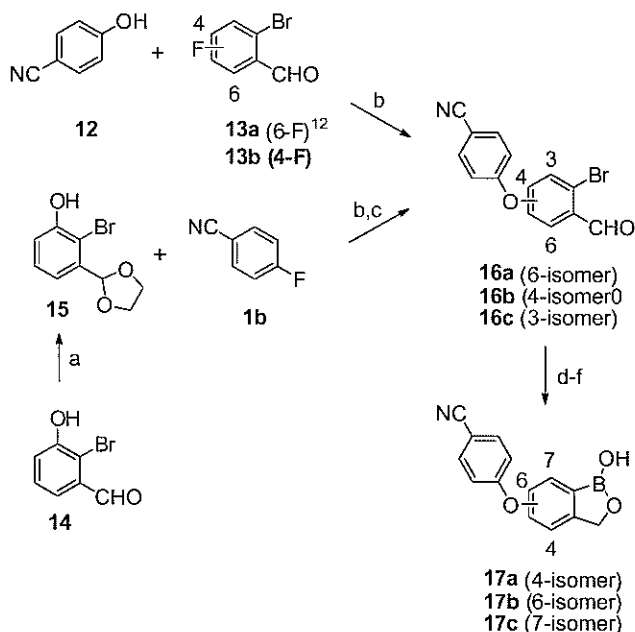
The compounds were tested against partially purified PDE4 from U937 cells¹³ and for inhibition of cytokine release from PBMCs stimulated by LPS or PHA.¹⁴ The results are summarized in Table 1. 5-Phenoxy derivative (**11a**) showed an IC_{50} value of 6.8 μ M against PDE4; however, it did not show significant inhibition against cytokine release ($IC_{50} > 10 \mu$ M). When various functional groups were introduced to the 5-phenoxy group, the activities changed drastically. 4-Cyano (**5b**), 4-morpholinocarbonyl (**5g**), and 4-trifluoromethyl (**11g**) derivatives showed potent activity against PDE4 (IC_{50} 0.49, 0.57 and 0.45 μ M, respectively), which were more potent than the classic PDE4 inhibitor rolipram in this system. Compounds **5b** and **5g** also showed a broad range of potent



Scheme 1. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, toluene, reflux, 6 h (quant.); (b) K₂CO₃, DMF, 100 °C, overnight (82–96%); (c) 3 M HCl, THF, reflux, 2 h (80–100%); (d) NaBH₄, MeOH, rt, 1 h (quant.); (e) 3,4-dihydro-2H-pyran, camphorsulfonic acid, CH₂Cl₂, rt, 2 h (quant.); (f) (*i*-PrO)₃B, *n*-BuLi, THF, –78 °C to rt, 3 h; (g) 6 M HCl, THF, rt, 3 h (37–44%); (h) 6 M NaOH, MeOH, 1,4-dioxane, reflux, 6 days (79%); (i) diethylamine (for **5f**) or morpholine (for **5g**), EDCl, HOBT, DMAP, DMF, rt, overnight (41–70%).



Scheme 2. Reagents and conditions: (a) K₂CO₃, DMF, 70 °C, overnight; (b) SnCl₂, HCl, EtOH, 50 °C, 2 h (2 steps 74–99%); (c) *t*-BuONO, HBr, CuCN, MeCN, H₂O, 0 °C to rt, overnight (22–86%); (d) NBS, AIBN, CCl₄, reflux, 2 h; (e) NaOAc, DMF, 70 °C, overnight; (f) 1 M NaOH, MeOH, rt, 1 h (3 steps 30–63%); (g) 3,4-dihydro-2H-pyran, camphorsulfonic acid, CH₂Cl₂, rt, 2 h (93–99%); (h) (*i*-PrO)₃B, *n*-BuLi, THF, –78 °C to rt, 3 h; (i) 6 M HCl, THF, rt, 3 h (22–57%).

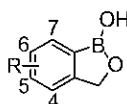


Scheme 3. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, toluene, reflux, 6 h (77%); (b) K₂CO₃, DMF, 100 °C, overnight (62–99%); (c) 3 M HCl, THF, reflux, 2 h (62%); (d) NaBH₄, MeOH, rt, 1 h; (e) 3,4-dihydro-2H-pyran, camphorsulfonic acid, CH₂Cl₂, rt, 2 h; (f) (*i*-PrO)₃B, *n*-BuLi, THF, –78 °C to rt, 3 h (3 steps 43–69%). (See above mentioned references for further information.)

cytokine release inhibition. Compound **5g** showed three to four fold higher IC_{50} values against TNF- α and IL-5, comparable potency against IL-2 and IFN- γ , and better activity against IL-10 compared to rolipram. The IC_{50} values of compound **5b** against the panel were three to six times less active than those of rolipram. 4-Fluorophenoxy derivative (**11c**) showed comparable PDE4 inhibition to **11a**, and slight inhibition against IL-2 and IL-5 (55 and 53% inhibition at 10 μ M, respectively). Introduction of chloro (**11d**), methyl (**11e**), and methoxy (**11f**) groups resulted in slight improvement of PDE4 inhibition. However, no significant cytokine release inhibition was observed. Activity was also diminished when the cyano group was relocated from *para* to *ortho* (**5a**) or *meta* (**11b**) positions.

Then the 4-cyanophenoxy group was relocated to other positions from the 5-position. The 4-position analog (**17a**) showed

Table 1
Inhibitory activity of benzoxaboroles against PDE4 and cytokine release



Compound	R	IC ₅₀ (μM) or inhibition% at 10 μM ^a					
		PDE4	TNF-α	IL-2	IFN-γ	IL-5	IL-10
5a	5-(2-CN-PhO)	3.7	8.5	4.0	4.1	7.8	8.0
5b	5-(4-CN-PhO)	0.49	0.54	0.61	0.83	2.4	5.3
5c	5-(4-SO ₂ NEt ₂ -PhO)	3.4	>10	>10	>10	>10	>10
5d	5-(4-SO ₂ N(CH ₂ CH ₂) ₂ NMe)	>30	>10	>10	>10	>10	>10
5e	5-(4-CO ₂ H-PhO)	6.4	>10	>10	>10	>10	>10
5f	5-(4-CONEt ₂ -PhO)	1.5	1.3	1.4	0.43	4.3	2.0
5g	5-(4-CON(CH ₂ CH ₂) ₂ O-PhO)	0.57	0.44	0.33	0.17	1.9	0.37
11a	5-PhO	6.8	>10	>10	>10	>10	>10
11b	5-(3-CN-PhO)	4.4	8.6	3.3	3.4	4.7	8.9
11c	5-(4-F-PhO)	7.7	>10	55%	15%	53%	>10
11d	5-(4-Cl-PhO)	1.3	>10	62%	29%	>10	>10
11e	5-(4-Me-PhO)	2.0	>10	>10	29%	>10	>10
11f	5-(4-OMe-PhO)	2.7	>10	>10	39%	>10	>10
11g	5-(4-CF ₃ -PhO)	0.45	6.1	3.6	2.2	12%	49%
17a	4-(4-CN-PhO)	6.0	>10	3.9	>10	8.2	>10
17b	6-(4-CN-PhO)	>30	>10	>10	>10	>10	>10
17c	7-(4-CN-PhO)	>30	>10	>10	>10	>10	>10
18	5-MeO		>10	>10	>10	>10	>10
Rolipram		0.86	0.16	0.23	0.23	0.50	0.88

^a IC₅₀ values and inhibition % are calculated from means of at least two experiments.

some inhibition against PDE4 (IC₅₀ 6.0 μM) as well as against several cytokines (IC₅₀s 3.9 μM against IL-2 and 8.2 μM against IL-5). However, other isomers (**17b,c**) did not show any activity in these systems. 5-Methoxy derivative (**18**)⁹ that lacks the pendant phenyl ring did not show any activity, either. Those data indicated the importance of a substituted phenoxy group at the 5-position for the inhibition against both PDE4 and cytokine release. As for the substituent, an electron withdrawing group (EWG), such as cyano, substituted carbamoyl, and trifluoromethyl groups, showed potent activity against PDE4. However sulfonamides (**5c,d**) did not, even though these are strong EWGs. The sulfonamide groups might be too bulky to be accommodated. Only cyano (**5b**) and morpholinocarbonyl (**5g**) derivatives showed sub-micro molar IC₅₀ values against TNF-α, IL-2, and IFN-γ. All these compounds did not show cytotoxicity against mouse fibroblast-derived L929 cells up to 100 μM (data not shown).

The most potent compounds (**5b** and **5g**) showed similar IC₅₀ values against both PDE4 (biochemical) and cytokine release (cell based) assays, suggesting these compounds have good cell membrane permeability. Interestingly, trifluoromethyl derivative (**11g**) was much less active in the cytokine assay, although it was equally potent as **5b** and **5g** against PDE4 enzyme. All the compounds synthesized and tested showed no significant inhibition against IL-1β and IL-4 release (data not shown). The same observation was seen with other non-oxaborole PDE4 inhibitors.¹⁵

In order to investigate the isozyme selectivity, compound **5b** was then tested against the panel of PDE isozymes (PDE1 through 11) by a modified two-step method using recombinant human PDE enzymes expressed in a baculoviral system.¹⁶ Compound **5b** showed the most potent activity against PDE4 catalytic domain, but it also showed inhibition against PDE1A3, PDE3Cat, and PDE7A1. The IC₅₀ values are summarized in Table 2. Compound **5b** did not show significant inhibition against other isozymes at 10 μM.

Compounds **5b** and **5g** were tested in phorbol ester-induced mouse ear edema model¹⁷ to determine in vivo efficacy and skin penetration by topical treatment. The results are summarized in

Table 2
Inhibitory activity of **5b** against PDE isozymes

Compound	IC ₅₀ (μM) ^a			
	PDE1A3 (cAMP)	PDE3Cat	PDE4Cat	PDE7A1
5b	6.1	6.4	0.11	0.73
Ref.	10 ^b	0.84 ^c	2.3 ^d	2.2 ^e

^a IC₅₀ values are calculated from means of three experiments. The substrate concentration was 1 μM except for PDE7A1 (0.1 μM).

^b 8-Methoxymethyl-IBMX.

^c Cilostazol.

^d Rolipram.

^e BRL-50481.

Table 3. Compounds **5b** and **5g** showed significant inhibition against the ear edema caused by phorbol ester after dosing at 1 mg/ear × 2 (78% and 68%, respectively). The efficacy was comparable to that of dexamethasone, suggesting that these compounds have good anti-inflammatory activity as well as skin penetration in vivo. Rolipram showed significant inhibition only at a higher concentration in this system.

In conclusion, we report the identification and SAR of a new series of phenoxybenzoxaborole anti-inflammatory agents. These compounds demonstrated both inhibitory activity against the PDE4 enzyme and inflammation-related cytokine release. The SAR illustrated that the substituted phenoxy group at the 5-position was important for both activities. Selected compounds **5b** and **5g** demonstrated in vivo efficacy in phorbol ester-induced mouse ear edema model by topical application. Compound **5b**

Table 3
Inhibitory activity of **5b** and **5g** against phorbol ester-induced mouse ear edema

Compound	Dose	Inhibition (%)
5b	1 mg/ear × 2	78
5g	1 mg/ear × 2	68
Dexamethasone	1 mg/ear × 2	72
Rolipram	1 mg/ear × 2	6
	3 mg/ear × 2	53

(AN2728) was chosen as the developmental candidate, and is currently in phase 2 clinical trials for the topical treatment of psoriasis and being pursued for the topical treatment of atopic dermatitis.

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- PDE4 assay: PDE4 was partially purified from human U-937 myeloid leukemia cells. The test article and/or vehicle was incubated with 0.2 mg of enzyme and 1 mM cAMP containing 0.01 mM [³H]cAMP in Tris buffer (pH 7.5) for 20 min at 25 °C. The reaction was terminated by boiling for 2 min and the resulting AMP was converted to adenosine by addition of 10 mg/ml snake venom nucleotidase and further incubation at 37 °C for 10 min. Unhydrolyzed cAMP was bound to AG1-X2 resin, and remaining [³H]Adenosine in the aqueous phase was quantitated by scintillation counting. Test articles were tested at 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 μM for IC₅₀ determination.
- Cytokine assay: Frozen human peripheral blood mononucleocytes (PBMC) were thawed and centrifuged. Cryopreservation media was aspirated off of the cell pellet, and the cells were resuspended in fresh culture media (CM) comprising RPMI 1640 and 10% FBS in 96 well plates. The test article was dissolved in DMSO to form a 10 mM sample (DMSO, 100%). The 10 mM samples were diluted to 100 μM in CM (DMSO, 1%), then further diluted to 10, 1, 0.1, and 0.01 μM final concentration (n = 3). Inducer (1 μg/mL LPS for TNF-α or 20 μg/mL PHA for IFNγ, IL-2, IL-5 and IL-10) plus vehicle (1% DMSO) was used as a control. Vehicle without inducer was used as a negative control. Cells were incubated at 37 °C, 5% CO₂. Supernatants were removed at 24 h (for TNF-α, IFNγ, and IL-2) or 48 h (for IL-5, and IL-10), and stored at -20 °C. The supernatants were thawed, and assayed for TNF-α, IFNγ, IL-2, IL-5, and IL-10 expression using the fluorochrome-labeled cytokine-specific beads and the Becton Dickinson FACSArray™.
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- Mouse ear edema model: phorbol 12-myristate 13-acetate (PMA, 5 μg in 20 μL of acetone) was applied topically to the anterior and posterior surface of the right ear to groups of 5 CD-1 (CrI.) derived mice (weighing 22 ± 2 g). Test substances (1 mg in 20 μL) and vehicle (acetone/ethanol/1:1, 20 μL/ear) were each applied 30 min before and 15 min after PMA challenge. Ear swelling was then measured by a Dyer model micrometer gauge at 6 h after PMA application as an index of inflammation. Percent inhibition was calculated according to the formula: $[(I_c - I_t)/I_c] \times 100\%$, where I_c and I_t refer to increase of ear thickness (mm) in control and treated mice, respectively.