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Treatment of Skin Inflammation with Benzoxaborole Phosphodiesterase Inhibitors: Selectivity, Cellular Activity, and Effect on Cytokines Associated with Skin Inflammation and Skin Architecture Changes[S]

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ABSTRACT

Psoriasis and atopic dermatitis are skin diseases affecting millions of patients. Here, we characterize benzoxaborole phosphodiesterase (PDE)-4 inhibitors, a new topical class that has demonstrated therapeutic benefit for psoriasis and atopic dermatitis in phase 2 or phase 3 studies. Crisaborole [AN2728, 4-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)benzonitrile], compd2 [2-ethoxy-6-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)oxy) nicotinonitrile], compd3 [6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborol-5-yl)oxy)-2-(2-isopropoxyethoxy)nicotinonitrile], and compd4 [5-chloro-6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborol-5-yl)oxy)-2-((4-oxopentyl)oxy)nicotinonitrile] are potent PDE4 inhibitors with similar affinity for PDE4 isoforms and equivalent inhibition on the catalytic domain and the full-length enzyme. These benzoxaboroles are less active on other PDE isozymes. Compd4 binds to the catalytic domain of PDE4B2 with the oxaborole group chelating the catalytic bimetal and overlapping with the phosphate in cAMP during substrate hydrolysis, and the interaction extends into the adenine pocket. In cell culture, benzoxaborole PDE4 inhibitors suppress the release of tumor necrosis factor-α, interleukin (IL)-23, IL-17, interferon-γ, IL-4, IL-5, IL-13, and IL-22, and these cytokines contribute to the pathologic changes in skin structure and barrier functions as well as immune dysregulation in atopic dermatitis and psoriasis. Treatment with compd3 or N^6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate increases cAMP response element binding protein phosphorylation in human monocytes and decreases extracellular signal-regulated kinase phosphorylation in human T cells; these changes lead to reduced cytokine production and are among the mechanisms by which compd3 blocks cytokine release. Topical compd3 penetrates the skin and suppresses phorbol myristate acetate–induced IL-13, IL-22, IL-17F, and IL-23 transcription and calcipotriol-induced thymic stromal lymphopoietin expression in mouse skin. Skin thinning is a major dose-limiting side effect of glucocorticoids. By contrast, repeated application of compd3 did not thin mouse skin. These findings show the potential benefits and safety of benzoxaborole PDE4 inhibitors for the treatment of psoriasis and atopic dermatitis.

Introduction

Psoriasis and atopic dermatitis (AD) are common skin diseases affecting millions of patients (Charman and Williams, 2002; Arkwright et al., 2013; Parisi et al., 2013). Leukocytes, keratinocytes, and inflammatory cytokines are crucial in initiating and perpetuating AD and psoriasis (Brandt and Sivaprasad, 2011; Gittler et al., 2012; Lowes et al., 2014). Reducing the levels of inflammatory cytokines and/or blocking their signaling pathways have been proven to benefit AD and psoriasis patients. Antibodies specific to tumor necrosis factor (TNF)-α, interleukin (IL)-12/23 p40, IL-17, and IL-4 receptor (IL-4R)/ IL-13 receptor (IL-13R) are clinically effective for moderate-to-severe psoriasis or AD (Crow, 2012; Beck et al., 2014; Thaçi et al., 2015). However, the majority of patients with mild-to-moderate disease are treated with topical corticosteroids, calcineurin inhibitors, or vitamin D analogs (Menter et al., 2009; Ring et al., 2012; Kivelevitch et al., 2013). Glucocorticoids induce skin atrophy and suppress the hypothalamic-pituitary-adrenal axis, and these side

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ABBREVIATIONS: AD, atopic dermatitis; AN2728, 4-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)benzonitrile; AP-1, activation protein 1; CBP, CREB binding protein; compd1, 4-((1-hydroxy-1,3,-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)phthalonitrile; compd2, 2-ethoxy-6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)nicotinonitrile; compd3, 6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)-2-(2-isopropoxyethoxy) nicotinonitrile; compd4, 5-chloro-6-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)oxy)-2-((4-oxopentyl)oxy)nicotinonitrile; CREB, cAMP response element binding protein; dBcAMP, N^6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate; ERK, extracellular signal-regulated kinase; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PBMC, peripheral blood mononuclear cell; PDE, phosphodiesterase; PHA, phytohemagglutinin; PKA, protein kinase A; PMA, phorbol myristate acetate; RS25344, 8-aza-1-(3-nitrophenyl)-3-(4-pyridylmethyl)-2,4-quinazoline dione; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; UCR, upstream conserved region.

effects are particularly concerning in children, who comprise the majority of patients with AD (Kivelevitch et al., 2013; Schäkel et al., 2014). Calcineurin inhibitors have additional potential risks of infection and cancer and have boxed warnings required by the U.S. Food and Drug Administration (Guttman-Yassky et al., 2011b; Ring et al., 2012). For these reasons, there is a need for novel, safe, and effective topical therapeutic agents for patients with mild-to-moderate AD or psoriasis.

Psoriasis is associated with hyperactivity of Th1 and Th17 immune responses. Psoriatic skin lesions exhibit prominent infiltrates of Th1 and Th17 T cells and dendritic cells (Guttman-Yassky et al., 2011a), as well as elevated levels of interferon (IFN)-γ, TNF-α, IL-17, and IL-23 (Guttman-Yassky et al., 2011b; Lowes et al., 2013; Martin et al., 2013). AD is characterized as a disorder of Th2 immunopathology. Pruritus is a prominent feature of AD and significantly affects the sleep and quality of life of patients with AD. AD lesional skin contains pronounced infiltrates of Th2 cells, Th22 cells, and dendritic cells, as well as increased numbers of eosinophils and mast cells (Guttman-Yassky et al., 2011a; Gittler et al., 2012).

The levels of cytokines associated with the Th2 phenotype, such as IL-4, IL-5, IL-13, and IL-31, are increased in both acute and chronic AD lesions (Leung et al., 2004; Gittler et al., 2012). When AD lesions become chronic, IFN-γ expression increases and the levels of Th2 cytokines remain high or even become further elevated (Gittler et al., 2012). The levels of itch mediators, such as thymic stromal lymphopoietin (TSLP) and IL-31, are increased in AD skin lesions (Soumelis et al., 2002; Bilsborough et al., 2006; Sonkoly et al., 2006). In both psoriasis and AD, IL-22 is highly expressed, and this cytokine prevents terminal differentiation of keratinocytes and promotes epidermal hyperplasia (Fujita, 2013).

Cyclic nucleotide-specific phosphodiesterases (PDEs) control the intracellular level of cAMP or cGMP and thus play critical roles in many cellular functions. Among the 11 families of PDEs, PDE4 has received great attention as a target for treating inflammatory diseases. Leukocytes are highly sensitive to PDE4 inhibition in part because PDE4 is a major PDE isozyme in immune cells, including T cells, B cells, eosinophils, neutrophils, monocytes, and macrophages (Torphy, 1998). PDE4 inhibitors block cytokine synthesis by elevating the cAMP level and subsequently activating protein kinase A (PKA), which negatively modulates signaling pathways that lead to cytokine secretion (Torgersen et al., 2002; Maurice et al., 2014). Continuing efforts have been devoted to the development of safer and more efficacious PDE4 inhibitors (Kumar et al., 2013; Maurice et al., 2014). Several novel PDE4 inhibitors have been tested in clinical trials for AD (Kumar et al., 2013; Maurice et al., 2014; Schäkel et al., 2014). Crisaborole [AN2728, 4-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborol-5-yl)oxy)benzonitrile] (Akama et al., 2009) has shown significant clinical benefit to patients with mild-to-moderate AD in phase 2 (Murrell et al., 2015; Stein Gold et al., 2015) and phase 3 (data on file, Anacor Pharmaceuticals, Inc., 2015) clinical trials.

Here, we characterize the anti-inflammatory properties of four benzoxaborole PDE4 inhibitors, including crisaborole. These molecules potently inhibited PDE4 enzymatic activity. Crystallography revealed that interaction of benzoxaboroles with the hydrophobic pocket in the PDE4 catalytic domain increased their affinity for PDE4. These benzoxaboroles strongly suppressed the secretion of cytokines associated with psoriasis and AD. We also explored the intracellular pathways modulated by cAMP/PKA and PDE4 to further elucidate the mechanism by which benzoxaborole PDE4 inhibitors suppress cytokine secretion. Finally, we demonstrated that a benzoxaborole PDE4 inhibitor reduced skin inflammation and inflammatory cytokine production in mice, without causing skin thinning. These findings provide a better understanding of the potential therapeutic benefits and safety of benzoxaborole PDE4 inhibitors.

Materials and Methods

Animals. Female CD-1 mice were purchased from Charles River Laboratories (Hollister, CA), and SKH-1 mice were obtained from Charles River Laboratories (Wilmington, MA). All animal studies were performed with approval from the Anacor Pharmaceuticals Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals.

Drugs. Crisaborole, compd2 [2-ethoxy-6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)nicotinonitrile], compd3 [6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)-2-(2-isopropoxyethoxy)nicotinonitrile], and compd4 [5-chloro-6-((1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yl)oxy)-2-((4-oxopentyl)oxy)nicotinonitrile] were synthesized by Anacor Pharmaceuticals, Inc. (Palo Alto, CA) and were $>$ 95% pure. Roflumilast [3-(cyclopropylmethoxy)-*N*-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide] and apremilast [(*S*)-*N*-(2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl)-1,3-dioxoisoindolin-4-yl) acetamide] were synthesized by Sundia (Shanghai, China).

Enzymes. The human recombinant PDE4B catalytic domain (amino acids 152–484) was purchased from Proteros (Regensburg, Germany). The full-length human recombinant PDE4 enzymes were purchased from BPS Bioscience (San Diego, CA).

PDE4 Assays. The PDE4-mediated cAMP enzymatic hydrolysis assay was performed as described by Saldou et al. (1998). The enzymatic activities of other PDEs were assessed by a fluorescent polarization assay by BPS Bioscience (San Diego, CA) using purified human recombinant full-length PDE enzymes. The IC_{50} value was calculated based on a four-parameter logistic equation, and the geometric mean of replicates is reported because the IC_{50} values are not normally distributed.

X-Ray Crystallography. The human recombinant PDE4B catalytic domain (amino acids 152–484) was used in the crystallization with compd4. Phase information, which was used to analyze the structure, was obtained by molecular replacement with a previously solved structure of PDE4B (Freund et al., 2012) as the search template. The model included residues Asn162 to Ile484 and was 99% complete with a resolution of 1.86 Å. The electron density in the boron-containing heterocyclic ring was weaker than that in the rest of the ligand. Additional information is available in the Supplemental Material. This structure is available in the Protein Data Bank under code 5K6J.

Cytokine Assays. Human peripheral blood mononuclear cells (PBMCs) were stimulated in vitro to produce TNF-α, IL-2, IFN-γ, IL-5, IL-4, IL-13, IL-17, and IL-22. Human PBMCs were stimulated with 1 μg/ml lipopolysaccharide (LPS) for 24 hours to produce TNF-α; stimulated with 20 μg/ml phytohemagglutinin (PHA)-L for 24 hours to induce IL-2 and IFN-γ, or for 48 hours to induce IL-5. IL-4, IL-13, and IL-17 were induced in human PBMCs with 20 μg/ml concanavalin A for 48 hours. To induce IL-22 production, PBMCs were stimulated for 24 hours with anti-CD2/CD3/CD28 beads (cell/bead = 1:1) (Miltenyi Biotec, Bergisch Gladbach, Germany). Human monocytes were isolated as described by Dong et al. (2013) with a purity of 85%–90% and were stimulated with 1 μg/ml LPS and 100 ng/ml IFN-γ for 24 hours to induce IL-23 production. The cell culture supernatants were collected

for cytokine analysis. The levels of TNF-α, IL-2, IL-4, IL-5, IL-13, IL-17, IL-23, and IFN-γ were determined using Cisbio HTRF cytokine determination kits (Cisbio, Bedford, MA); the level of IL-22 was quantified by an enzyme-linked immunosorbent assay (eBioscience, San Diego, CA). The results are not normally distributed and are thus presented as the geometric mean of the IC_{50} values from multiple independent experiments.

Protein Phosphorylation and Western Blot Analysis. To evaluate the effect of the benzoxaborole PDE4 inhibitors on cAMP response element binding protein (CREB) phosphorylation, human monocytes were incubated at 37°C for 3 hours and then either not treated or treated with 1 μM compd3 or 100 μM N^6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate (dBcAMP) (Sigma, St. Louis, MO), a cell-permeant cAMP analog, for 15 minutes prior to stimulation with 1 μg/ml LPS. The nuclear fraction was isolated using a nuclear extract kit (Active Motif, Carlsbad, CA), separated by SDS-PAGE, and transferred to a polyvinylidene fluoride membrane. Phospho-CREB was detected by an antibody specific to phosphoserine (S133) (Santa Cruz Biotechnology, Santa Cruz, CA). To address the effect of the benzoxaborole PDE4 inhibitors on extracellular signal-regulated kinase (ERK) phosphorylation, human T cells were isolated as described by Dong et al. (2013) with a purity of $\geq$ 90%. The T cells were incubated at 37°C for 2 hours and then either not treated or treated with compd3 or dBcAMP for 15 minutes prior to stimulation with human T-activator CD3/CD28 beads (Thermo Fisher Scientific, Grand Island, NY). Total cell lysates were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Phospho-ERK was detected by an antibody specific to phospho-threonine (T202) and phospho-tyrosine (Y204) (Cell Signaling Technology, Danvers, MA). The experiments were performed three times using cells from different donors, and two representative results are reported here.

Topical Phorbol Myristate Acetate–Induced Contact Dermatitis. Ten female CD-1 mice (20–30 g) per group were used in this experiment, which was based on a protocol described by Kuchera et al. (1993) with a few modifications as described by Dong et al. (2013). Three milligrams of compd3 dissolved in acetone/ethanol [1:1 (v/v)] was applied 30 minutes before and 15 minutes after the phorbol myristate acetate (PMA) challenge. The thickness of the ears was measured at 6 hours after PMA application, and 8-mm ear punches were collected and preserved in RNAlater (Thermo Fisher Scientific). Total RNA was isolated from the ear punches using an RNeasy Plus Universal Tissue Kit (Qiagen, Valencia, CA). The following primers used for reverse-transcription polymerase chain reaction were purchased from Qiagen: IL-22 (QT00128324), IL-17F (QT00144347), IL-23 (p19) (QT01663613), IL-13 (QT00099554), and the reference gene hypoxanthine phosphoribosyltransferase 1 (QT00166768). Statistical significance was analyzed using Kruskal–Wallis one-way analysis of variance and Dunn's post-test for computation of multiplicity-adjusted *P* values as recommended by Yuan et al. (2006).

Topical Calcipotriol-Stimulated TSLP Secretion. Six female CD-1 mice per group were used in this experiment, which was modified from the protocol of Li et al. (2006). To induce TSLP expression, 20 μl of 200 μM calcipotriol in 95% ethanol was applied to a mouse ear. Compd3 in acetone/ethanol [1:1 (v/v)] was applied to the same area 30 minutes before and 15 minutes after the calcipotriol challenge. At 24 hours postchallenge, skin punches were collected, and total RNA was isolated as described above. TSLP mRNA was quantified by reverse-transcription polymerase chain reaction using primers from Qiagen (QT02416155). In parallel, skin tissues were homogenized as described by Dong et al. (2013), and the concentration of TSLP in the homogenates was determined by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Glucocorticoid Receptor Binding Assay. A [^{3}H]-dexamethasone displacement assay based on the report by Honer et al. (2003) was conducted by Eurofins Panlabs Taiwan (Taipei, Taiwan) using the human recombinant glucocorticoid receptor ligand-binding domain (Thermo Fisher Scientific).

In Vivo Skin Thinning Assay. The effect of each compound on mouse skin thickness was evaluated using hairless SKH-1 mice (eight mice per group) generally following the protocol of Kirby and Munro (1976), with the addition of histopathology as reviewed by Schoepe et al. (2006). The baseline thickness of the dorsal skin was measured using calipers prior to drug treatment. Each mouse received 30 μl of a topical drug once a day for 16 consecutive days on a 1-cm^2 area of the dorsal skin between the shoulder blades near the neck. The compounds were dissolved in acetone/ethanol [1:1 (v/v)]. The thickness of the dorsal skin was measured on days 1, 7, and 16. At the end of the treatment period, the dorsal skin was removed and fixed in neutral buffered formalin, followed by hematoxylin and eosin staining. The thicknesses of the epidermis and dermis was quantified by measuring three representative areas. Statistical significance was analyzed using one-way analysis of variance and Dunnett's multiple comparisons test.

Results

Benzoxaborole PDE Inhibitors Potently Inhibit PDE4. Crisaborole, compd2, compd3, and compd4 (Supplemental Fig. 1) inhibited the activity of the catalytic domain of PDE4B2, with IC_{50} values of 75, 1.8, 3.0, and 0.32 nM, respectively (Table 1). Although some types of PDE4 inhibitors interact with both the catalytic domain and the upstream conserved region (UCR) 2 region (Saldou et al., 1998; Burgin et al., 2010), the benzoxaborole PDE4 inhibitors described here displayed essentially equal affinity for the full-length form and the catalytic domain of PDE4 (Table 1). Crisaborole displayed IC_{50} values of 61 nM and 75 nM against the full-length PDE4B1 and the PDE4B2 catalytic domain, respectively, and compd4 showed equivalent activities on the full-length and truncated forms of PDE4B2, with IC_{50} values

TABLE 1
Affinities of crisaborole, compd2, compd3, compd4, apremilast, and roflumilast for PDE4 isoforms

Compound	IC_{50} Value (No. of Independent Experiments)					
	PDE4B2_cat	PDE4A1A	PDE4B1	PDE4B2	PDE4C1	PDE4D7
			nM			
Crisaborole	75 (2)	55 (5)	61 (5)	NT	340 (4)	170 (5)
Compd2	1.8 (2)	6.4 (2)	6.8 (2)	4.4 (2)	71 (2)	12 (2)
Compd3	3.0 (2)	6.5 (2)	3.1 (2)	2.1 (2)	8.4 (2)	4.6 (2)
Compd4	0.32 (10)	2.3 (2)	0.15 (2)	0.22 (2)	4.4 (2)	0.36 (2)
Apremilast	39 (1)	8.9 (5)	16 (4)	23 (2)	48 (4)	12 (5)
Roflumilast	0.47 (6)	0.33 (2)	0.28 (2)	15 (2)	0.95 (2)	0.53 (2)

PDE4B2_cat is the catalytic domain of human PDE4B2; the sequences of the catalytic domain of PDE4B1 and PDE4B2 are identical. The others are full-length human PDE4 enzymes. In the assays, 1.5 μM [^{3}H]-cAMP was used as a substrate. NT, not tested.

of 0.22 nM and 0.32 nM, respectively. Similar to benzoxaborole inhibitors, apremilast and roflumilast displayed equal affinity for full-length PDE4 and its catalytic domain. Thus, crisaborole, compd2, compd3, and compd4 bound to the catalytic domain but did not interact with other regulatory regions of PDE4.

Four *PDE4* genes and their alternatively spliced mRNAs encode more than 20 PDE4 proteins (Houslay et al., 2007). The effects of benzoxaboroles on some of these PDE4 isoforms were further evaluated. Crisaborole, compd2, compd3, and compd4 exhibited comparable activity for PDE4A1A, PDE4B1, PDE4B2, PDE4C1, and PDE4D7 (Table 1), which was mirrored by apremilast and roflumilast. Thus, the examined benzoxaborole PDE4 inhibitors, apremilast and roflumilast, were not selective among PDE4 isozymes.

In addition to PDE4, 10 other homologous gene families have been identified in mammals; these genes constitute the PDE superfamily, classified as PDE1–PDE11 (Maurice et al., 2014). These PDE family proteins exhibit selectivity for cAMP or cGMP, and have diverse regulatory mechanisms, subcellular localization, and tissue expression (Maurice et al., 2014). The effects of the tested benzoxaborole PDE4 inhibitors, as well as apremilast and roflumilast, on the 10 PDE families other than PDE4 were explored (Table 2). Apremilast and roflumilast were more selective for PDE4 and displayed no appreciable inhibitory activity on other PDE isoenzymes. Crisaborole, compd2, compd3, and compd4 showed moderate inhibitory activity on PDE enzymes outside the PDE4 family, with IC_{50} values ranging from 24 to 9400 nM, the majority of which were > 500 nM.

X-Ray Crystal Structures of Compd4 and PDE4B. Compd4 was cocrystallized with the human recombinant PDE4B catalytic domain. Similar to the crystallographic structure of compd1 (3O0J) (Freund et al., 2012), a close analog of crisaborole, the benzoxaborole moiety of compd4 interacted with the two metal ions at the catalytic site in a tetrahedral configuration. The substituted pyridine ring of the benzoxaborole core was tightly sandwiched between Phe446 and Phe414, whereas the nitrile substituent at C(5) of the pyridine ring was positioned to form a key H-bond with Gln443. The 4-(keto)pentyloxy side chain occupied an enclosed hydrophobic pocket formed by Ile410, Tyr233, Asn395, Tyr403, and Pro396. Collectively, these interactions likely contribute to the substantially increased affinity of compd4 (0.32 nM) relative to crisaborole (75 nM) (Fig. 1).

Cytokine Inhibition. Cytokines play key roles in initiating, promoting, and maintaining the pathology of psoriasis and AD (Brandt and Sivaprasad, 2011; Guttman-Yassky et al., 2011b; Gittler et al., 2012; Fujita, 2013; Lowes et al., 2014). The levels of TNF-α, IL-23, and IL-17 are elevated in psoriatic lesions, and these cytokines are central to the pathogenesis of this disorder. As shown in Table 3, crisaborole, compd2, compd3, and compd4 potently inhibited LPS-induced TNF-α secretion from human PBMCs, with IC_{50} values of 170, 24, 3.4, and 0.56 nM, respectively. Crisaborole also blocked TNF-α secretion from monocyte-derived dendritic cells, with an IC_{50} value of 890 nM; and compd3 reduced TNF-α secretion from human primary monocytes, with an IC_{50} of 8 nM (Supplemental Table 1A). Crisaborole, compd2, compd3, and compd4 were potent inhibitors of LPS/IFN-γ–induced IL-23 secretion from human primary monocytes, with IC_{50} values of 2400, 32, 7.7, and 73 nM, respectively. In addition, these compounds inhibited IL-17 release from human PBMCs. Similarly, apremilast and roflumilast inhibited TNF-α, IL-23, and IL-17 production. The IC_{50} values of apremilast against TNF-α, IL-23, and IL-17 were 15, 22, and 1500 nM, respectively, and those of roflumilast were 0.49, 0.39, and 520 nM, respectively.

Th2 cytokines contribute to the pathogenesis of AD (Biedermann et al., 2004; Brandt and Sivaprasad, 2011; Guttman-Yassky et al., 2011a; Gittler et al., 2012). Therefore, we determined the effects of the benzoxaboroles crisaborole, compd2, compd3, and compd4, as well as those of apremilast and roflumilast, on Th2 cytokine production. Crisaborole inhibited IL-4, IL-5, and IL-13 secretion, with IC_{50} values of 480, 2000, and 22,000 nM, respectively (Table 3). Compd3 and

TABLE 2
Affinities of crisaborole, compd2, compd3, compd4, apremilast, and roflumilast for other PDE isozymes

PDE Isozyme	IC_{50} Value					
	Crisaborole	Compd2	Compd3	Compd4	Apremilast[c]	Roflumilast[c]
	nM					
PDE1A1	[a]	260	1500	6400		
PDE1B	3700	90	800	5500		
PDE1C	[b]	81	520	5700		
PDE2A1	6700	400	970	580		
PDE3A	5100	130	1200	1400		
PDE3B	4500	310	1700	1400		
PDE5A1	[c]	1700	1100	3100		
PDE6C	6700	5000	7500	2700		
PDE7A1	[c]	24	260	470		
PDE7B	9400	330	650	710		
PDE8A1	[c]	140	910	1500		
PDE9A2	[c]	[c]	[c]	[c]		
PDE10A1	[c]	450	580	1800		
PDE10A2	[c]	380	480	1600		
PDE11A4	[c]	2700	2400	3200		

A fluorescent polarization assay was performed using full-length human PDE enzymes and 0.1 μM FAM-cAMP or FAM-cGMP as a substrate. FAM, 5-(and-6)-carboxyfluorescein.
[a]54% inhibition at 10 μM.
[b]80% inhibition at 10 μM.
[c]Less than 50% inhibition at 10 μM. For apremilast and roflumilast, inhibition was $< 50\%$ at 10 μM for all PDE isozymes.

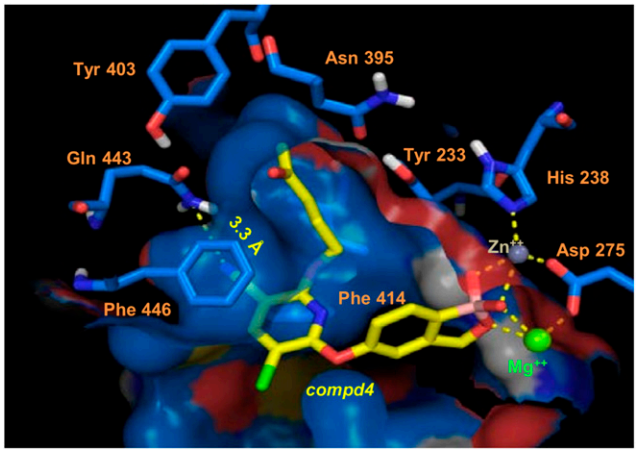

Fig. 1. A surface view of compd4 complexed with the PDE4B catalytic domain.

compd4 were more potent than crisaborole in reducing the levels of IL-4, IL-5, and IL-13 (Table 3). These inhibitory properties were not unique to benzoxaborole PDE4 inhibitors, because apremilast and roflumilast also suppressed IL-4 and IL-5 secretion (Table 3).

Psoriatic lesions have increased numbers of Th1 cells, which produce high amounts of IFN-γ (Austin et al., 1999; Biedermann et al., 2004). IFN-γ is also significantly upregulated in chronic AD lesions (Gittler et al., 2012). Crisaborole, compd2, compd3, and compd4, as well as apremilast and roflumilast, potently blocked PHA-induced IL-2 and IFN-γ release from human PBMCs, with IC_{50} values ranging from 0.10 to 700 nM (Table 3). IL-22 is upregulated in both psoriasis and AD skin lesions, and this cytokine promotes keratinocyte hyperplasia and Th2 polarization in AD (Fujita, 2013; Lowes et al., 2014). Compd2, compd3, compd4, apremilast, and roflumilast inhibited anti-CD2/CD3/CD28–induced IL-22 secretion, with IC_{50} values of 51, 14, 1.8, 86, and 5.9 nM, respectively (Table 3).

The relationship between inhibition of PDE4 and suppression of cytokine secretion was explored by correlating the IC_{50} for PDE4 inhibition with the IC_{50} for cytokine suppression by up to 183 benzoxaborole and nonbenzoxaborole PDE4 inhibitors. A strong relationship would be shown by a slope close to 1 when plotted with log (IC_{50} cytokine) versus log (IC_{50} PDE4). Inhibition of PDE4 enzymatic activity was strongly correlated with the suppression of LPS-induced TNF-α and IL-23, as well as PHA-stimulated IFN-γ, IL-2, and IL-5, with slopes of 0.70–0.80 (Supplemental Table 2). These results indicate that PDE4 is critical in the LPS and PHA signaling pathways that lead to the synthesis of these cytokines. There was a weak relationship between inhibition of PDE4 and suppression of concanavalin A–induced IL-17, IL-4, and IL-13 secretion, with slopes of 0.043–0.33 (Supplemental Table 2).

Effect of Compd3 on the Phosphorylation of CREB and ERK1/2 in Human Primary Leukocytes. Inhibiting PDE4 increases the intracellular level of cAMP, which then activates PKA (Giembycz et al., 1996; Torphy, 1998). PKA activation subsequently leads to the phosphorylation of target proteins, some of which are involved in the control of cytokine production (Supplemental Fig. 4), such as CREB (Gonzalez and Montminy, 1989; Wen et al., 2010), nuclear factor-κB (NF-κB) (Hou et al., 2003; Kwak et al., 2005), nuclear factor of activated T cells (Chow and Davis, 2000), Rap1 (Stork and Dillon, 2005), and Csk (Mosenden and Taskén, 2011).

In HL-60 promyelocytic leukemia cells, increasing the cAMP level prevents cytokine production, and this effect is attributed to the suppression of NF-κB activity by increased phospho-CREB (Parry and Mackman, 1997). To explore whether benzoxaborole PDE4 inhibitors use the same mechanism to inhibit LPS-induced cytokine production, the effect of compd3 on CREB phosphorylation in human primary monocytes was assessed. As shown in Fig. 2A, compd3 increased the phosphorylation of CREB in cells compared with LPS stimulation alone, and the same effect was observed in cells treated with dBcAMP. These results indicate that suppression of LPS-induced cytokines by a PDE4 inhibitor is at least partially mediated by increasing the phospho-CREB level in human monocytes. However, compd3 had no effect on anti-CD3/CD28–induced CREB phosphorylation in human primary T cells (data not shown).

cAMP inhibits activation of Ras and its downstream effector ERK in human primary T cells (Grader-Beck et al., 2003; Stork and Dillon, 2005; Kortum et al., 2013) (Supplemental Fig. 4). The effect is thought to be mediated by increasing phosphorylation of Rap1, which then suppresses the association of Ras and Raf-1 (Stork and Dillon, 2005). ERK regulates

TABLE 3
Effect of crisaborole, compd2, compd3, compd4, apremilast, and roflumilast on cytokine secretion from human leukocytes in vitro

Compound	IC_{50} Value (No. of Independent Experiments)								
	TNF-α[a]	IL-23[b]	IL-17[c]	IL-4[c]	IL-13[c]	IL-5[d]	IL-2[e]	IFN-γ[e]	IL-22[f]
					nM				
Crisaborole	170 (14)	2400 (3)	8300 (2)	480 (4)	22,000 (10)	2000 (7)	210 (11)	700 (12)	NT
Compd2	24 (3)	32 (2)	8200 (2)	8100 (2)	8200 (2)	37 (2)	31 (3)	32 (3)	51 (2)
Compd3	3.4 (8)	7.7 (6)	240 (2)	410 (4)	1400 (3)	31 (2)	5.8 (5)	4.2 (5)	14 (9)
Compd4	0.56 (13)	73 (2)	1500 (2)	190 (5)	1800 (5)	8.4 (3)	0.86 (8)	1.9 (6)	1.8 (3)
Apremilast	15 (8)	22 (3)	1500 (2)	7100 (4)	30,000 (4)	202 (2)	31 (4)	21 (3)	86 (4)
Roflumilast	0.49 (7)	0.39 (2)	520 (2)	7.6 (2)	>100,000 (4)	10 (2)	0.68 (4)	0.10 (3)	5.9 (4)

NT, not tested.
[a]LPS stimulation for 24 hours.
[b]LPS/IFN-γ stimulation for 24 hours.
[c]Concanavalin A stimulation for 48 hours.
[d]PHA-L stimulation for 48 hours.
[e]PHA stimulation for 24 hours.
[f]Anti-CD2/CD3/CD28 stimulation for 24 hours.

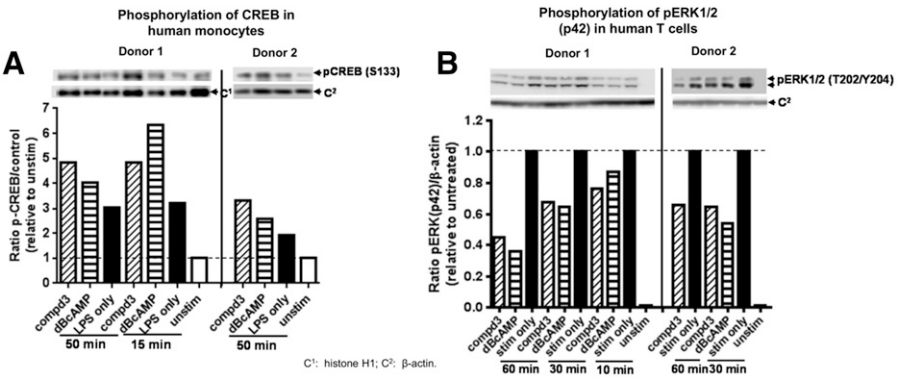

Fig. 2. Compd3 increased CREB phosphorylation in human monocytes and decreased ERK1/2 phosphorylation in human T cells. (A) CREB phosphorylation in nuclear extract from LPS-stimulated human monocytes. (B) ERK1/2 phosphorylation in anti-CD3/CD28–stimulated human T cells. stim, stimulated; unstim, unstimulated.

the expression and stability of c-Fos, a subunit of the activator protein 1 (AP-1) complex, and inhibiting ERK activity results in a reduction in AP-1–mediated cytokine transcription (Schade and Levine, 2004). Because inhibition of PDE4 increases the cAMP level in T cells, we assessed the effect of compd3 on ERK phosphorylation in human primary T cells. As shown in Fig. 2B, ERK was phosphorylated in activated T cells, and treatment with compd3 or dBcAMP reduced anti-CD3/CD28–induced ERK phosphorylation. Thus, the suppression of ERK phosphorylation in T cells is at least partially responsible for the inhibition of T cell receptor–mediated cytokine production by compd3 (Supplemental Table 1B).

Effect of Topical Benzoxaborole PDE4 Inhibitors on Acute Contact Dermatitis. To extend our in vitro observations, we studied the in vivo effect of benzoxaborole PDE4 inhibitors on the production of cytokines associated with AD and psoriasis during skin inflammatory reactions to contact irritants.

Contact irritation induced by PMA is a widely used acute contact dermatitis model. Topical application of PMA triggers prominent neutrophil migration and macrophage activation in the skin. TNF-α, IL-6, and IL-1β, along with proinflammatory lipids such as prostaglandin E2 and leukotriene B4, are induced by topical PMA (Raederstorff et al., 1996; Jang and Pezzuto 1998; Dong et al., 2013). We examined the effect of compd3 on the mRNA levels of IL-23, IL-17F, IL-13, and IL-22 in this model. Challenge with topical PMA upregulated the transcription of IL-23, IL-17F, IL-13, and IL-22 in mouse skin; however, the mRNA levels of IL-4 and IL-5 were not significantly increased by PMA. The mRNA levels of IL-23, IL-17F, IL-13, and IL-22 were low, with cycle threshold values of 32, 33, 34, and 32, respectively. The cycle threshold values of induced IL-6 and the housekeeping gene hypoxanthine phosphoribosyltransferase 1 were 28 and 25, respectively. The levels of PMA-induced IL-23, IL-17F, IL-13, and IL-22 mRNA were low but significant. In accordance with the low mRNA level, we were not able to detect IL-22 protein in PMA-treated ear punches. Compd3 dose-dependently inhibited PMA-induced IL-22 and IL-23 transcription in mouse skin (Supplemental Fig. 2), and 3 mg compd3 completely blocked the upregulation of IL-23, IL-17F, IL-13, and IL-22 mRNA in mouse ears (Fig. 3A). The topical benzoxaborole PDE4 inhibitors crisaborole, compd2, and compd3 significantly suppressed PMA-induced ear swelling in this model: 0.3 mg of these compounds reduced the thickness of

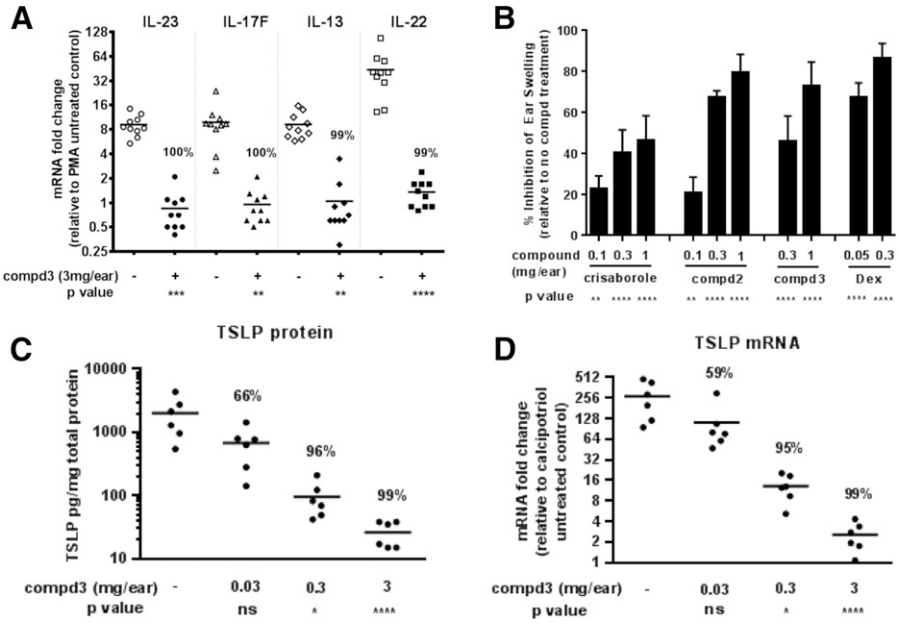

Fig. 3. Effect of compd3 on cytokine production in mouse models. (A) Compd3 inhibited PMA-induced cytokine transcription in mouse skin. (B) Crisaborole, compd2, and compd3 decreased PMA-induced mouse ear swelling. (C and D) Compd3 reduced calcipotriol-induced TSLP expression in mouse skin. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. Dex, dexamethasone.

PMA-treated ears by 40%, 68%, and 46%, respectively, and 1 mg decreased ear swelling by 46%, 78%, and 72%, respectively (Fig. 3B).

Compd3 Inhibited Calcipotriol-Induced TSLP Expression. Increased expression of TSLP is observed in skin lesions of patients with AD (Soumelis et al., 2002; Sano et al., 2013). Mice overexpressing TSLP in keratinocytes develop AD-like symptoms (Li et al., 2005; Yoo et al., 2005). TSLP promotes Th2 polarization (Ziegler and Artis, 2010), and injection of TSLP into mice evokes scratching, indicating that TSLP is an itch mediator (Wilson et al., 2013). To address the effect of a PDE4 inhibitor on TSLP expression, we developed a calcipotriol-induced TSLP murine model based on the studies by Li et al. (2005, 2006) with a shorter calcipotriol treatment time. Topical calcipotriol induced TSLP expression in mouse ears in a dose- and time-dependent manner (Supplemental Fig. 3). Treatment with compd3 significantly inhibited TSLP production at both mRNA and protein levels in a dose-dependent manner: 0.03, 0.3, or 3 mg compd3 reduced TSLP protein levels by 66%, 96%, and 99% (Fig. 3C) and TSLP mRNA levels by 59%, 95%, and 99% (Fig. 3D), respectively.

Skin Thinning. Skin atrophy is a major adverse effect of topical glucocorticoids, which are mainstays of therapy for both AD and psoriasis. We thus investigated the ability of a benzoxaborole PDE4 inhibitor to cause skin thinning.

An in vitro competition binding assay was conducted to assess the ability of compd3 to interact with glucocorticoid receptors. Dexamethasone, progesterone, and clobetasol bound to the glucocorticoid receptor with IC_{50} values of 4.64, 240, and 1.35 nM, respectively. However, compd3 at up to 10 μM was unable to compete with dexamethasone for binding to the glucocorticoid receptor.

The effect of compd3 on mouse skin was further investigated using hairless SKH-1 mice. The mice were topically treated with 30 μl 1% compd3 for 16 consecutive days, and no significant change in dorsal skin thickness was observed at the end of treatment (Fig. 4A). By contrast, 30 μl 0.25% dexamethasone and 30 μl 0.05% clobetasol significantly decreased skin thickness by 41.5% and 19.3%, respectively, on day 16 (Fig. 4A). Histopathologic assessment of the dorsal skin collected on day 16 also indicated that treatment with compd3 did not significantly alter the thickness of the epidermis or dermis compared with vehicle treatment. By contrast, dexamethasone significantly reduced the thickness of the epidermis and dermis, and clobetasol moderately decreased epidermal and dermal thicknesses (Fig. 4, B and C).

Discussion

Several benzoxaborole PDE4 inhibitors, including crisaborole, were characterized for their PDE selectivity and effect on cytokines associated with skin inflammation and architecture in psoriasis and AD. Crisaborole has shown clinical benefit in phase 2 (Murrell et al., 2015; Stein Gold et al., 2015) and phase 3 (data on file, Anacor Pharmaceuticals, Inc., 2015) studies for patients with mild-to-moderate AD.

Eleven PDE families have been identified in mammals. PDE4, PDE7, and PDE8 selectively hydrolyze cAMP; PDE5, PDE6, and PDE9 specifically hydrolyze cGMP; and PDE1, PDE2, PDE3, PDE10, and PDE11 cleave both cAMP and cGMP (Maurice et al., 2014). These PDEs differ in their regulatory mechanisms and tissue and cellular distributions. PDE4 is the major PDE family in primary leukocytes, rendering these cells susceptible to PDE4 inhibition (Torphy, 1998). Thus, PDE4 is an important therapeutic target for inflammatory and autoimmune diseases. Four benzoxaboroles (crisaborole, compd2, compd3, and compd4) were potent PDE4 inhibitors, and most of them displayed an IC_{50} value < 10 nM for PDE4 (Table 1). These compounds also displayed moderate activities against other PDE isozymes, with IC_{50} values generally in the micromolar range (Table 2). Roflumilast and apremilast were more selective for PDE4 than the examined benzoxaboroles and had no activity on other PDE isoforms. The additional activities of benzoxaboroles on PDEs aside from PDE4 may enhance their

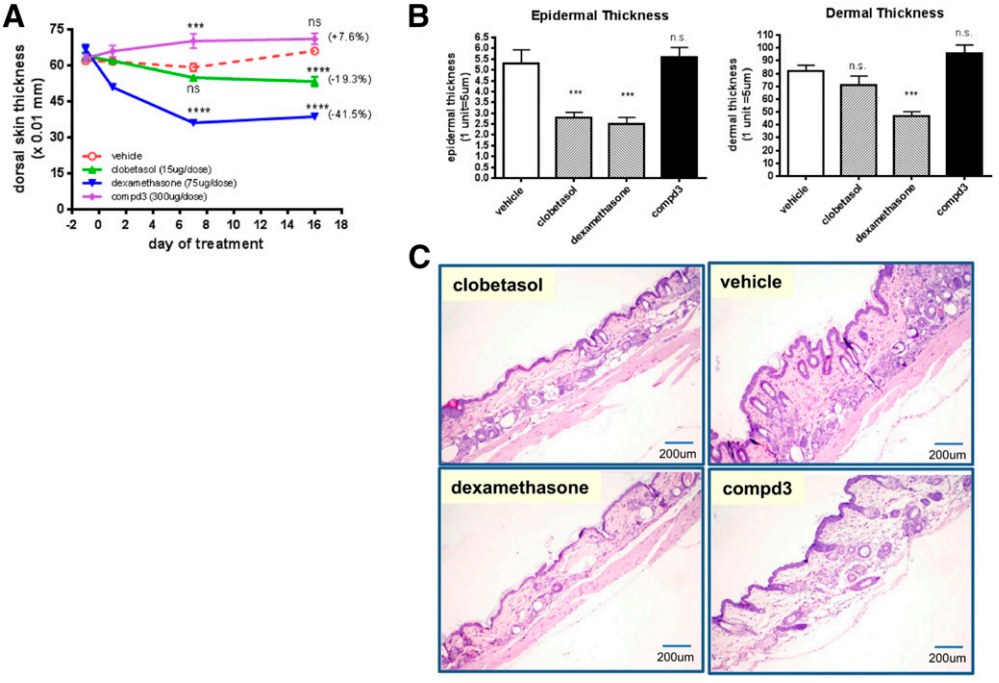

Fig. 4. Effect of compd3 on mouse skin thickness. (A) Thickness of mouse dorsal skin after repeated topical application of compd3, clobetasol, or dexamethasone. (B) Quantitation of epidermal and dermal thickness on day 16 after consecutive treatment with compd3, clobetasol, or dexamethasone. (C) Hematoxylin and eosin staining of mouse dorsal skin on day 16 after topical treatment with compd3, clobetasol, or dexamethasone. ***$P < 0.001$; ****$P < 0.0001$. n.s., not significant.

anti-inflammatory effect, since other PDEs (e.g., PDE1 and PDE7) are involved in regulating immune cell functions (Kanda and Watanabe, 2001; Smith et al., 2004).

PDE4 family proteins contain a C-terminal catalytic domain and N-terminal regulatory regions termed UCR1 and UCR2. Regulatory proteins bind to or modify these UCR domains to modulate PDE4 catalytic activity (Torphy, 1998; Maurice et al., 2014). Some PDE4 inhibitors, such as rolipram, RS25344 [1-(3-nitrophenyl)-3-(4-pyridylmethyl)pyrido[2,3-d]pyrimidine-2,3(1H,3H)-dione], and PMNPQ [6-(4-pyridylmethyl)-8-(3-nitrophenyl)quinoline], bind to both the catalytic domain and the UCR2 and exert their actions by not only regulating catalytic activity but also modifying the functions of other domains (Saldou et al., 1998; Burgin et al., 2010). Crisaborole, compd2, compd3, and compd4 displayed similar activity for full-length PDE4 and its catalytic domain (Table 1), suggesting that these benzoxaboroles bind only to the catalytic domain and unlikely interact with the UCR domains of PDE4. Therefore, the effects of these compounds were mediated by inhibiting catalytic activity and elevating the cAMP levels, not by regulating the functions of other PDE4 regions.

The crystallography of PDE4 complexed with compd4 (Fig. 1) was determined in part to understand the mechanism underlying the increased affinity of compd4 to PDE4 compared with that of crisaborole (Table 1). Similar to crisaborole (unpublished observations) and its analog compd1 (Freund et al., 2012), compd4 interacted with the catalytic bimetal of PDE4 via the oxaborole group. The side chain of compd4 occupied a hydrophobic pocket in the catalytic domain, which increases the affinity of compd4 to PDE4 (Supplemental Fig. 1). The catechol PDE4 inhibitor roflumilast displayed equal affinity for PDE4 as compd4 (Table 1), and the crystal structure of roflumilast (1XOQ) (Card et al., 2004) shows that the difluoromethyl moiety fills the same hydrophobic pocket as the compd4 side chain. Thus, this interaction is important for the high affinities of roflumilast and compd4 for PDE4. In contrast with benzoxaborole PDE4 inhibitors, roflumilast does not directly interact with the bimetal center of PDE4. Because all PDEs have the bimetal center, the interaction of boron with the bimetal center contributes to the weak inhibition of benzoxaboroles to other PDEs.

Several cytokines are associated with skin inflammatory diseases. IL-23, IL-17, and TNF-α drive the pathology of psoriasis (Lowes et al., 2014), and elevation of Th2 cytokines such as IL-4, IL-13, and IL-5 is important in acute AD (Guttman-Yassky et al., 2011b). IFN-γ is elevated in both psoriasis and chronic AD and contributes to the immunopathology of both diseases (Guttman-Yassky et al., 2011b; Gittler et al., 2012). Crisaborole, compd2, compd3, and compd4 suppressed the production of these cytokines associated with psoriasis and AD in cell culture (Table 3; Supplemental Table 1). The broad anti-inflammatory activities of PDE4 inhibitors and the strong relationship between cytokine inhibition and PDE4 affinity (Supplemental Table 2) reflect the central role of cAMP in regulating the signaling pathways leading to cytokine synthesis. Interestingly, crisaborole is more active on inhibiting IL-4 release, whereas apremilast has better activity on suppressing TNF-α, IL-23, and IL-17 secretion in vitro. The different cytokine inhibitory profiles of crisaborole and apremilast may partially explain their clinical effect in AD or psoriasis. Moreover, topical compd3 decreased PMA-induced IL-23, IL-17F, IL-13, and IL-22 mRNA expression in mouse skin (Fig. 3A). IL-22 is elevated in skin lesions in psoriasis and AD. Overproduction of IL-22 stimulates the proliferation of keratinocytes and prevents their terminal differentiation, thus contributing to epidermal hyperplasia (Boniface et al., 2005; Sa et al., 2007; Nograles et al., 2009; Guttman-Yassky et al., 2011b; Lowes, et al., 2013; Martin et al., 2013). PDE4 inhibitors also blocked IL-22 secretion from human leukocytes (Table 3). These findings suggest that blockade of PDE4 would reduce IL-22–mediated epidermal hyperplasia in psoriasis and AD.

Itch is a major symptom of AD (Guttman-Yassky et al., 2011a). TSLP evokes itch and is increased in AD skin lesions (Soumelis et al., 2002; Sano et al., 2013). Injection of TSLP into mice triggers acute itch by directly activating sensory neurons (Wilson et al., 2013). TSLP also promotes Th2 polarization and initiates the Th2 phenotype, which is associated with allergic inflammation (Soumelis et al., 2002; Ziegler and Artis, 2010). Topical compd3 dose-dependently suppressed calcipotriol-induced TSLP expression in mouse skin (Fig. 3, C and D), suggesting that PDE4 inhibitors may inhibit TSLP expression in patients with AD and providing a potential explanation for pruritus suppression observed upon treatment with crisaborole (Murrell et al., 2015).

Benzoxaborole PDE4 inhibitors blocked Toll-like receptor–mediated TNF-α secretion from human PBMCs, monocytes, and monocyte-derived dendritic cells (Table 3; Supplemental Table 1). NF-κB is a transcription activator that modulates TNF-α synthesis. Optimal activity of NF-κB requires the association of its p65 subunit with CBP/p300 at the KIX region, to which phospho-CREB also binds (Fraser et al., 2007). In HL-60 promyelocytic leukemia cells, cAMP increases the phosphorylation of CREB, and phospho-CREB competes with NF-κB for a limited amount of CREB binding protein (CBP)/p300, thus inhibiting TNF-α synthesis (Parry and Mackman, 1997). Compd3 or dBcAMP increased CREB phosphorylation in LPS-stimulated human monocytes (Fig. 2A), and this effect likely reduces the association of p65 with CBP/p300 and blocks NF-κB–mediated TNF-α transcription.

Dysregulation of T cell–mediated immune responses drives the pathogenesis of psoriasis and AD (Leung et al., 2004; Guttman-Yassky et al., 2011b; Lowes et al., 2013). Optimal T-cell activation requires the CD28 costimulatory signal, which recruits the protein kinase B/β-arrestin/PDE4 complex to the proximity of plasma membrane and allows PDE4 to hydrolyze cAMP to overcome the inhibitory signal elicited by cAMP (Bjørgo et al., 2010). Increase of cAMP and activation of PKA as results of PDE4 inhibition directly or indirectly modulate the activities of NF-κB, AP-1, and nuclear factor of activated T cells, which regulate transcription of cytokines in T cells (Supplemental Fig. 4) (Torgersen et al., 2002). PKA phosphorylates Rap1 (Takahashi et al., 2013), and phospho-Rap1 suppresses the association of Ras with Raf-1 and inhibits the activation of ERK (Dumaz and Marais, 2005; Stork and Dillon, 2005; Kortum et al., 2013). ERK phosphorylates several proteins, including Elk-l, a transcription factor involved in c-Fos transcription (Karin, 1995). ERK also regulates the stability of c-Fos in T cells (Schade and Levine, 2004). Inhibition of PDE4 by compd3 or the addition of dBcAMP reduced the phosphorylation of ERK in activated T cells (Fig. 2B), and decreasing ERK activity results in reduced c-Fos expression and inhibition of AP-1–mediated cytokine production. Thus, a decrease in ERK phosphorylation at least

partially contributes to the suppression of cytokine expression by PDE4 inhibitors in human activated T cells.

Topical glucocorticoids are the first-line treatment of mild-to-moderate psoriasis and AD (Menter, et al., 2009; Ring et al., 2012). Skin atrophy is one side effect preventing the long-term topical application of glucocorticoids, especially to sensitive skin regions (Schoepe et al., 2006; Ring et al., 2012). A hairless mouse model is considered to be predictive of human skin responses and is used to evaluate the effect on skin atrophy (Schoepe et al., 2006). Topical dexamethasone or clobetasol resulted in substantial skin thinning, but repeated application of compd3 did not significantly affect skin thickness (Fig. 4A). These effects were confirmed by histopathologic evaluation of skin biopsies, in which dexamethasone and clobetasol, but not compd3, reduced epidermal and dermal thicknesses (Fig. 4, B and C). These results indicate that long-term topical application of benzoxaborole PDE4 inhibitors has low potential to cause skin atrophy.

These studies demonstrated that benzoxaborole PDE4 inhibitors crisaborole, compd2, compd3, and compd4 preferentially inhibited PDE4. As exemplified by compd4, benzoxaborole PDE4 inhibitors directly interact with the bimetal center using the boron atom as a phosphomimetic, and compd4 gains additional affinity by binding to the adenine pocket of PDE4. These benzoxaborole PDE4 inhibitors suppressed TNF-α, IL-23, IL-17, IFN-γ, IL-2, IL-4, IL-5, IL-13, and IL-22 secretion from human leukocytes. This inhibitory effect is partially attributed to the altered phosphorylation of CREB and ERK and the subsequent reduction in the transcriptional activities of NF-κB and AP-1. Compd3 penetrated mouse skin, diminished skin inflammation, and decreased PMA-induced IL-13, IL-17F, IL-22, and IL-23 transcription and calcipotriol-induced TSLP expression. Repeated topical application of compd3 did not induce skin atrophy, in contrast with the significant effect of glucocorticoids. Benzoxaborole PDE4 inhibitors such as crisaborole could serve as a non-steroidal topical treatment of skin inflammatory diseases, including AD and psoriasis.

Authorship Contributions

Participated in research design: Dong, Rock, Jarnagin.

Conducted experiments: Dong, Virtucio, Zemska, Baltazar, Baia, Jones-Iatauro, Sexton, Martin, Dee, Mak, Meewan.

Contributed new reagents or analytic tools: Akama.

Performed data analysis: Dong, Virtucio, Zemska, Baltazar, Zhou, Baia, Jones-Iatauro, Dee, Mak, Meewan, Jarnagin.

Wrote or contributed to the writing of the manuscript: Dong, Zemska, Jarnagin.

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