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Water-soluble PDE4 inhibitors for the treatment of dry eye

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ABSTRACT

PDE4 inhibitors have the potential to alleviate the symptoms and underlying inflammation associated with dry eye. Disclosed herein is the development of a novel series of water-soluble PDE4 inhibitors. Our studies led to the discovery of coumarin **18**, which is effective in a rabbit model of dry eye and a tear secretion test in rats.

Dry eye is a disease of the tears and ocular surface.[1] Numerous factors contribute to the onset of the disease, but once dry eye has developed, inflammation of various ocular surface tissues propagates the disease as both cause and consequence of ocular surface damage.[2] Individuals with dry eye suffer from ocular discomfort (dry, gritty feeling; itching; stinging/burning; pain/soreness) and blurred vision.[3] Improvement in these symptoms can be affected by administration of artificial tears, but the relief is transitory as the underlying inflammation persists.[4] Therefore, an agent capable of reducing inflammation and inducing tear secretion should be an effective therapy for dry eye.

The phosphodiesterase 4 (PDE4) enzymes regulate a host of biological processes by degrading the intracellular second messenger cAMP.[5] PDE4 inhibitors have been intensively investigated as anti-inflammatory therapies because increases in cAMP levels are known to attenuate inflammatory responses in multiple cell types.[6] Other agents that increase cAMP have been shown to induce tear secretion.[7] Therefore, PDE4 inhibitors should serve the dual role of reducing inflammation and inducing tear secretion providing an effective treatment for dry eye.

In this letter, we describe our efforts to create a novel, potent series of PDE4 inhibitors with aqueous solubility compatible with topical ocular delivery. Furthermore, we disclose efficacy in an in vivo model for dry eye and an in vivo test for tear secretion.

Piclamilast (**1**) is a potent, selective PDE4 inhibitor.[8] The cocrystal structure of piclamilast bound to PDE4B reveals a binding mode within the active site that results in an exposed region of piclamilast encompassing the amide carbonyl and C2 of the phenyl ring (Fig. 1).[9] We postulated that appending a flat, fused ring in this region would provide novel PDE4 inhibitors (**2**, Fig. 2). Furthermore, substituents at C2 of **2** would have minimal interaction with the protein which should allow for a variety of solubilizing groups to be incorporated without adversely impacting potency. Indeed, a related series of compounds, represented by **3**, has been reported,[10] but substitution at N2 is not possible with this flat, unsaturated phthalazine ring.

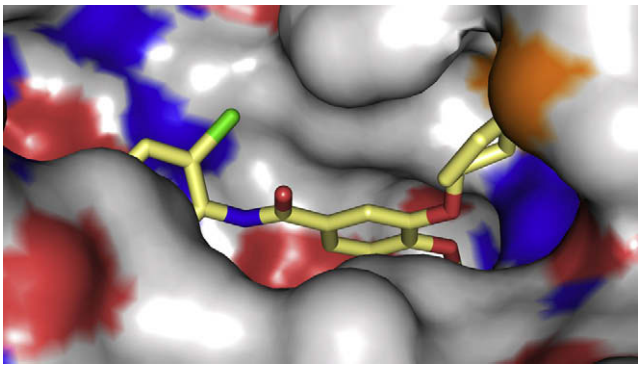

Figure 1. Structure of piclamilast (**1**) bound to PDE4B (pdb:1XM4).

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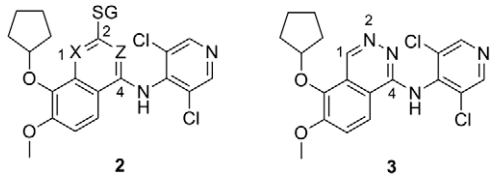
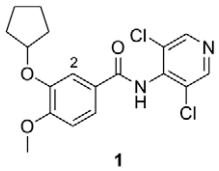


Figure 2. Generic structure **2** (X, Z = CH, N, O, or a bond; SG = solubilizing group) and phthalazine **3**.

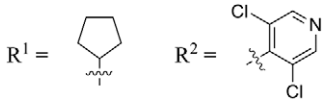


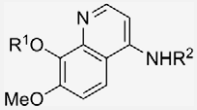
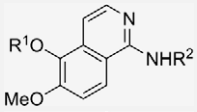
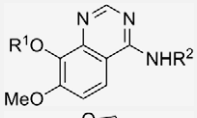
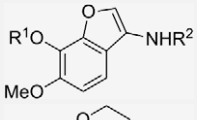
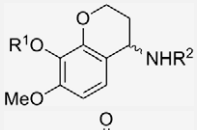
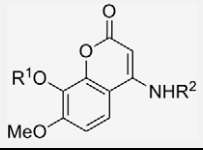
New scaffolds were prepared as detailed in Table 1. Compounds were assayed[11] for their intrinsic potency against human PDE4B enzyme,[12] as well as their ability to inhibit cAMP hydrolysis in a cell-based biosensor assay.[13] Our initial efforts focused on simple nitrogen-containing heterocycles where SG = H (**4**–**6**). Disappointingly, a dramatic loss in potency (2 nM → 0.5–3.3 μM) discouraged further exploration. Under the presumption that the polar, basic nitrogens were at the root of potency loss, compounds with oxygen-containing rings were prepared. For benzofuran **7**, we expected that the spatial positioning of the catechol motif and pyridine ring would not be ideal, but the possibility of C2 substitution prompted us to prepare the compound. As expected, **7** is a very weak PDE4 inhibitor. Initial attempts to prepare analogs with oxygen-containing six-membered rings proved challenging, but eventually, chroman **8** was accessed. The lack of activity is not unexpected when considering the deviation from planarity that exists within the dihydropyran ring. Coumarin **9** was prepared to restore planarity. With a biochemical potency of 5 nM and a cell-based potency of 28 nM, coumarin **9** represents a novel scaffold of PDE4 inhibitors that is comparable to piclamilast.

Although we were successful in appending a flat, fused ring, the carbonyl of coumarin **9** is positioned where we initially proposed to append solubilizing groups. In an effort to evaluate other opportunities for substitution, we cocrystallized coumarin **9** with PDE4B (Fig. 3).[14] In this complex, the PDE4B enzyme adopts the same conformation as that observed in the piclamilast complex.[15] Furthermore, **9** binds to the enzyme in an orientation that is nearly identical to that of piclamilast and more importantly, exposes one side of the cyclopentyl group. We postulated that if the cyclopentane was replaced with an alkyl chain of sufficient length to exit the hydrophobic pocket and further attached to a polar, solubilizing group, the resultant PDE4 inhibitors would be both potent and water soluble.

The results of exploring chain length with a carboxylic acid as the solubilizing group are shown in Table 2. Initially, acids **10**–**13** were prepared, and it became immediately clear that our strategy was feasible. As the length of the alkyl chain was extended from $n = 1$ to $n = 5$ (**10**–**13**), the compounds became increasingly more potent in both the biochemical (1.2 μM → 17 nM) and cell-based

Table 1
In vitro potency data for novel scaffolds



Compound	Structure	PDE4B IC_{50}, μM[a]	CNG IC_{50}, μM[a]
1	—	0.002	0.013
3	—	0.4	1.1
4		3.3	8.2
5		0.5	na
6		2.3	10
7		16	>30
8		>30	>30
9		0.005	0.028

[a] Value is mean of two or more experiments (na: not active at 70 μM).

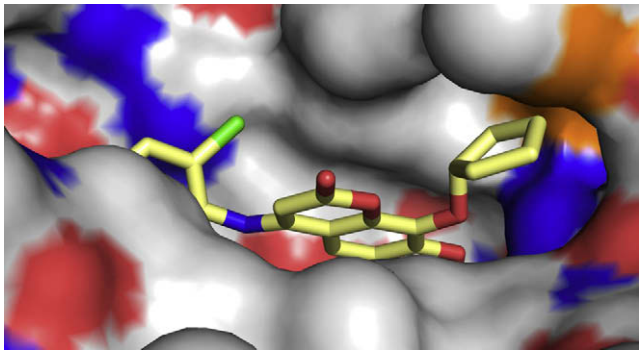
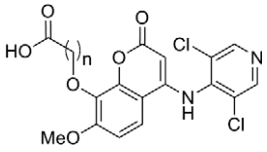


Figure 3. Structure of coumarin **9** bound to PDE4B (pdb: 3LY2).

Table 2
In vitro potency and solubility data for acid derivatives



Compound	*n*	PDE4B IC_{50}, μM[a]	CNG IC_{50}, μM[a]	Solubility mg/mL[b]
9	—	0.005	0.03	0.0003
10	1	1.180	5.35	
11	3	0.080	0.89	
12	4	0.043	0.73	0.5
13	5	0.017	0.28	0.9
14	6	0.002	0.12	1.4
15	7	0.002	0.04	0.3
16	8	0.001	0.03	0.3

[a] Value is mean of three or more experiments except **10** ($n = 2$).
[b] Shake-flask solubility (0.1 M phosphate buffer; pH = 7.4).

assays. Because the potency was still increasing at $n = 5$, the alkyl chain was extended further (**14**–**16**), and the potency continued to improve (17 → 1 nM). With respect to aqueous solubility, compounds **12**–**16** had moderate to good solubility, but no clear trend was observed. Overall, the alkanoic acid side chain with $n = 5$–6 (**13** and **14**) provides for potent PDE4 inhibitors that have good water solubility (0.9, 1.4 mg/mL).

We also employed a basic amine as a solubilizing group. The results of exploring chain length with a dimethylamino group are shown in Table 3. As seen with the acids, an improvement in potency was observed as the alkyl chain was lengthened. There was a dramatic potency enhancement (670 → 5 nM) from $n = 3$ to $n = 6$ (**17**–**20**), but further improvement was minimal (5 → 2 nM) from $n = 6$ to $n = 8$ (**20**–**22**). With respect to aqueous solubility, amine **18** ($n = 4$) had very good solubility (1.8 mg/mL), but there was a significant drop-off (0.06 mg/mL) for **19** ($n = 5$). Within this subseries, the dimethylaminobutyl side chain provides for a potent, soluble PDE4 inhibitor.

Having identified a novel series of water soluble PDE4 inhibitors, we sought to demonstrate in vivo efficacy. To explore the anti-inflammatory nature of our compounds, we utilized the rabbit model of lacrimal gland inflammation-induced dry eye, in which corneal staining was used to measure ocular surface health.[16] Of the many new PDE4 inhibitors screened in this model (data not shown), compound **18** looked to be the most promising, so a full dose response study was run (Fig. 4). Compound **18** was extremely effective at protecting the eye, equivalent to the corticosteroid dexamethasone from 10 ng/mL to 10 μg/mL. To explore the ability of our compounds to induce tear secretion, we utilized the phenol red thread test in rats (Fig. 5).[17] At doses of 10 μg/mL and 100 μg/mL, compound **18** was effective at inducing tear secretion. Thus, compound **18** demonstrates the potential effectiveness of PDE4 inhibitors for the treatment of dry eye.

In summary, we have disclosed the development of a novel series of water-soluble PDE4 inhibitors. Our studies led to the discovery of coumarin **18**, which is effective in a rabbit model of dry eye and a tear secretion test in rats. Coumarin **18** substantiates the idea that PDE4 inhibitors can serve the dual role of reducing inflammation and inducing tear secretion providing an effective treatment for alleviating the symptoms and underlying inflammation associated with dry eye.

Chemistry:[18] Compounds **4**–**6** were synthesized as illustrated in Scheme 1. Aniline **24** was prepared by nitration, alkylation, and then reduction of guiacol (**23**). Heating **24** with Meldrum's acid and trimethylorthoformate gave an intermediate enamine that cyclized to quinolinone **25** by thermolysis in diphenyl ether. Alkylation of **26** followed by condensation with malonic acid gave acrylic acid **27**. Formation of the acyl azide and thermolysis in diphenylmethane gave isoquinolinone **28**. Nitration of **29** followed by alkylation gave **30**. Reduction of the nitro group gave the aniline which cyclized to quinazolinone **31** upon heating in formamide. Intermediates **25**, **28**, and **31** were transformed into their respective chlorides by treatment with phosphoryl chloride. These chlorides were converted into the desired compounds **4**, **5**, and **6** by displacement with 4-amino-3,5-dichloropyridine.

Compounds **7**–**9** were synthesized as illustrated in Scheme 2. Bromination of ketone **32** gave an α-bromo ketone. Treatment with base followed by bromocyclopentane resulted in first, cyclization onto the adjacent hydroxyl and then, alkylation of the remaining hydroxyl. The resultant furanone was reduced with sodium borohydride to give **33**. Elimination to the benzofuran was spontaneous when the hydroxyl of **33** was activated. Bromination followed by coupling with 4-amino-3,5-dichloropyridine gave the desired benzofuran **7**. Towards **8**, **32** was transformed into a chromenone by treatment with triethylorthoformate in perchloric acid. Alkylation and reduction gave chromanone **34**. Treatment of **34** with hydrox-

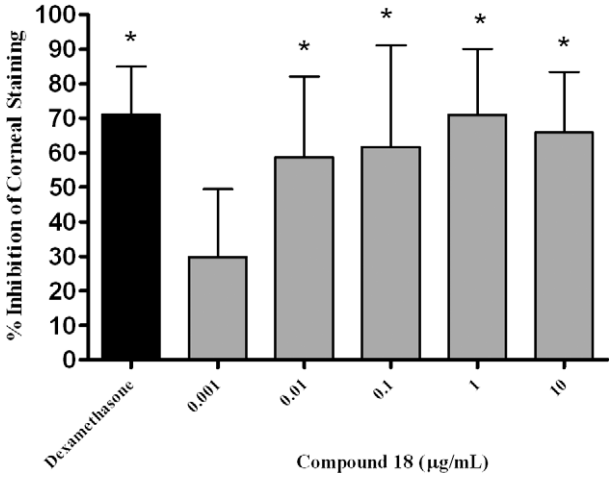


Figure 4. Inhibition of corneal staining in the rabbit model of lacrimal gland inflammation-induced dry eye. Inhibition of corneal staining corresponds with protection of the ocular surface. Percent inhibition is relative to vehicle (*p <0.05, ANOVA, Dunnett's post-test). Compounds were administered topically b.i.d. for 4 days. Positive control is the corticosteroid dexamethasone (100 μg/mL).

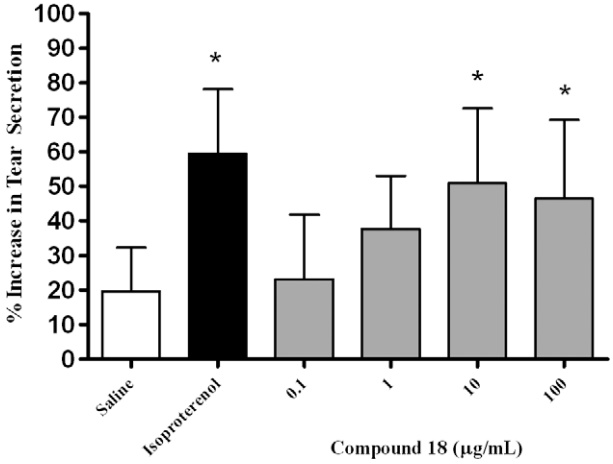
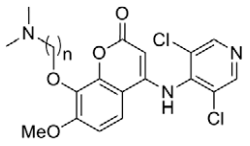


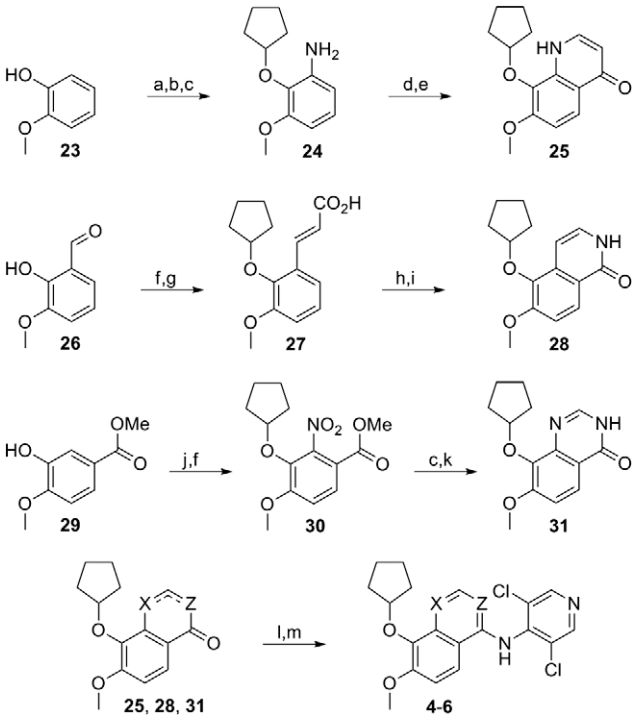
Figure 5. Increase in tear secretion in rats. Tear secretion was measured by the phenol red thread test 10 min after topical administration of compound. Percent increase is relative to untreated animals. Statistical significance is relative to saline (*p <0.05, ANOVA, Dunnett's post-test). Positive control is the beta-adrenergic agonist isoproterenol (10 μg/mL).

Table 3
In vitro potency and solubility data for Me_2N derivatives

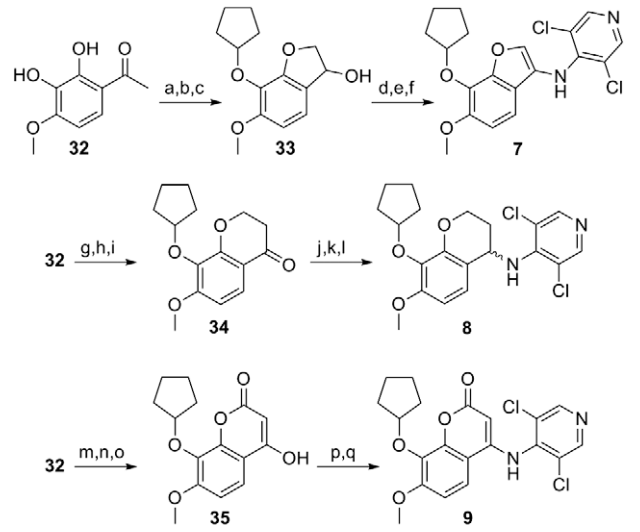


Compound	n	PDE4B IC_{50}, μM[a]	CNG IC_{50}, μM[a]	Solubility mg/mL[b]
17	3	0.670	>30	
18	4	0.045	1.82	1.8
19	5	0.021	0.66	0.06
20	6	0.005	0.18	
21	7	0.003	0.15	
22	8	0.002	0.15	

[a] Value is mean of three or more experiments.
[b] Shake-flask solubility (0.1 M phosphate buffer; pH = 7.4).

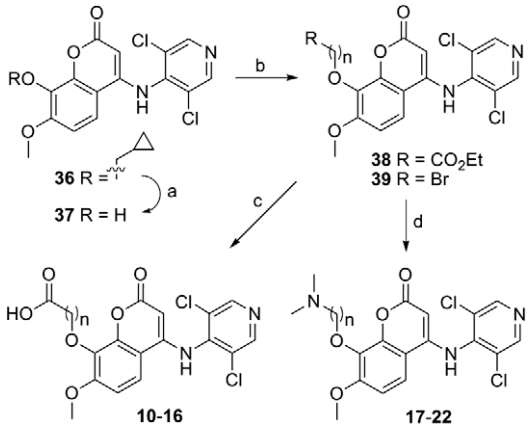


Scheme 1. Reagents and conditions: (a) $NaNO_3$, cat. $NaNO_2$, H_2SO_4, Et_2O, rt, 14 h; (b) bromocyclopentane, Cs_2CO_3, CH_3CN, reflux, 40 h; (c) 1 atm H_2, 10% Pd/C, MeOH, 21 h; (d) Meldrum's acid, $HC(OMe)_3$, reflux 5 h; (e) Ph_2O, 250 °C, 15 min; (f) bromocyclopentane, K_2CO_3, CH_3CN, reflux, 17 h; (g) malonic acid, piperidine, pyridine, 100 °C, 2.5 h; (h) $Ph_2P(O)N_3$, Et_3N, PhMe, rt, 3 h; (i) Ph_2CH_2, 200–225 °C, 5 h; (j) *i*PrONO$_2$, *n*Bu$_4$NHSO$_4$, H_2SO_4, CH_2Cl_2, 0 °C to rt, 45 min; (k) formamide, 190 °C, 6 h; (l) $POCl_3$, cat. DMF, 1,2-dichloroethane, reflux, 15 h; (m) 4-amino-3,5-dichloropyridine, NaH, DMF, 120 °C, 15 h from **25** and **28**, or rt, 30 min from **31**.



Scheme 2. Reagents and conditions: (a) $CuBr_2$, EtOAc, $CHCl_3$, reflux, 16 h; (b) K_2CO_3, CH_3CN, rt, 2 h, then bromocyclopentane, reflux, 16 h; (c) $NaBH_4$, THF, MeOH, rt, 1 h; (d) CBr_4, PPh_3, CH_2Cl_2, 0 °C to rt, 30 min; (e) Br_2, CS_2, 0 °C, 1 h, then NaOEt, EtOH, rt, 16 h; (f) 4-amino-3,5-dichloropyridine, $Pd_2(dba)_3$, X-phos, NaO*t*Bu, PhMe, 110 °C, 2 h; (g) $HClO_4$, $HC(OEt)_3$, rt, 30 min, filter, then H_2O, 90 °C, 5 min; (h) bromocyclopentane, K_2CO_3, DMF, 100 °C, 3 h; (i) 1 atm H_2, 10% Pd/C, PhMe, 7 d; (j) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH, 80 °C, 6 h; (k) 1 atm H_2, 10% Pd/C, THF, MeOH, 3.5 d; (l) *n*BuLi, THF, −78 to 0 °C, then 3,4,5-trichloropyridine, reflux, 2 h; (m) bromocyclopentane, K_2CO_3, DMF, 110 °C, 17 h; (n) NaH, Et_2CO_3, reflux, 1 h; (o) KO*t*Bu, *t*BuOH, reflux, 4 d; (p) NH_4OAc, *m*-xylene, reflux, 3 h; (q) NaH, 3,4,5-trichloropyridine, DMSO, rt, 4 h.

ylamine followed by hydrogenation gave an aminochroman which was reacted with 3,4,5-trichloropyridine to give the desired chroman **8**. Towards **9**, **32** was alkylated with bromocyclopentane, acylated with diethylcarbonate, and then cyclized to hydroxycoumarin **35**. Heating **35** with ammonium acetate in *m*-xylene provided an aminocoumarin which was reacted with 3,4,5-trichloropyridine to give the desired coumarin **9**.



Scheme 3. Reagents and conditions: (a) concd HCl, rt, 1 h; (b) Br(CH$_2$)$_n$CO$_2$Et or Br(CH$_2$)$_n$Br, NaH, DMSO, rt, 3–9 h; (c) LiOH, THF, MeOH, rt, 2–4 h; (d) 2 M Me_2NH in THF, DMSO, rt, 1–5 h.

Compounds **10**–**22** were synthesized as illustrated in Scheme 3. Dealkylation of **36** (prepared in analogous fashion as **9**) with concd HCl gave phenol **37**. Subsequent alkylation with an ethyl bromoalkanoate or with a 1,*n*-dibromoalkane gave intermediates **38** and **39**, respectively. Saponification of esters **38** gave the desired acids **10**–**16**. Displacement of bromides **39** with dimethylamine gave amines **17**–**22**.

References and notes

1. International Dry Eye WorkShop (2007). Report of the Definition and Classification Subcommittee. *Ocul. Surf.* **2007**, *5*, 75.
2. (a) Baudouin, C. *Surv. Ophthalmol.* **2001**, *45*, S211; (b) Stern, M. E.; Pflugfelder, S. C. *Ocul. Surf.* **2004**, *2*, 124.
3. Perry, H. D. *Am. J. Manag. Care* **2008**, *14*, S79.
4. International Dry Eye WorkShop (2007). Report of the Management and Therapy Subcommittee. *Ocul. Surf.* **2007**, *5*, 163.
5. (a) Conti, M.; Richter, W.; Mehats, C.; Livera, G.; Park, J.-Y.; Jin, C. *J. Biol. Chem.* **2003**, *278*, 5493; (b) Houslay, M. D.; Adams, D. R. *Biochem. J.* **2003**, *370*, 1.
6. (a) Spina, D. *Br. J. Pharmacol.* **2008**, *155*, 308; (b) Kodimuthali, A.; Jabaris, S. S. L.; Patel, M. *J. Med. Chem.* **2008**, *51*, 5471.
7. Gilbard, J. P.; Rossi, S. R.; Heyda, K. G.; Dartt, D. A. *Invest. Ophthalmol. Vis. Sci.* **1990**, *31*, 1381.
8. Ashton, M. J.; Cook, D. C.; Fenton, G.; Karlsson, J.-A.; Palfreyman, M. N.; Raeburn, D.; Ratcliffe, A. J.; Souness, J. E.; Thurairatnam, S.; Vicker, N. *J. Med. Chem.* **1994**, *37*, 1696.
9. Card, G. L.; England, B. P.; Suzuki, Y.; Fong, D.; Powell, B.; Lee, B.; Luu, C.; Tabrizizad, M.; Gillette, S.; Ibrahim, P. N.; Artis, D. R.; Bollag, G.; Milburn, M. V.; Kim, S.-H.; Schlessinger, J.; Zhang, K. Y. J. *Structure* **2004**, *12*, 2233.
10. Napoletano, M.; Norcini, G.; Pellacini, F.; Marchini, F.; Morazzoni, G.; Ferlenga, P.; Pradella, L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2235.
11. All compounds were tested to a minimum of $n = 2$ (Table 1) or $n = 3$ (Tables 2 and 3; except **10** ($n = 2$)). The difference in IC_{50} was generally <twofold with respect to the values quoted in the tables.
12. Bioluminescent biochemical assay: Govek, S. P.; Shiau, A. K.; Noble, S. A.; Thomas, D. J. WO 2008/006051 A2, Jan 10, 2008.
13. HEK-293 cells stably expressing a mutant cyclic nucleotide-gated (CNG) channel (BD ACTOne, BDBiosciences) were plated at 1×10^6 cells/mL (25 μL) in black 384-well, clear-bottom plates. Cells were incubated at 37 °C for 16 h. Cells were then loaded by adding 25 μL of FLIPR membrane potential dye (R8034, Molecular Devices) per manufacturer's instructions for 4 h at room temperature (rt). Compounds, previously arrayed in 11 or 21 point 1/2 log dilutions, were then added as 10 μL solutions containing 1% DMSO in Dulbecco's phosphate-buffered saline. After a 15 min incubation at rt, 10 μL of NECA (5′-(*N*-ethylcarboxamido)adenosine, Sigma) in phosphate-buffered saline was added to stimulate production of cAMP. The concentration of NECA was titrated to yield an EC_{10} for maximal cAMP production (generally about 0.2 μM). After an additional 45 min at rt, fluorescence intensity was read on a Molecular Devices Acquest plate reader. PDE4 inhibition results in higher fluorescence signal due to decreased cAMP hydrolysis. Raw fluorescence data

were normalized to negative (DMSO) and positive (Roflumilast, a known PDE4 inhibitor, IC_{50} = 1 nM) controls. Data analysis was performed using Spotfire (Spotfire, Inc.) and Kalypsys proprietary software.

14. An N-terminally His_6-tagged catalytically active fragment of human PDE4B was constructed by inserting the sequence for residues 152-487 into pET-28a. The protein was expressed in *Escherichia coli* (BL21(DE3)) and purified using metal affinity (Ni-NTA resin) and anion exchange (HiTrap Q column) chromatography. Protein (14 mg/mL) was mixed with 1 mM compound in DMSO and incubated on ice for 1 h. Equal volumes of the protein stock and well buffer (1.8–2.0 M ammonium sulfate, 1–2% PEG 400, 100 mM sodium cacodylate pH 6.0) were mixed and then suspended over 1 mL well buffer reservoirs at 4 °C. Crystals were soaked in a cryoprotecting solution (well buffer with the PEG concentration increased to 30%) prior to freezing in liquid nitrogen. Data were collected to 2.60 Å (R_{sym} = 12.1%) at the Advanced Light Source beam line 5.0.3 (Lawrence Berkeley National Laboratory). The crystals lie in the space group $C222_1$ with cell constants a = 214.8 Å, b = 234.0 Å, c = 165.3 Å. The structure was determined by molecular replacement as implemented in PHASER[a] using the protein component of the PDE4B roflumilast complex (PDB ID:1XMU) as the search model. The model was rebuilt using Moloc[b] and refined using non-crystallographic symmetry constraints and a maximum-likelihood target (CNX)[c] to an R/R_{free} = 20.5/23.3. The coordinates and structure factors have been deposited in the Protein Data Bank (accession code 3LY2). (a) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. *J. Appl. Crystallogr.* **2007**, *40*, 658. (b) Muller, K.; Amman, H. J.; Doran, D. M.; Gerber, P. R.; Gubernator, K.; Schrepfer, G. *Bull. Soc. Chim. Belg.* **1998**, *97*, 655. (c) Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren G. L. *Acta Crystallogr., Sect. D* **1998**, *54*, 905.
15. The conformational similarity of PDE4B bound to **9** and piclamilast was assessed by calculating the root mean square deviation (rmsd) from a least squares-based superimposition of the relevant coordinates of the alpha carbon atoms of each amino acid. The rmsd was 0.57 Å.
16. Nagelhout, T. J.; Gamache, D. A.; Roberts, L.; Brady, M. T.; Yanni, J. M. *J. Ocul. Pharmacol. Ther.* **2005**, *21*, 139.
17. The PRT test was performed on one eye of ophthalmologically normal, male Lewis rats (4–6 weeks old, 10 per group). For a description of PRT methodology in normal guinea pigs see: Trost, K.; Skalicky, M.; Nell, B. *Vet. Ophthalmol.* **2007**, *10*, 143.
18. All final compounds displayed spectral data (NMR, MS) that were consistent with the assigned structure. Experimental details can be found in Ref. 12.