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Quinolines as a novel structural class of potent and selective PDE4 inhibitors. Optimisation for inhaled administration

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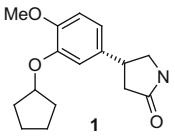
ABSTRACT

Crystallography driven optimisation of a lead derived from similarity searching of the GSK compound collection resulted in the discovery of quinoline-3-carboxamides as highly potent and selective inhibitors of phosphodiesterase 4B. This series has been optimized to GSK256066, a potent PDE4B inhibitor which also inhibits LPS induced production of TNF-α from isolated human peripheral blood mononuclear cells with a pIC_{50} of 11.1. GSK256066 also has a suitable profile for inhaled dosing.

Inhibition of phosphodiesterase 4 (PDE4) has been shown clinically to have an anti-inflammatory effect,[1] and is well established as a target with potential to treat conditions such as asthma and COPD.[2] First generation PDE4 inhibitors (e.g., *R-Rolipram*, **1**) cause emesis at effective anti-inflammatory doses.[3] Second generation inhibitors such as Roflumilast **2** show efficacy against COPD, but this is limited by nausea and emesis at high doses.[4]

Our goal was to design a third generation of PDE4 inhibitors with an increased therapeutic index, enabling a robust anti-inflammatory action without being dose limited by emetic side effects.



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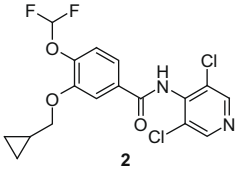

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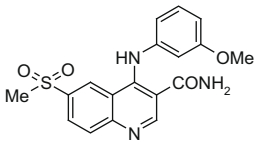
One hypothesis that we wished to test was that delivery of the PDE4 inhibitor directly to the site of action in the lung might improve the therapeutic index, in a similar manner to inhaled glucocorticoids.[5] A lower dose would be required than in an oral drug, and the compound need not be systemically bioavailable. This would result in a dramatically lower systemic exposure, and hence a reduced level of emesis.

We have recently described the discovery of 3-aminocarboxy-4-anilino quinolines (e.g., **3**).[6] In this Letter we describe the optimization of **3** into GSK256066 (**4**), a highly potent PDE4 inhibitor suitable for inhaled administration. In parallel with this approach the series was also optimised for oral administration.[7]



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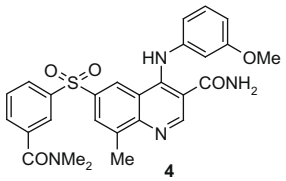

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Quinoline **3** is a relatively potent and selective PDE4 inhibitor (PDE4B pIC_{50} 8.4, for other data see Table 5). Our strategy was to seek to increase the PDE4 potency, whilst keeping the aqueous solubility and oral bioavailability low. One method of doing this was to target a relatively high molecular weight lipophilic compound. Since PDE4 is an intracellular target we aimed to keep permeability high, and to judge this by cellular activity.

A crystal structure of our lead **3** bound to PDE4B was determined to 1.7 Å resolution (Fig. 1).[7] This showed the core of our molecule to be a good fit with the binding site, but revealed the presence of a large solvent filled pocket (A) that could be accessed from the 6-position of the quinoline, as well as a small pocket (B) at the 8-position of the molecule that could be exploited to increase binding interactions. We focused on these areas to increase the PDE4 activity of this series.

We initially looked to modify the 6-position of the quinoline ring. The R^6 substituent was introduced at the start of the synthesis with the appropriate aniline **5**, which was condensed with diethyl ethoxymethylenemalonate, and then cyclised at 250 °C in diphenyl ether to give ester **6** (Scheme 1). This was hydrolysed with sodium hydroxide and the resulting acid dichlorinated with thionyl chloride, at both the acid moiety and the 4-position, then quenched with aqueous ammonia to afford the primary carboxamide **7**. The functionality at R^4 was introduced by refluxing **7** in acetonitrile with 3-methoxyaniline to give the desired compounds **8a–d**.[9] As can be seen in Table 1, increased steric bulk at this position was tolerated, in particular aryl sulfones such as **8a**. The observed tolerance of the phenyl sulfone at the quinoline 6-position of **8a** was rationalized by the phenyl group pointing towards the large pocket (A) seen on the crystal structure (Fig. 1). Removal of the sulfone here was detrimental (**8c**).

The linear nature of the initial route limited the diversity that could be introduced at the 6-position. Modification allowed sulfone variation to be introduced at a late stage via the iodoquinoline **8d** (Scheme 1). Thus **8d** (prepared from **5d**) was reacted with aryl thiols under palladium catalysed coupling conditions[10] to give the sulfide **9**.

In addition, chemistry was devised to allow the sulfone linker to be varied to amide or sulfonamide (Scheme 2). Carbonylation of iodoquinoline **8d** gave the corresponding ethyl ester. Basic hydrolysis then coupling with an amine under standard conditions gave amides **10–11**. Sulfonamide linked compounds were obtained starting from iodoquinoline **7d**, which was coupled under Stille conditions with $^tBuSSnBu_3$ to give the *tert*-butyl sulfide, and oxi-

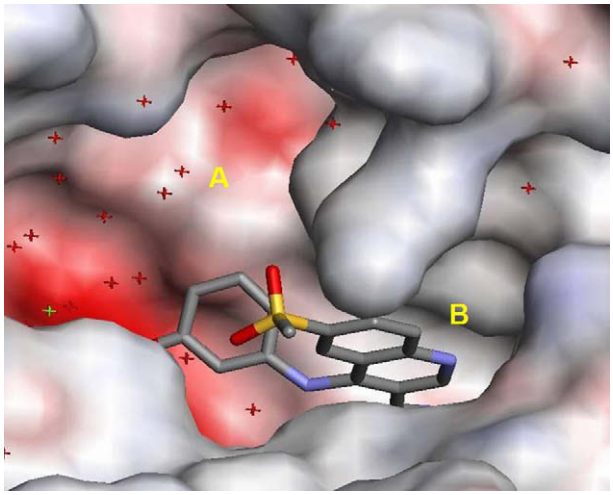
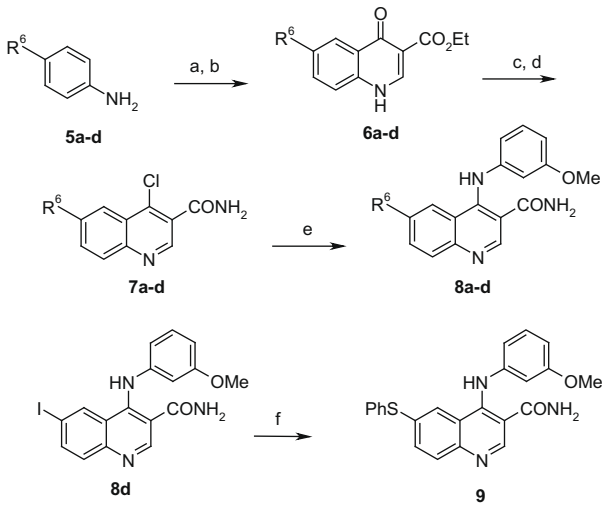
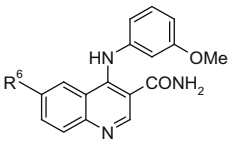


Figure 1. Crystal structure of PDE4B + **3**, indicating 2 pockets, A + B, for additional binding interactions.

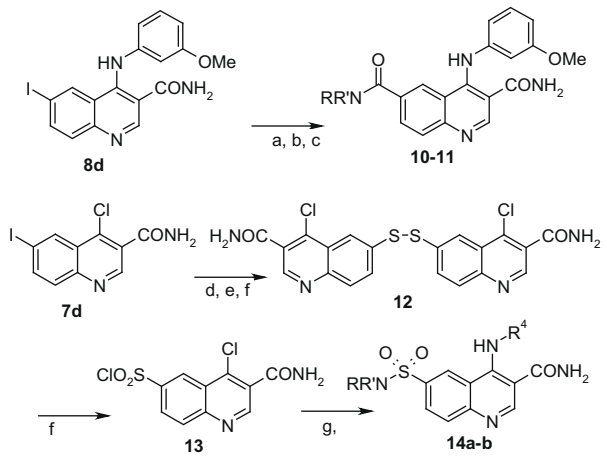


Scheme 1. Reagents and conditions: (a) $EtOCH{=}C(CO_2Et)_2$, 80 °C; (b) Ph_2O, 250 °C; (c) NaOH, EtOH, relux; (d) (i) $SOCl_2$, DMF, 80 °C; (ii) NH_4OH, rt; (e) R^4NH_2, MeCN, 80 °C; (f) PhSH, $Pd_2(dba)_3$, DPEphos, KO*t*Bu, toluene, 100–120 °C or PhSH, CuI, K_3PO_4, DMPU, *N*,*N*-diethylsalicylamide, 100 °C.

Table 1
Initial SAR of 6-position



Compds	R^6–	PDE4B pIC_{50}[12]
R-Rolipram, **1**	—	7.4 (9)
Roflumilast, **2**	—	9.4 (308)
3	$MeSO_2$–	8.4 (7)
8a	$PhSO_2$–	8.8 (2)
8b	$^cPentylSO_2$–	8.5 (3)
8c	H–	6.7 (3)
9	PhS–	6.8 (2)
10	$Me_2NC(O)$–	7.5 (3)
11	MorpholineC(O)–	7.6 (3)
14a	Me_2NSO_2–	9.1 (2)
14b	Morpholine-SO_2–	8.7 (2)



Scheme 2. Reagents and conditions: (a) CO, $Pd(PPh_3)_2Cl_2$, Et_3N, EtOH, 80 °C; (b) NaOH, EtOH, rt; (c) TBTU, RR′NH, DMF, rt; (d) $^tBuSSnBu_3$, $Pd(PPh_3)_4$, toluene, reflux; (e) $MeSiCl_3$, Ph_2SO, TFA, 0–5 °C; (f) Cl_2, AcOH, H_2O, rt; (g) RR′NH, DIPEA, CH_2Cl_2, rt; (h) 3-methoxyaniline, MeCN, 80 °C.

dized with methyltrichlorosilane to the symmetrical disulfide **12**. This was cleaved with chlorine gas to the sulfonyl chloride **13**, then treated with an amine under standard conditions to form a sulfonamide and finally refluxed with 3-methoxyaniline to afford **14a–b**.

To thoroughly explore the SAR, the substituent was varied from a sulfone to sulfide (**9**) and amide (**10–11**), but none showed improvements to PDE4B activity (Table 1). Our hypothesis for lack of affinity of sulfide **9** is that the sulfone (**8a**) is pre-oriented in the bio-active conformation[11a] whereas the sulfide loses entropy in attaining this conformation. In addition, both aromatic rings connected to the sulfur in **8a** and **9** are involved in face-to-face π-stacking interactions which greatly benefit[11b] from electron-poor aromatic systems. Sulfonamides (**14a–b**) maintained a similar level of PDE4B inhibition to sulfone linked compounds.

With stocks of phenyl sulfone intermediate 7**a** ($R^6 = PhSO_2$) in hand a second round of optimization was carried out at the quinoline 4-position, using the same chemistry described in Scheme 1, but varying the amine R^4NH_2 (addition of diisopropylethylamine as base was required with an aliphatic amine in the final step) to give **15a–g**. Whilst several groups were of similar potency versus PDE4B (Table 2), none proved superior to the initial 3-methoxyphenyl group (**8a**). Substitution at this position with an aliphatic group gave a drop in activity (**15d**). The SAR can be rationalized from the crystal structure in Figure 1 as the 3-methoxyphenyl is a very good fit to the protein surface and we were unable to improve activity at this position.

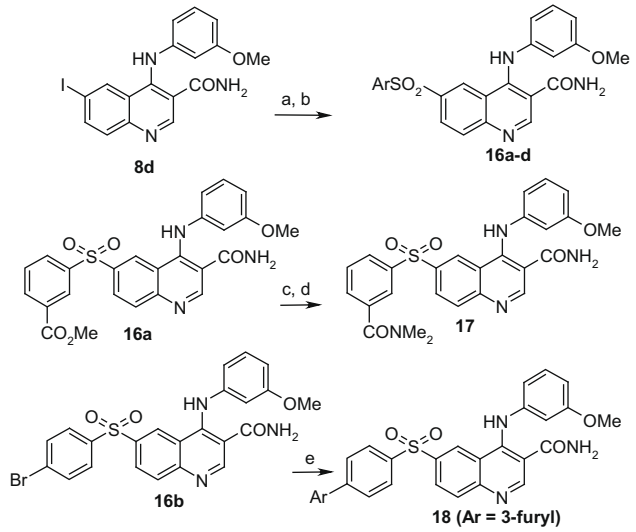
We rationalized earlier that the phenylsulfone group pointed towards the large pocket (A) seen on the crystal structure (Fig. 1), and we used this to assist in our design of further molecules. It appeared that there was space here for either *meta* or *para* substitution. This functionality was introduced from iodide **8d** via the corresponding sulfide (prepared under either palladium[10] or copper[13] catalysed coupling conditions). The sulfide could be cleanly oxidised with Oxone™ to the appropriate sulfone **16a–d**. Amide substituents were introduced by hydrolysis of the ester functionality under basic conditions and then standard amide coupling with the appropriate amine to give **17** (Scheme 3).

As is shown in Table 3, *para* substituents gave up to a 10-fold increase in PDE4B activity, but *meta* substituted amide **17** was preferable, possibly due to improved Van der Waals contact of the dimethylcarboxamide with Ser-282, and a desolvation effect by displacing trapped water molecules. A secondary assay measuring inhibition of LPS induced TNF-α production from isolated human peripheral blood mononuclear cells[14] (PBMC) was used to determine the activity of these compounds in a relevant cellular system. It was most encouraging that the majority of these compounds maintained good activity in this assay, with no more than 1 log dropoff from the PDE4B enzyme data except for **16d** (Table 3). This shows the good cellular penetration of this series of compounds which may be due to their relatively high lipophilicity (e.g., **16c** clog $P = 4.8$). Further elaboration to explore the SAR even more widely at this position was exemplified by Suzuki coupling at the *para* position of the 6-phenylsulfone (seeking to maximize the space-filling of the large pocket A shown in Fig. 1) to give compounds such as **18** (Scheme 3) but these showed no potency increase over **8a**.

Having made these changes, we looked to increase the potency of these compounds by making further modifications. We decided to exploit the small pocket **B** that we had seen in the crystal structure (Fig. 1), and found that we could introduce a small methyl group at the 8-position of the ring, starting from 2-methyl-4-(phenylsulfonyl)aniline to give **19** by the same route shown in Scheme 1. As can be seen in Table 4, this led to a dramatic leap in potency.

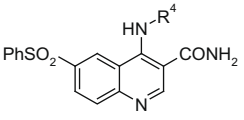
Finally, the *para*-OMe and *meta*-$CONMe_2$ substituents on the 6-aryl ring that were preferred in the 8-H series were combined with the 8-methyl substituent, to give **20** and **4** by the routes described earlier starting from 4-iodo-2-methylaniline. The PDE4B potencies of the 6- and 8- substitution were found to be additive and activity was maintained in the cellular assay. This afforded GSK256066 (Compound **4**), which is among the most potent PDE4 inhibitors described in the literature.

GSK256066 (**4**) is an exceedingly potent PDE4 inhibitor, with picomolar activity in both enzyme and cellular assays and activity



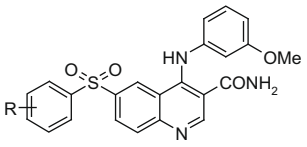
Scheme 3. Reagents and conditions: (a) ArSH, $Pd_2(dba)_3$, DPEphos, KO*t*Bu, toluene, 100–120 °C or ArSH, CuI, K_3PO_4, DMPU, *N*,*N*-diethylsalicylamide, 100 °C; (b) oxone, DMF, rt; (c) NaOH, MeOH, 80 °C; (d) Me_2NH, HATU, DIPEA, DMF, rt; (e) 3-furylboronic acid, $Pd(Ph_3)_4$, Na_2CO_3, DME, 100 °C.

Table 2
SAR of 4-position



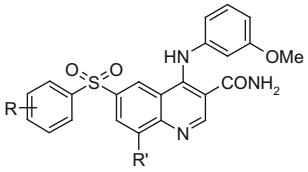
Compds	R^4	PDE4B pIC_{50}[12]
8a	3-OMe phenyl	8.8 (2)
15a	3-Et phenyl	8.0 (2)
15b	3-CN phenyl	8.5 (3)
15c	Phenyl	8.2 (2)
15d	Cyclohexyl	7.4 (2)
15e	3-Pyridyl	7.4 (4)
15f	6-Benzothiazolyl	8.6 (2)
15g	4-(3-Me) tetrahydrobenzofuryl	7.9 (2)

Table 3
Further SAR of 6-position



Compds	Phenyl substituent	PDE4B pIC_{50}[12]	PBMC pIC_{50}[14]
8a	H	8.8 (2)	8.2 (8)
16c	4-OMe	9.9 (3)	8.9 (6)
16d	4-^{t}Bu	9.2 (2)	7.8 (4)
17	3-$CONMe_2$	11.0 (2)	10.0 (8)
18	4-(3-Furyl)	8.8 (2)	7.8 (4)

Table 4
SAR of 8-Me compounds



Compds	R	R′	PDE4B pIC_{50}[12]	PBMC pIC_{50}[14]
8a	H	H	8.8 (2)	8.2 (8)
19	H	Me	10.0 (2)	9.3 (6)
20	4-OMe	Me	10.7 (2)	10.5 (6)
4	3-$CONMe_2$	Me	11.1 (6)	11.0 (18)

at 0.1nM concentration in human whole blood.[14] It is >1000-fold selective versus PDEs 1–3 and 5–7.[12] The properties of GSK256066 (Table 5) are highly appropriate for administration via the inhaled route, with negligible oral bioavailability in rat.

The crystal structure[8] of GSK256066 bound to PDE4B was obtained to 1.75 Å resolution (Fig. 2). This shows an excellent fit with the surface of the enzyme, occupying both pockets that were identified in the crystal structure of compound **3** bound to PDE4B. Whilst there are no direct hydrogen bonding interactions from the quinoline 6- and 8-substituents, there are significant Van Der Waals contacts, particularly by Ser-282 with the *meta*-$CONMe_2$ group and by Met-431 with the 8-methyl group.

Table 5
In vitro, physicochemical and pharmacokinetic properties of **4** compared with initial lead molecule **3**

Property		Value	
		4	**3**
PDE4B pIC_{50}[12]		11.1	8.4
Selectivity versus PDE3,5,6[12]		>1000-fold	>1000-fold
PBMC TNF- α pIC_{50}[14]		11.0	—
Human whole blood TNF-α pIC_{50}[14]		9.9	7.4
Aqueous solubility at pH 6.4 (mg/mL)		<0.001	0.005
Rat pharmacokinetics:	Cl (mL/min/kg)	39	10
1 mg/kg	Vd (L/kg)	0.8	0.3
	po F%	0%	10
	iv $t_{1/2}$ (h)	1.1	1.1

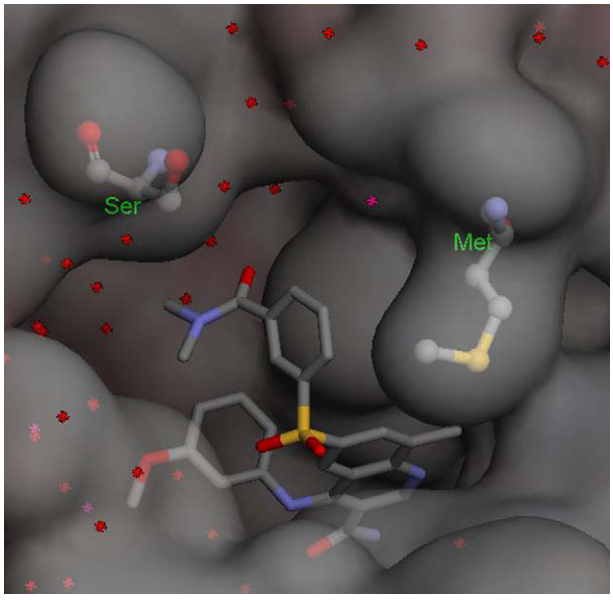


Figure 2. Crystal structure of PDE4B + **4**.

Table 6
Lung and plasma levels of GSK256066 after intratracheal administration in rats as an aqueous suspension at a dose of 30 μg/kg

Time (h)	Lung concentration (ng/g)	Plasma concentration (ng/g)	Lung:plasma ratio
0.08	2831	1.5	1887
6	2649	0.6	4415

GSK256066 was optimized for inhaled administration in order to increase therapeutic index, so a key pharmacokinetic measure of success was having high lung and low plasma levels after inhaled administration. Even 6 h after dosing to rats as an intratracheal aqueous suspension there was a significant concentration of GSK256066 in the lung, and exceedingly low plasma levels (Table 6). Whilst this experiment does not describe the precise location of the material in the lung it is clear that there are substantial quantities present at the 6 h timepoint indicating the potential for a long duration of action.

In conclusion a novel and exceedingly potent series of PDE4 inhibitors has been identified. This has been optimized to afford GSK256066, a compound with picomolar activity in vitro and properties suitable for inhaled dosing. More detailed investigations of the in vitro and in vivo properties of GSK256066, including its duration of action and therapeutic index, have been carried out[15] and a clinical evaluation of this compound in asthma and COPD is underway.

Acknowledgements

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References and notes

1. (a) Houslay, M. D.; Schafer, P.; Zhang, K. Y. J. *Drug Discovery Today* **2005**, *10*, 1503; (b) Soto, F. J.; Hanania, N. A. *Curr. Opin. Pulm. Med.* **2005**, *11*, 129.
2. For recent reviews see: McKenna, J. M.; Muller, G. W. *Cyclic Nucleotide Phosphodiesterases Health Disease* **2007**, 667; Dyke, H. J. *Exp. Opin. Ther. Patents* **2007**, *17*, 1183; Smith, V. B.; Spina, D. *Curr. Opin. Invest. Drugs* **2005**, *6*, 1136.
3. Horowski, R.; Sastre-Y-Hernandez, M. *Curr. Ther. Res.* **1985**, *38*, 23.
4. Spina, D. *Drugs* **2003**, *63*, 2575.
5. (a) Hogger, P. *Curr. Opin. Pulm. Med.* **2003**, *9*, 1; (b) Mollmann, H.; Wagner, M.; Meibohm, B.; Hochhaus, G.; Barth, J.; Stockmann, R.; Krieg, M.; Weisser, H.; Falcoz, C.; Derendorf, H. *Eur. J. Clin. Pharmacol.* **1998**, *53*, 459.
6. Kranz, M.; Lindvall, M. K.; Hamblin, J. N.; Jones, P. S.; Neu, M. Y.; Somers, D. O. in preparation.
7. Lunniss, C. J.; Cooper, A. W. J.; Eldred, C. D.; Kranz, M.; Lindvall, M.; Lucas, F. S.; Neu, A.; Preston, A. G. S.; Ranshaw, L. E.; Redgrave, A. J.; Robinson, J. E.; Shipley, T. J.; Solanke, Y. E.; Somers, D. O.; Wiseman, J. O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1380.
8. Methods as described in Ref. 15 of Hamblin, J. N.; Angell, T. D. R.; Ballantine, S. P.; Cook, C. M.; Cooper, A. W. J.; Dawson, J.; Delves, C. J.; Jones, P. S.; Lindvall, M.; Lucas, F. S.; Mitchell, C. J.; Neu, M. Y.; Ranshaw, L. E.; Solanke, Y. E.; Somers, D. O.; Wiseman J. O. Bioorg. Med. Chem. Let., 2008, 14, 4237, except compound **4** was soaked at 1 mM for ~1 day. Compound **4** was refined with partial occupancy due to incomplete substitution of an Arsenate species that normally occupies the inhibitor binding site and is derived from the crystallisation conditions (Xu, R. X.; Hassell, A. M.; Vanderwall, D.; Science 2000, 288, 1822). The final R-factor achieved for complex **4** following structure refinement was 20.5% (Rfree = 25.0%) and the coordinates deposited in the PDB as entry 3GWT. Note that red crosses in Figures 1 and 2 show the position of water molecules.
9. All compounds were characterized by LC/MS and ^{1}H NMR analysis.
10. Schopfer, U.; Schlapbach, A. *Tetrahedron* **2001**, *57*, 3069.
11. (a) Analysis of CCDC X-ray structures for bis-aryl sulfones show a strong preference for a 90° torsional angle whereas the sulfides show no torsional preference.; (b) Mignon, P.; Loverix, S.; Geerlings, P. *Chem. Phys. Lett.* **2005**, *401*, 40–46.
12. The ability of compounds to inhibit catalytic activity at PDE4B (human recombinant), PDE3 (from bovine aorta) and PDE5 (human recombinant) was

determined by Scintillation Proximity Assay (SPA) in 96-well format as described in WO2004024728. Values are means of at least two experiments (*n* shown in parentheses).
13. Kwong, F. Y.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 793. Copper catalysed conditions were preferred for products with ester functionality, palladium was used in all other cases.
14. Inhibition of LPS induced TNF-α production from isolated human peripheral blood mononuclear cells (PBMC) or human whole blood using IGEN technology was carried out as described in patent WO2007036733. Values are means of at least four experiments (*n* shown in parentheses).
15. (a) Nials, A.T. in preparation.; (b) Tralau-Stewart, C.J. in preparation.