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2-Cycloalkyl phenoxyacetic acid CRTh2 receptor antagonists

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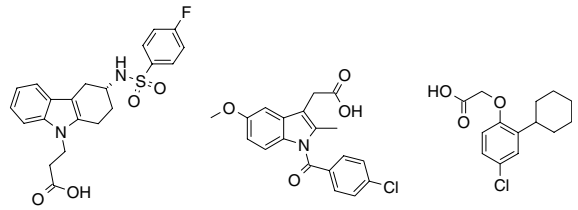
Abstract—High throughput screening identified a phenoxyacetic acid scaffold as a novel CRTh2 receptor antagonist chemotype, which could be optimised to furnish a compound with functional potency for inhibition of human eosinophil shape change and oral bioavailability in the rat.

Prostaglandin D_2 (PGD_2) is a product of the arachidonic acid cascade and is synthesized primarily by mast cells, as well as by macrophages and Th2 lymphocytes. PGD_2 has long been associated with the allergic inflammatory response, being found at high concentrations in the lungs of asthmatic patients.[1] PGD_2 was initially shown to act via a G-protein coupled receptor known as DP which shows significant homology with other prostanoid receptors. More recently, a second receptor for PGD_2, known as CRTh2 or DP_2, has been identified.[2] CRTh2 shows minimal homology with DP and is expressed on inflammatory cells, in particular showing selective expression on Th2 over Th1 cells. Using specific agonists, it has been demonstrated that the PGD_2-mediated activation and migration of eosinophils, basophils and Th2 cells in vitro proceeds via CRTh2 rather than DP receptor activation. Taken together with in vivo data on pulmonary eosinophil influx in rodents mediated by CRTh2 specific agonists, a CRTh2 antagonist may be an attractive therapeutic target for allergic diseases such as asthma.[2] This premise has been recently supported by the observation that a CRTh2 antagonist can inhibit pulmonary tissue eosinophilia in a mouse model of asthma.[3]

Ramatroban **1** is a TP receptor antagonist used for treatment of allergic rhinitis which was shown to also exhibit CRTh2 antagonist activity.[4] Recently, close analogues of **1** have been reported which are selective antagonists for



1 **2** **3**

CRTh2 ligands

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CRTh2 over TP.[5] The COX-1/COX-2 inhibitor indomethacin **2** has also been shown to function as a CRTh2 agonist and the indole substituents have been modified to deliver CRTh2 selective antagonists devoid of COX activity.[6] In this paper, we describe the discovery and exploration of an alternative phenoxyacetic acid chemotype[7] which functions as a CRTh2 antagonist.

Following a high throughput screen (HTS) of the Novartis compound archive, the known phenoxyacetic acid **3**[8] was identified as a potent ligand for the CRTh2 receptor in a [^{3}H]-PGD_2 radioligand binding assay using CHO cells stably transfected with the human CRTh2 receptor. Functional activity was assessed using an eosinophil shape change assay.[9] Leukocyte shape change responses are mediated through rearrangements of the cellular cytoskeleton and are essential to the process of migration from the microcirculation to sites of inflammation. Follow-up profiling (Table 1) confirmed **3** as an antagonist of the CRTh2 receptor, exhibiting potent inhibition of shape change in isolated human eosinophils stimulated by the CRTh2 specific agonist[10] 13,14-dihydro-15-ketoprostaglandin D_2 (DK-PGD_2). In addition, **3** showed selectivity over the DP, EP_2 and TP prostanoid receptors and was also tested in a panel of 45 additional receptors and 25 kinases, where no inhibition >50% at 10 μM concentration was found.

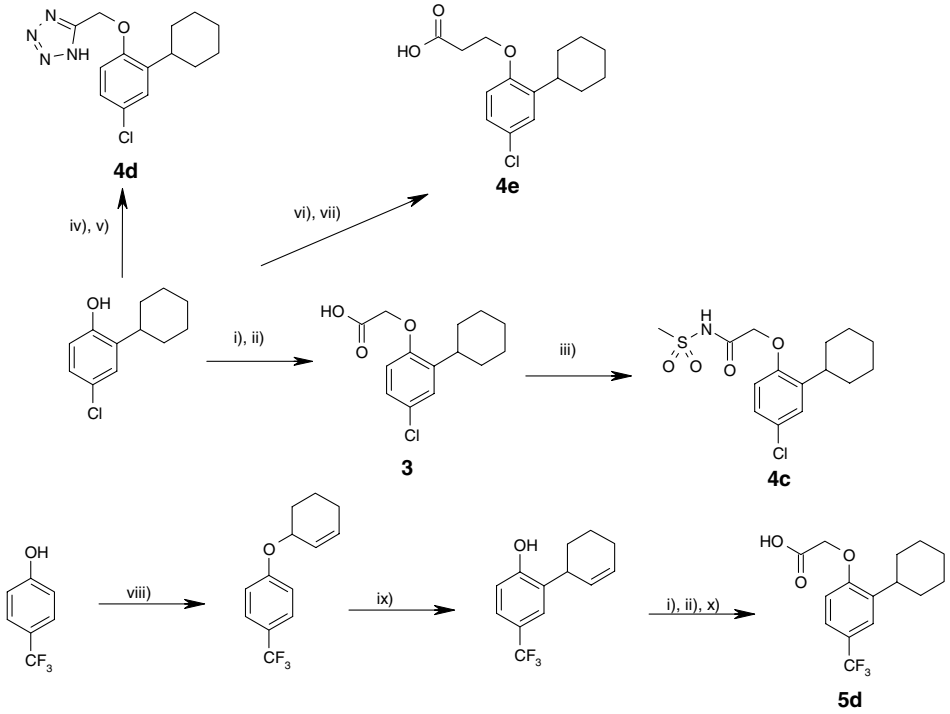
Table 1. Initial profiling of HTS hit **3**

CRTh2 binding K_i (μM)	Eosinophil shape change IC_{50} (μM)	DP binding K_i (μM)	EP_2 binding K_i (μM)	TP binding K_i (μM)
0.154	0.093	7.6	3.0	>10

Values represent means of at least two experiments.

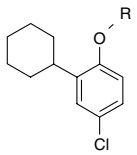
Encouraged by the profile of **3**, we initiated an optimisation programme to explore the CRTh2 structure–activity relationship (SAR) of the 2-cycloalkyl phenoxyacetic acid scaffold. Representative synthetic methods employed are depicted in Scheme 1. Commercially available 2-cyclohexyl-4-chlorophenol could be functionalised by standard methods. The cyclopentyl and cycloheptyl analogues were prepared by $AlCl_3$-mediated Friedel-Crafts alkylation of 4-chlorophenol using the appropriate cycloalkene and the quaternary analogue was prepared by treatment of the phenol with 1-methylcyclohexanol and H_3PO_4. In the case of 4-trifluoromethyl analogue, the Friedel-Crafts methodology was unsuccessful and an alternative Claisen rearrangement was employed. Using the 4-bromo analogue as a handle, further functionality could be introduced by transition metal-mediated coupling reactions, to furnish either the 4-cyano or the 4-(4-fluorophenyl) derivatives.

We initially focused on the acetic acid moiety (Table 2, compounds **4a**–**4h**) confirming the acid itself was required and could not be replaced by either tetrazole or acylsulfonamide isosteres **4c** or **4d**. The acetic acid itself was shown to provide the optimal length of linker to the aromatic core, with extended analogues **4e** and **4f** being less potent. Substitution α- to the acid gave a racemic compound of similar potency to **3**, which on resolution by preparative chiral HPLC[11] showed 30-fold potency difference between the enantiomers **4g** and **4h.**

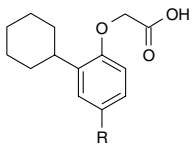


Scheme 1. Representative syntheses. Reagents and conditions: (i) ethyl bromoacetate, Cs_2CO_3, DMF, rt (90%); (ii) aq NaOH, MeOH, rt (85%); (iii) $MeSO_2NH_2$, water soluble carbodiimide, DMAP, 4 Å sieves, CH_2Cl_2 (27%); (iv) chloroacetonitrile, K_2CO_3, acetone, reflux (81%); (v) NaN_3, AcOH, 1-butanol, reflux (76%); (vi) Triton-B, acrylonitrile, reflux (37%); (vii) concd HCl, AcOH, rt (65%); (viii) 3-bromocyclohexene, K_2CO_3, acetone, rt (80%); (ix) 160 °C, neat (crude); (x) H_2, Pd/C, EtOH (96%).

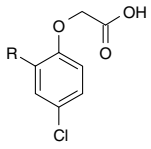
Table 2. CRTh2 and DP binding activities



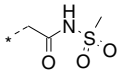
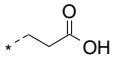
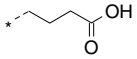
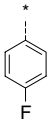
4a - 4i



5a - 5h



6a - 6e

	R	CRTh2 K_i	DP K_i
4a		3.28	8.9
4b		>10	>10
4c		>10	>10
4d		>10	>10
4e		0.799	>10
4f		1.99	>10
4g[a]		0.075	4.49
4h[a]		2.25	>10
5a	–H	0.311	>10
5b	–Br	0.083	5.9
5c	–F	0.446	>10
5d	$-CF_3$	0.020	>10
5e	–CN	0.230	>10
5f	$-CH_3$	0.353	>10
5g	$-OCH_3$	0.782	>10
5h		0.099	6.4
6a		0.150	4.8
6b		0.059	1.88
6c		0.090	1.21
6d		5.13	4.74
6e		>10	>10

K_i values are reported in μM and represent the mean of at least two experiments.

[a] Absolute configurations arbitrarily assigned.

Having established the requirement for the phenoxy acetic acid moiety, we next investigated variation of the para substituent (Table 2, compounds **5a**–**5h**). Electron-deficient substituents favoured binding activity, although the low activity of fluoro derivative **4c** suggested an additional steric contribution. The trifluoromethyl analogue **5d** gave the optimal combination of binding activity with improved selectivity over DP. In contrast, electron rich substituents such as **5g** gave reduced binding potency. Interestingly, incorporation of an aromatic group maintained binding potency in **5h**. We next explored the ortho cycloalkyl group (**6a**–**6e**), taking the 4-chlorophenyl moiety of **3** as the optimal substituent. Ring contraction to **6a** resulted in a reduction in binding potency, whereas the novel cycloheptyl analogue **6b**[12] was somewhat more potent than the original hit, albeit with a slightly reduced selectivity over the DP receptor. Notably, the 2-allyl derivative **6d** showed dual, albeit weak, activity at CRTh2 and DP.

Table 3. Functional potency of selected compounds

	5b	**5d**	**5e**	**6a**	**6b**	**6c**
Eosinophil shape change IC_{50}(μM)	0.448	0.172	0.052	1.013	0.248	0.451

IC_{50} values represent means of at least two experiments.

With CRTh2 binding SAR established we next profiled selected compounds for human eosinophil shape change functional activity, as indicated in Table 3. All compounds were found to be antagonists, with some variation in the binding-functional potency correlation. For compound **6b**, the eosinophil assay was also run using the CCR-3 receptor agonist eotaxin[13] as a shape change stimulus. No inhibition was found up to 10 μM under these conditions, supporting the concept of a CRTh2 specific effect for the compound.

Compound **6b**[14] was selected for further profiling, and showed $IC_{50} > 10$ μM in a panel of cytochrome P450 (CYP) enzyme isoforms 3A4, 2D6 1A2, 2C19 and 2C9. Table 4 shows the results of in vitro and in vivo ADME profiling. Moderate to high microsomal clearance and good permeability were observed, along with a high level of plasma protein binding, consistent with the carboxylic acid functionality. In the rat, good exposure was observed after oral dosing of the compound.

In conclusion, HTS identified a novel phenoxyacetic acid chemotype for selective CRTh2 antagonists. The SAR of the scaffold was explored, resulting in identification of compound **6b** which is a functional antagonist of the human CRTh2 receptor and shows oral bioavailability in the rat.

Table 4. ADME profile of compound **6b**

Human microsomes, Cl_{int}	109 μl/min/mg
Rat microsomes Cl_{int}	205 μl/min/mg
Caco-2 permeability, A–B	8.4×10^{-6} cm/s
Caco-2 permeability, B–A	21.4×10^{-6} cm/s
Human plasma protein binding	>99.9%
$T_{1/2}$ (iv, 0.5 mg/kg)	71 min
Cl	7.3 ml/min/kg
V_{ss}	0.52 L/kg
Bioavailability (po, 2 mg/kg)	58%
AUC_{all}	694.9 μM min
C_{max}	4.7 μM

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14. Selected data for **6b**: δ_H (400 MHz, DMSO) 13.00 (1H, br s), 7.15 (2H, m), 6.80 (1H, d), 4.70 (2H, s), 3.10 (1H, t), 1.4–1.8 (12H, m); *m/z* (−ve) (APCI) 281; $C_{15}H_{19}ClO_3 \cdot 0.5\ H_2O$ requires C, 61.75; H, 6.91%. Found: C, 61.68; H 6.80%.