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Discovery of potent CRTh2 (DP_2) receptor antagonists

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Abstract—Starting with the weak agonist indomethacin, a series of potent, selective CRTh2 (DP_2) antagonists have been discovered as potential treatments for asthma, allergic rhinitis and other inflammatory diseases.

Prostaglandin D_2 (PGD_2) is involved in a number of inflammatory conditions and is an important mediator in allergic reactions, including asthma and allergic rhinitis. A single receptor, DP_1, has been known for many years.[1] Recently, a second PGD_2 receptor, CRTh2 (DP_2),[2,3] has been identified which is a G-protein coupled 7-transmembrane protein found on human Th2 cells, eosinophils and basophils. Endogenous agonists for CRTh2 include PGD_2 and a number of its metabolites, notably 13,14-dihydro-15-keto PGD_2 (DK-PGD_2), which is selective for CRTh2 over DP_1.[2] Activation of CRTh2 promotes chemotaxis of Th2 cells, eosinophils and basophils,[2,3] as well as degranulation of eosinophils[4] and cytokine release from Th2 cells.[5] This profile suggests that an antagonist of CRTh2 may have potential as a therapy in allergic disorders.

Indomethacin has been shown to be a CRTh2 agonist,[6] but potency at CRTh2 is more than 10-fold lower than for inhibition of cyclooxygenase (COX) 1 or 2 enzyme activity.[6] Starting from indomethacin, we have identified a series of potent, selective CRTh2 (DP_2) antagonists with good selectivity against both COX enzymes.

Compounds, selected by structural similarity to indomethacin, were screened for both agonist and antagonist activity at CRTh2 using a functional response.[7] This approach identified agonists of varying efficacies with compound **2** clearly exhibiting partial agonism (Fig. 1).

This suggested that antagonists for CRTh2 could be obtained by reducing the number of heteroatoms and increasing the lipophilicity of the N1 substituent.

Following this hypothesis, compound **3** was identified as a CRTh2 antagonist (Fig. 2).

Compounds **2** and **3** had modest potency at CRTh2 as measured in a binding assay[8] displacing [^{3}H]PGD_2 from CRTh2 with IC_{50} values of 5200 nM and 178 nM, respectively. Compound **3** was initially synthesised during investigation into the SAR of indomethacin as a COX-1 inhibitor,[9,10] and has properties which are acceptable for a moderately lipophilic acid ($\log D_{7.4} = 1.7$). It is soluble in physiological buffer at 47 mg/mL and is 99% bound to human plasma protein. Dosing in rats showed moderate clearance of 11 mL/min/kg, a higher than expected volume of distribution of 2.3 L/kg and consequently an acceptable terminal half-life of 2.2 h with a bioavailability of 62%.

Keywords: CRTh2; DP_2; Inflammation; Asthma; Indomethacin; Indole; Quinoline.
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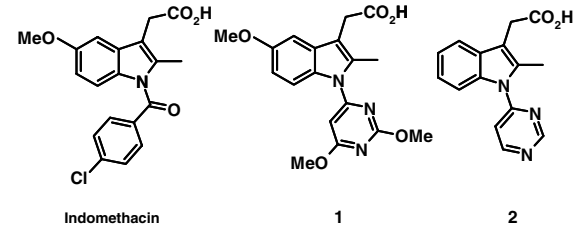


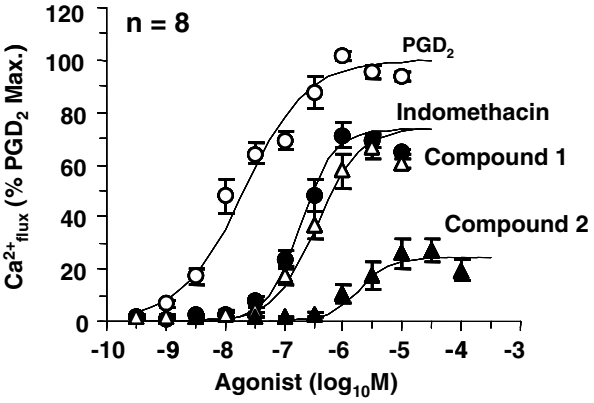


Figure 1. Induction of Ca^{2+} flux in CRTh2-expressing cells by agonists discovered through substructure searching.

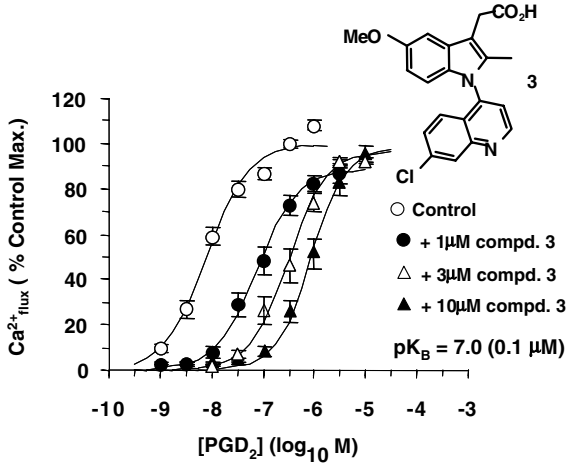


Figure 2. Inhibition of PGD_2-induced Ca^{2+} flux in CRTh2-expressing cells by the initial antagonist hit, compound **3**.

Compound **3** also showed significant inhibition of recombinant human CYP 2C19 (IC_{50} = 1.6 μM).[11] However, compound **3** still possessed significant inhibitory activity against COX-1 with an IC_{50} of 40 nM in a functional assay of COX-1-mediated platelet aggregation.[12]

Inversion of an indole template maintains the same relative spatial distribution of the 1.3-substituents[13] and applying this strategy to **3** led to compounds with similar properties at CRTh2. Compounds **4**, **5** and **6** (Fig. 3) all had improved potency at CRTh2 compared to compound **3** (Table 1).

Besides a 20-fold improved potency at CRTh2, compound **6** had 10-fold less activity at COX-1 (IC_{50} = 400 nM). Plasma protein binding remains high

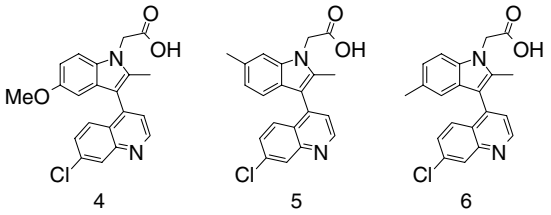


Figure 3. Compounds **4**–**6** on inverted indole scaffold.

Table 1. Activities of inverted indole scaffold compounds

Compound	CRTh2 binding IC_{50}[a] (nM)	Cl_{int} (μL/min/10^6 cells)		Hu (ppb)
		Rat hepatocyte[b]	Hu hepatocyte[b]	
3	180	6.5	1.4	99.0
4	50	5.0		99.7
5	125			
6	8	6.0	3.8	99.8

[a] Radiometric displacement binding assay,[8] mean of at least two measurements.
[b] Fresh.

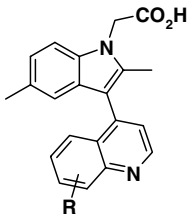
as would be expected for an acidic compound (99.8% bound to human plasma protein and 98% for rat) but in vivo clearance in the rat after iv dosing is relatively low (6 mL/min/kg), and, with a volume of distribution (V_{ss}) of 1.4 L/kg, a consequently long half-life of 6.7 h is observed. The large volume and long half-life are due to recirculation of the glucuronide; a process that we could not rely on happening in man and was not seen in less lipophilic analogues.

A series of analogues of the lead compound **6** (Table 2)[14] was prepared with the aim of reducing plasma protein binding (to increase the potential activity in whole blood, which was used as a surrogate for in vivo potency), whilst maintaining or improving on intrinsic clearance in human hepatocytes (needed to counter the increased metabolism due to increased free fraction).

A substituent at the 7- or 8-position of the quinoline gives an increase in potency of up to 13-fold compared to the unsubstituted compound (**7**). A substituent at the 8- (or 2-) position also conferred greater metabolic stability, probably by hindering metabolism at the quinoline nitrogen. No compound has activity greater than 6 μM[15] at the hERG channel or DP_1 receptor, or any activity as a CRTh2 agonist.

The 8-methyl analogue (**12**) has a preferred profile. The combination of potency and human plasma protein binding gives good predicted potency in whole blood (Table 2) due to its higher potency and lower human plasma protein binding than compound (**6**). In rat and dog, it has acceptable pharmacokinetic properties with, in rat, plasma protein binding of 98%, an iv clearance of 3 mL/min/kg, bioavailability of 76%, half-life of 1.7 h and a volume of distribution of 1.4 L/kg, and in dog, a clearance of 1 mL/min/kg, bioavailability of 100%, half-life of 5.3 h and a volume of distribution of 0.2 L/kg. The closely related compound (**13**) has very similar

Table 2. CRTh2 antagonist potencies and biological data



Compound	Quinoline substituent	CRTh2 binding IC_{50}[a] (nM)	Cl_{int} (μL/min/10^6 cells)		$\log D_{7.4}$	Hu (ppb)	Calculated whole blood potency[b] (nM)
			Rat hepatocyte	Hu hepatocyte			
6	7-Cl	8	6.0	3.8	1.6	99.8	3800
7	None	21	10.7	3.0			
8	2-Me	18	5.0		2.9	99.6	4500
9	6-CF_3	68	16		1.8		
10	7-CF_3	5.4	17	2.6	1.8	99.6	1350
11	8-CF_3	6.0	6.5	2.4	1.3	99.6	1500
12	8-Me	2.6	5.5	1.9	1.3	99.5	520
13	8-Cl	2.3	<3	2.0	1.3	99.4	380
14	8-F	7.1	<1	1.6	0.5	99.1	790
15	8-OMe	11	7.0		0.5	98.2	610
16	8-SO_2Me	23	3.0	<1	−0.3	93.6	360
17	8-CN	18	<3			98.3	1060

[a] Radiometric binding assay,[8] mean of at least two measurements.
[b] CRTh2 binding IC_{50}/(1 − (ppb/100)).

properties with the main difference being a shorter half-life of 2.4 h in the dog. The more polar compounds (**15**) and (**16**) had poor bioavailabilities (13% and 5%, respectively).

The substituents on the indole ring are important (Fig. 4). The analogue of compound **6** without the 2-methyl group (**18**) was 5-fold less potent and chemically less stable. The 2,4-dimethyl analogue (**19**) was equipotent, while the 2,6-dimethyl (**5**) was 15-fold less potent. In general substitution at the 6-position led to loss of potency and higher COX-1 activity.

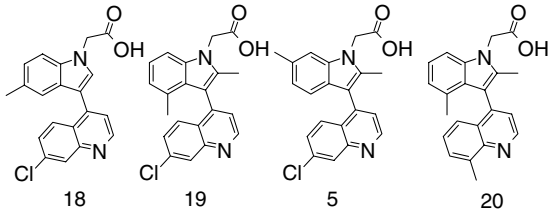
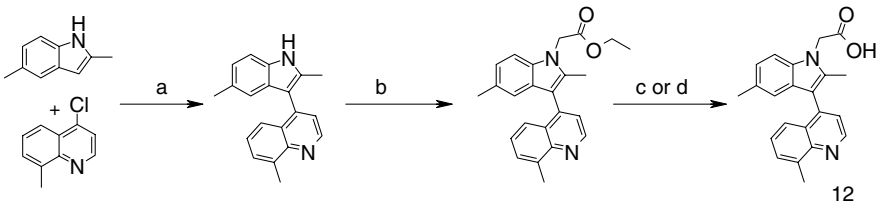


Figure 4. Methyl substituents on indole ring.

Compound **12** has been tested on a bank of 153 screens[18] and the only activities seen were inhibition of rat aldose reductase (IC_{50} = 150 nM) and the serotonin transporter (IC_{50} = 2 μM) with no measurable activity against both COX-1 and COX-2 at 3 μM. In addition, compound **12** weakly inhibited CYP 2C19 (IC_{50} = 2.5 μM) and 2C9 (IC_{50} = 5 μM).

The compounds were prepared, either as the sodium salts or as the free acids, from 2,5-dimethylindoles and the 4-chloroquinolines (either commercially available or prepared by literature methods,[16]) by the route exemplified in Scheme 1 for compound (**12**). The fusion step could be carried out in the absence of a solvent, but addition of a small amount of NMP, DMF or dioxane (with or without a trace of HCl as catalyst) gave cleaner, faster reactions at lower temperatures; particularly on a large scale. This represents a novel synthesis of this type of structure, and is tolerant of a wide variety of substituents on both indole and chloroquinoline moieties.[17]

The 2,4-dimethyl analogue (**20**) of compound (**12**) exhibits atropisomerism by reason of restricted rotation



Scheme 1. Reagents and conditions: (a) trace of NMP, 140 °C melt (100%); (b) ethyl bromoacetate, Cs_2CO_3, acetone, reflux (60%); (c) 1 equiv NaOH, aq MeOH (100%); (d) as method (c) followed by addition of 1 equiv HCl (95%).

around the biaryl bond.[19] The isomers could be separated by chiral-phase HPLC at the ester stage and shown to be configurationally stable for more than one week. Absolute configurations were not determined. Surprisingly, there was only a 12-fold difference in the potencies of the two isomers (IC_{50} = 1.4 and 17 nM). By contrast the racemisation half-life of compound (**6**) was estimated at ca. 8 min by ^1H NMR; the signals for the methylene protons of a number of compounds showed some splitting ('AB' quartets) suggesting that the two protons were non-equivalent on the NMR time scale.

In summary, starting from the CRTh2 agonist indomethacin we have prepared a series of potent, novel, CRTh2 (DP_2)-selective antagonists with good pharmacokinetic properties.

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7. HEK 293 cells stably expressing human CRTh2 and Gα16 were plated in 96-well plates and loaded with Fluo 3AM for 1 h at 37 °C. Ca^{2+} flux in response to various agonists was measured using a FLIPR. A baseline signal was established for 10 s and changes in fluorescence were followed for 3 min after the addition of agonist. For antagonist studies, Fluo 3AM-loaded cells were pre-treated with compound for 5 min at 37 °C.
8. Membranes were isolated from HEK 293 cells stably expressing human CRTh2 and Gα16. Binding assays were performed in a 96-well SPA format. Membranes were prebound to Wheat Germ Agglutinin-coated PVT-SPA beads (Amersham) for 18 h at 4 °C. Each well contained membrane coated beads, ^3H-PGD_2 (2.5 nM) and test compounds in a final volume of 50 μL. Nonspecific binding was determined in the presence of 100 μM DK-PGD_2. Plates were incubated for 2 h at room temperature and bead-associated radioactivity was measured using a Wallac Microbeta counter.
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18. Panel of 153 enzyme and receptor assays (including COX-1 and COX-2) performed by MDS Pharma Services.