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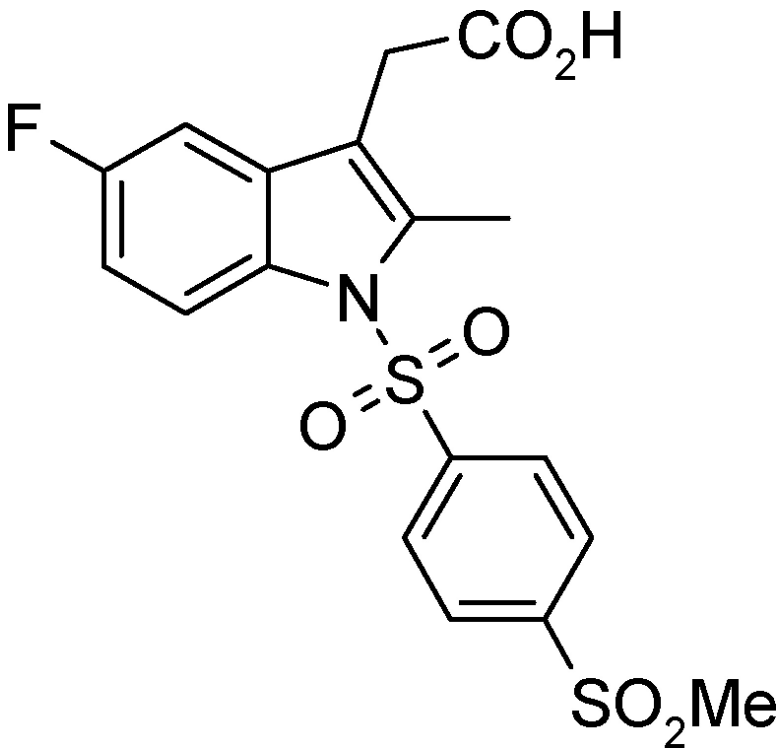

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Indole-3-acetic Acid Antagonists of the Prostaglandin D_2 Receptor CRTH2

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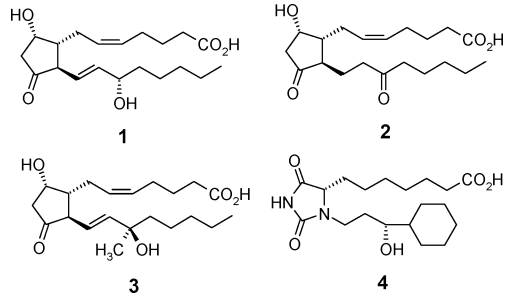
Abstract: Prostaglandin D_2 (PGD_2) acting at the CRTH2 receptor (chemoattractant receptor-homologous molecule expressed on Th2 cells) has been linked with a variety of allergic and other inflammatory diseases. We describe a family of indole-1-sulfonyl-3-acetic acids that are potent and selective CRTH2 antagonists that possess good oral bioavailability. The compounds may serve as novel starting points for the development of treatments of inflammatory disease such as asthma, allergic rhinitis, and atopic dermatitis.

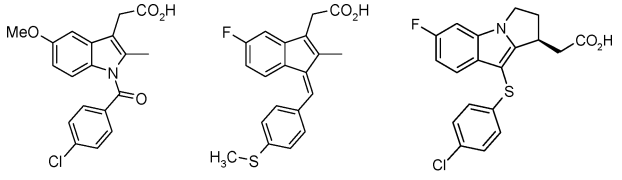
Prostaglandin D_2 (PGD_2) (**1**) is an eicosanoid, a class of chemical mediator synthesized by cells in response to local tissue damage and hormonal and immunological stimuli. Eicosanoids bind to specific cell surface receptors on a wide variety of tissues throughout the body and mediate various effects in these tissues. PGD_2 is the major prostanoid produced by mast cells and is also synthesized by macrophages and Th2 lymphocytes. It was detected in high concentrations in the airways of asthmatic patients challenged with antigen,[1] and instillation of PGD_2 into airways can provoke many features of the asthmatic response including bronchoconstriction[2] and eosinophil accumulation.[3]



The potential of PGD_2 to induce inflammatory responses was confirmed by the use of transgenic mice overexpressing human PGD_2 synthase. These mice exhibit exaggerated eosinophilic lung inflammation and Th2 cytokine production in response to antigen.[4]

The first PGD_2 specific receptor to be discovered was the DP receptor, which is linked to elevation of the intracellular levels of cAMP. However, the effects of PGD_2 on leukocyte activation are thought to be mediated by an action on CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), which is expressed by Th2 lymphocytes, eosinophils, and basophils.[5] It seems clear that the effect of PGD_2 on the activation of Th2 lymphocytes and eosinophils is mediated through CRTH2 because the selective CRTH2 agonists 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) (**2**) and 15*R*-methyl-PGD_2 (**3**) can elicit this response and the effects of PGD_2 are blocked by an anti-CRTH2 antibody.[5a,6] In contrast, the selective DP agonist BW245C (**4**) does not promote migration of Th2 lymphocytes or eosinophils.[5a,7] On the basis of this evidence, antagonizing PGD_2 at the CRTH2 receptor should be an attractive approach to treat the inflammatory component of Th2-dependent allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis.

Indomethacin (**5**) is a nonsteroidal antiinflammatory drug (NSAID) that achieves analgesic and antipyretic activity through the inhibition of cylcooxygenases. It was previously shown to bind to the human CRTH2 receptor with values ranging from 25 nM to 8 μM and to act in an agonistic fashion in vitro with a potency of approximately 15−50 nM.[8] Sulindac sulfide (**6**), another NSAID that has a similar spatial arrangement of acetic acid and aromatic groups, was also shown to bind with moderate affinity to the human CRTH2 receptor (K_i = 3.5 μM)[8b] and more recently to act agonistically on murine CRTH2.[9] More recent studies describe a range of indolylacetic acid analogue type ligands for human CRTH2; however, no pharmacokinetic and only limited human cellular activity data were presented for selective antagonist compounds.[10] L-888,607 (**7**), which also possesses a fluoroindolecarboxylic acid template, has been reported to be a potent (hCRTH2 binding K_i = 0.8 ± 0.4 nM) and orally bioavailable CRTH2 agonist.[10d] On the basis of the earlier observations around indomethacin and sulindac sulfide,[8] we hypothesized that it is possible to discover novel molecules based on indole acetic acid structures that show antagonistic activity at the human CRTH2 receptor.



Our founding hypothesis was that the carboxylic acid group was important for binding human CRTH2 because the endogenous agonist, indomethacin, and sulindac sulfide contain this moiety and the human CRTH2 receptor is rich in basic residues in the transmembrane region. Preliminary studies indicated that the (5-fluoro-2-methyl-1*H*-indolyl-3-yl)acetic acid template (with a substitution pattern similar to that found in sulindac sulfide (**6**) and L-888,607 (**7**)) was readily synthetically accessible and that small SAR probing compound libraries derived from this template retained moderate

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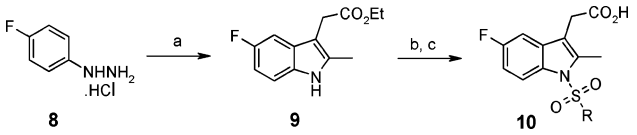
Scheme 1[a]



8 9 10

[a] Reagents and conditions: (a) ethyl levulinate, DCE/TFA (6:4), 43%; (b) RSO_2Cl, KO^{t}Bu, 18-crown-6, THF; (c) LiOH, H_2O, THF.

binding potency with good microsomal metabolic stability. As a consequence, the (5-fluoro-2-methyl-1*H*-indolyl-3-yl)acetic acid core was kept constant for the remainder of the study. The most significant improvements in hCRTH2 binding were achieved with modification of the indole N-group. In particular, *N*-sulfonyl analogues were found to be particularly potent and selective compounds in ligand binding experiments. The indole *N*-sulfonyl analogue **10a** (R = 4-chlorophenyl) was prepared using the conditions described in Scheme 1.

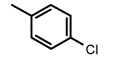
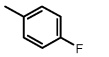
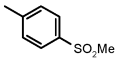
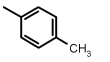
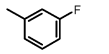
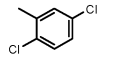
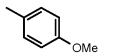
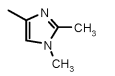
(5-Fluoro-2-methyl-1*H*-indol-3-yl)acetic acid ethyl ester (**9**) was obtained via a Fischer indole synthesis using 4-fluorophenylhydrazine hydrochloride (**8**) and ethyl levulinate.[11] The indole *N*-sulfonyl group was introduced from reaction with the corresponding sulfonyl chloride with potassium *tert*-butoxide and 18-crown-6. Hydrolysis was achieved using lithium hydroxide in aqueous tetrahydrofuran to give the free acid.

10a displayed potent binding to hCRTH2 (K_i = 29 nM) with excellent selectivity over the DP receptor (membranes from Chinese hamster ovary (CHO) cells expressing recombinant human CRTH2 or DP were used in these experiments). With binding of **10a** to hCRTH2 established, it was pivotal to ascertain that the interaction was resulting in agonism or the desired antagonism of the receptor. This was determined through measurement of the compound's ability to inhibit PGD_2-stimulated Ca^{2+} flux in intact CHO cells expressing human CRTH2; IC_{50} values for this assay are shown in Table 1. **10a** on its own did not induce calcium mobilization in CHO/CRTH2 cells.

With increasing concentration of **10a**, a dose-dependent and parallel shift of the PGD_2 dose response curve in CHO/hCRTH2 cells was observed, thereby indicating that the compound was indeed a competitive hCRTH2 antagonist ($pA_2 = 7.48 \pm 0.07$). In addition, the antagonistic effect of the compound appeared to be hCRTH2 selective, since no inhibitory effect was seen with ATP-stimulated Ca^{2+} flux. This important result led us to prepare a series of analogues of **10a** and to evaluate them for selective binding to hCRTH2 over the DP receptor. The test compounds were prepared according to the conditions in Scheme 1.

The structure−activity relationship data presented in Table 1 demonstrate the importance of a phenylsulfonamide group substituted at the para position with an electron-withdrawing group to give optimal potency and selectivity. Replacing the electron-withdrawing group with a neutral (**10d**) or donating moiety (**10h**) leads to reduced affinity at the hCRTH2 receptor. When the electron-withdrawing group is present at the meta position (**10f**), affinity for hCRTH2 is reduced. The pendent phenyl group appears to be essential for activity because replacement with a heterocyclic group (**10i**) or an alkyl chain (**10j**) leads to a decrease in affinity. In all cases the selectivity for hCRTH2 over the DP

Table 1. Assay Data for the Indole *N*-Sulfonyl Compounds

Comp 10	R	hCRTH2 Binding K_i (nM) ± SEM[a]	hDP Binding K_i (nM) ± SEM	Ca^{2+} Flux IC_{50}(nM)[b] ± SEM
a		29±7	8460± 1864	26±8
b		71±16	>10000	158±39
c		68±6	>10000	19±12
d		250±18	4860± 640	987±488
e		457±239	>10000	2184±800
f		225±11	6890± 1023	639±57
g		19±4	1872± 884	44±16
h		299±73	7710± 2160	318±85
i		2979±453	>10000	>10000
j		634±90	>10000	842±452

[a] Standard error of the mean. [b] Carried out at 10 nM PGD_2.

Table 2. Cellular Assay Data

compd	eosinophil shape change IC_{50} (nM)[a]	Th2 cell chemotaxis IC_{50} (nM)
10a	908	not tested
10c	74	67

[a] Forward scatter measured by fluorescence-activated cell sorting carried out in the presence of 10% fetal calf serum.

receptor was high with >160-fold selectivity being achieved for compound **10c**. Interestingly, the dichloro analogue **(10g)** demonstrated a small increase in affinity for hCRTH2 despite lacking an electron-withdrawing group at the para position; however, its functional potency and selectivity over DP were not optimal.

The most potent and selective compounds (**10a** and **10c**) were assessed in secondary assays to ensure that the observed antagonism of hCRTH2 gave rise to activity in primary human eosinophils and Th2 cells that express endogenous CRTH2. The ability of the compounds to inhibit PGD_2 induced eosinophil or Th2 cell activation was assessed by shape change or chemotaxis; both compounds demonstrated an effect with **10c** showing particularly potent inhibition (Table 2). The weaker effect of **10a** on eosinophil shape change in the presence of serum may be due to the poorer solubility (0.05 mg/mL for **10a** compared to 0.08 mg/mL for **10c**) and therefore putative higher protein binding for this compound. The antagonistic effect of **10c** appears to be hCRTH2 selective, since no inhibitory effect was seen when other chemoattractant compounds were used, including eotaxin, 5-oxo-ETE, IL-5, C5a, and LTB4.[12]

The potency of **10c** made it a candidate for further evaluation, and so the potential for metabolic and toxic liabilities was assessed. The data for these assays are shown in Table 3. **10c** showed no inhibition of the five major cytochrome P450 (CYP450) isoforms and no

Table 3. Metabolic and Toxicity Assay Data for **10c**

assay type	
cytochrome P450 inhibition (μM)[a]	
3A4 (ketoconazole)	>50
2D6 (quinidine)	>50
2C9 (sulfaphenazole)	>50
2C19 (tranylcypromine)	>50
1A2 (furafylline)	>50
cytochrome P450 induction (% at 1 μM)[b]	
3A4 (midazolam)	-8 ± 18
1A (ethoxyresorufin)	-7 ± 11
2C9 (tolbutamide)	-18 ± 9
microsomal stability $t_{1/2}$ (min)	
human	>60
rat	>60
hERG block (μM)	>50
HepG2 cytotoxicity (μM)[c]	>50

[a] Using the fluorogenic substrates with standard inhibitors stated in the table. [b] Comparison of turnover of standard substrates stated in the table compared to 50 μM positive control of dexamethasone (CYP3A4), omeprazole (CYP1A), and rifampicin (CYP2C9). [c] Viability reading using the Alamar Blue redox tracer.

Table 4. Pharmacokinetic Data for **10c** in Rats

dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{inf} (ng/(mL·h))	Cl ((mL/min)/kg)	$t_{1/2}$ (h)	F (%)
1 (iv)	1801		619	40	2.2	
10 (po)	332	1.8	3480	na	5.5	56

induction of three CYP450 compounds at 1 μM. No microsomal metabolism was observed with rat and human microsomes. No functional activity was seen in a human ether-a-go-go-related (hERG) potentiomentric dye fluorescence based functional potassium channel assay, and no cytotoxicity was observed in a HepG2 hepatocarcinoma cell line. **10c** was negative in an Ames and CHO clastogenicity assay and did not show any activity up to 1 μM against a panel of >85 enzymes (including COX-1 and COX-2), receptors, and ion channels. The clean profile that **10c** demonstrated in these assays led to the pharmacokinetic profile of the compound being determined (Table 4).

10c gave a good pharmacokinetic profile in rats; oral and iv administration led to acceptable plasma levels with a good half-life and area under the curve; bioavailability was also favorable. These data suggest that **10c** may be suitable for oral administration for the treatment of inflammatory disorders.

In summary, a novel series of indole *N*-sulfonyl compounds that are potent and selective hCRTH2 antagonists is reported. **10c** showed activity in cellular assays and has good oral pharmacokinetic properties in rats. The molecule is under further biological evaluation and should prove to be a useful tool for understanding of the pharmacological role of the CRTH2 receptor in vivo.

Acknowledgment. The authors thank Carole Gayet, Chris Palmer, and Frank Schroer for synthesis of the test compounds.

Supporting Information Available: Experimental procedures, compound characterization data, assay methods with additional information, and broad selectivity screening information. This material is available free of charge via the Internet at http://pubs.acs.org.

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