

Identification of a Potent and Selective Synthetic Agonist at the CRTH2 Receptor

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ABSTRACT

The chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells (CRTH2) is a G protein-coupled receptor whose function in vivo has been incompletely characterized. One of the reasons is that its current known ligands, prostaglandin D₂ and some of its metabolites, have either poor selectivity for CRTH2 or are metabolically unstable in vivo. In this study, we describe the biological and pharmacological properties of L-888,607, the first synthetic potent and

selective CRTH2 agonist. We show that L-888,607 exhibits 1) subnanomolar affinity for the human CRTH2 receptor, 2) high selectivity over all other prostanoid receptors and other receptors tested, 3) agonistic activity on recombinant and endogenously expressed CRTH2 receptor, and 4) relative stability in vivo. L-888,607 thus represents a suitable tool to investigate the in vivo function of CRTH2.

Chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells (CRTH2) is a G-protein-coupled receptor closely related to chemoattractant receptors for the *N*-formyl peptide, the complement peptides C3a and C5a, and the leukotriene B₄. It was first reported to be selectively expressed on human T-helper type 2 cells (Nagata et al., 1999b). It is also expressed on T-cytotoxic type 2 cells, eosinophils, and basophils (Nagata et al., 1999a; Cosmi et al., 2000; Sawyer et al., 2002). CRTH2 mRNA is found in various adult tissues in the digestive tract, heart, thymus, spleen, and brain (Sawyer et al., 2002). The roles played by this

receptor throughout the body are currently unknown. However, it has been demonstrated that its activation by prostaglandin (PG) D₂ can increase eosinophil, basophil, and Th2 cell motility (Gervais et al., 2001; Hirai et al., 2001; Monneret et al., 2001). Activation of CRTH2 has also been shown to modulate eosinophil morphology and degranulation (Gervais et al., 2001), basophil degranulation (Yoshimura-Uchiyama et al., 2004), and Th2 cell cytokines secretion (Tanaka et al., 2004). The currently known high-affinity ligands for CRTH2 are the agonists PGD₂, some PGD₂ metabolites [13-14-dihydro-15-keto PGD₂ (DK-PGD₂), 15-deoxy-Δ^{12,14}-PGJ₂, PGJ₂, and Δ¹²-PGJ₂] (Sawyer et al., 2002), and indomethacin (Hirai et al., 2002) and the antagonist ramatroban (Sugimoto et al., 2003). PGD₂ is an arachidonic acid metabolite implicated in a wide range of physiological events, including sleep induction, goblet cell depletion, vasodilation, increased microvas-

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ABBREVIATIONS: CRTH2, chemoattractant receptor-homologous molecule expressed on T-helper cells; PG, prostaglandin; DK-PGD₂, 13-14-dihydro-15-keto-PGD₂; L-883,595, {9-[(4-chlorophenyl)thio]-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl}acetic acid; compound A [9-(4-chlorobenzyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; compound B, [10-(4-chlorobenzyl)-6,7,8,9-tetrahydropyridol[1,2-*a*]indol-9-yl] acetic acid; compound C, [10-(4-chlorophenyl)thio]-6,7,8,9-tetrahydropyridol[1,2-*a*]indol-9-yl] acetic acid; compound D, {8-bromo-9-[(4-chlorophenyl)thio]-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; compound E, {9-[(4-chlorophenyl)thio]-8-ethyl-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; compound F, {9-[(4-chlorophenyl)thio]-8-cyclopropyl-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; compound G, {9-[(4-chlorophenyl)thio]-8-cyclopentyl-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; compound H, {9-[(4-chlorophenyl)thio]-6-cyano-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl] acetic acid; Ro 20-1724, 4-[(3-butoxy-4-methoxyphenyl)-methyl]-2-imidazolidinone; DMSO, dimethyl sulfoxide; HEK, human embryonic kidney; FBS, fetal bovine serum; hCRTH2, human chemoattractant receptor-homologous molecule expressed on T-helper cells; MES, 2-(*N*-morpholino)ethanesulfonic acid; BW245C, (4*S*)-(3-[(*R,S*)-3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo-4-imidazolidineheptanoic acid; L-888,607, (S)-{[9-[(4-chlorophenyl)thio]-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl]acetic acid; L-888,291, (*R*)-{[9-[(4-chlorophenyl)thio]-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl]acetic acid; SQ-29548, [1*S*-(1 α , 2 α), 3 α , 4 α]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; L-888,607, (S)-{[9-[(4-chlorophenyl)thio]-6-fluoro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-yl]acetic acid.

cular permeability, eosinophil infiltration, smooth muscle relaxation, contraction of the myometrium, inhibition of platelet aggregation, cell survival, control of intraocular pressure, and allergic responses (Nagata and Hirai, 2003). PGD₂ binds with equivalent affinity to DP and with lower affinities to FP and EP₃, all members of the prostanoid receptor family. Because of the nonselectivity of PGD₂ and its instability in vivo, it has been difficult to determine which receptor is responsible for each effect mediated by PGD₂ (Liston and Roberts, 1985; Robinson et al., 1989). The discovery of BW245C, a selective and stable synthetic agonist of DP, has allowed the identification of DP-specific effects mediated by PGD₂ such as vasodilation, relaxation of smooth muscle, and platelet aggregation (Whittle et al., 1983; Wright et al., 1998). However, a number of PGD₂ functions could not be mimicked by BW245C, including increased microvascular permeability, eosinophil infiltration, and goblet cell depletion (Woodward et al., 1990; Fernandes and Crankshaw, 1995). To investigate whether these functions are mediated through CRTH2, investigators have used the selective agonist DK-PGD₂, a metabolic product of PGD₂. Unfortunately, given the inherent metabolic instability of PGD₂ and some of its metabolic intermediates, one must interpret functional data with caution. Therefore, given that PGD₂ and potentially DK-PGD₂ can be metabolized and that some intermediates may serve as activators of receptors other than CRTH2 (Bocher et al., 2002), a synthetic, stable CRTH2 ligand is needed to investigate the role of this receptor in vivo. Here, we describe the biological and pharmacological properties of L-888,607, the first synthetic, potent, and selective CRTH2 agonist.

Materials and Methods

Chemicals. L-883,595, compound A, compound B, compound C, compound D, compound E, compound F, compound G, and compound H are racemic compounds that were synthesized at Merck Frosst (Montreal, QC, Canada). The two enantiomers L-888,607 and L-888,291 (*S*- and *R*-enantiomers, respectively) were isolated from L-883,595. PGD₂, DK-PGD₂, and BW245C were purchased from Cayman Chemical (Ann Arbor, MI).

Radioligand Binding Assay. Prostanoid receptor binding assays were performed at room temperature in a final volume of 0.2 ml in 10 mM HEPES/KOH, pH 7.4 (CRTH2, DP, and IP), or 10 mM MES/KOH, pH 6.0 (EP subtypes, FP, and TP), containing 1 mM EDTA and 10 mM MnCl₂ (CRTH2) or 10 mM MnCl₂ only (DP, FP, IP, and TP) or 1 mM EDTA and 10 mM MgCl₂ (EP subtypes) and radioligand (0.4 nM [³H]PGD₂, 172 Ci/mmol for CRTH2 and DP, 0.5 nM [³H]PGE₂, 181 Ci/mmol for EP subtypes, 0.95 nM [³H]PGF_{2α}, 170 Ci/mmol for FP, 5 nM [³H]iloprost, 16 Ci/mmol for IP and 1.8 nM [³H]SQ-29548, 46 Ci/mmol for TP). EP₃ assays also contained 100 μM guanosine 5'-O-(3-thio)triphosphate. Competing ligands (from BIOMOL Research Laboratories, Plymouth Meeting, PA, and Cayman Chemical) were diluted in dimethyl sulfoxide (DMSO) that was kept constant at 1% (v/v) of the final incubation volume. Nonspecific binding was determined in the presence of 1 μM concentrations of the corresponding nonradioactive prostanoid. The reaction was initiated by the addition of membrane proteins from HEK293 (EBNA) cells stably expressing the appropriate receptor (23 μg of membrane proteins for CRTH2; 30 μg for DP and EP₁; 20 μg for EP₂; 2 μg for EP₃; 10 μg for EP₄, TP, and IP; and 60 μg for FP). Incubations were conducted for 60 min at room temperature and terminated by rapid filtration through a 96-well Unifilter GF/C (PerkinElmer Life and Analytical Sciences, Boston, MA) using a Tomtec MachIII semiautomated harvester. The filters were then washed with 4 ml of the same buffer, and residual radioligand bound to the filter was determined

by liquid scintillation counting after equilibration in 50 μl of Ultima Gold F (Unifilter) (PerkinElmer Life and Analytical Sciences) using a 1450 MicroBeta (PerkinElmer Wallac, Gaithersburg, MD).

[cAMP] Measurements. The intracellular concentration of cAMP was determined using the ¹²⁵I-cAMP scintillation proximity assay (Amersham Biosciences Inc., Piscataway, NJ) as described previously (Sawyer et al., 2002). In brief, cells were in Hanks' balanced salt solution containing 25 mM HEPES, pH 7.4. The assay was performed in 0.2 ml of Hanks' balanced salt solution/HEPES containing 5 μM forskolin (Sigma-Aldrich, St. Louis, MO), 100 μM Ro 20-1724 (BIOMOL Research Laboratories) and 2 μl of test compound. The reaction was initiated by the addition of 100,000 cells and left to proceed for 10 min at 37°C. The reaction was stopped by a 3-min incubation in a boiling water bath. The samples were centrifuged for 10 min at 500g and the cAMP content in the supernatant was determined using a ¹²⁵I-cAMP scintillation proximity assay (Amersham Biosciences Inc.). All compounds were prepared in DMSO kept constant at 1% (v/v) of the final incubation volume.

Eosinophil Purification. Circulating eosinophils were isolated from heparinized venous blood from healthy volunteers as described previously (Gervais et al., 2001). In brief, erythrocytes were removed by addition of Dextran-T500 (Amersham Biosciences), and mononuclear cells were removed by means of centrifugation over Ficoll-Paque (Amersham Biosciences). Remaining erythrocytes were lysed by brief incubation in water, and the eosinophils were isolated from the granulocyte fraction by negative depletion using immunomagnetic beads directed against CD16 (Miltenyi Biotec Inc., Auburn, CA). The purity of the eosinophil fraction was evaluated by flow cytometry on a CELL-DYN 3700 system (Abbott Diagnostics, Abbott Park, IL) on the basis of size, granularity, and lobularity. In general, the populations were composed of more than 90 to 95% eosinophils with 5 to 10% contaminating neutrophils and lymphocytes.

Immunofluorescence Microscopy. Purified eosinophils were resuspended in RPMI 1640 medium supplemented with 0.5% (v/v) FBS (RPMI-FBS). Poly-D-lysine-coated culture slides (BD Biosciences, San Jose, CA) were seeded at 150,000 cells/well and incubated with test compounds for 20 min at 37°C in a humidified atmosphere (6% CO₂). Cells were washed with phosphate-buffered saline and fixed with ice-cold 70% ethanol for 30 min. The purified eosinophils were stained with anti-actin antibody (Sigma-Aldrich), washed with phosphate-buffered saline, and stained with Alexa-594 goat anti-rabbit antibody (Molecular Probes, Eugene, OR) before visualization on an Axioplan2 fluorescence microscope (Carl Zeiss, Thornwood, NY).

Eosinophil Chemotaxis. Purified eosinophils were resuspended at 3.0 × 10⁶ cells/ml of RPMI-FBS, and 0.1 ml was deposited in the top half of a Transwell chamber (6.5-mm Transwell, 3.0-μm polycarbonate membrane; Costar, Cambridge, MA). Test compounds (100 nM DK-PGD₂ or 100 nM L-888,607) or DMSO vehicle was added to 0.6 ml of RPMI-FBS to the bottom chamber to a final vehicle concentration of 0.1% (v/v). After 30 min in a CO₂ chamber, the upper chamber was removed and the eosinophils that had migrated to the lower chamber were photographed with a 35-mm SLR camera (Contax, Reading, UK) mounted on an Axiovert25 microscope (Carl Zeiss). Individual cells were counted and the mean of two chambers was determined for each test condition. Chemotaxis efficiency is expressed as the number of transmigrating cells with the agent, divided by the number of transmigrating cells in presence of vehicle only (fold-increase over background).

Pharmacokinetic Profile of L-888,607. Male (ICR)BR mice with an average weight of 42 g were obtained from Charles River Breeding Laboratories (Portage, MI). All procedures were approved by the Animal Care Committee at Merck Frosst Canada.

A single dose [5 mg/kg in 60% (v/v) polyethylene glycol 200] of L-888607 was given intravenously via the saphenous vein or a single dose (20 mg/kg in 60% (v/v) polyethylene glycol 400) was given orally by gavage. No obvious side effects were observed (*n* of four in each case).

Blood (10 μ l) was taken from the jugular vein at each time point indicated and added to 30 μ l of 0.1 M aqueous trisodium citrate. The mixture was kept at -20°C until analysis. To the mixture was added 60 μ l of acetonitrile, and the samples were mixed for 20 s before centrifugation at 9000 rpm for 20 min. The supernatant was removed and analyzed by liquid chromatography/mass spectrometry on an APCI 2000 instrument equipped with a Luna $50 \times 2\text{-mm}$ column $5 \mu\text{m}$ and using a 10 to 90% gradient of $\text{CH}_3\text{CN}/20\text{MM}$ NH_4OAc . The chromatography/mass spectrometry analysis was done by selective ion monitoring in negative mode.

Results

Identification of a Synthetic Human CRTH2 Receptor Ligand. L-883,595 a racemic compound with some affinity for the human DP receptor ($K_i = 211 \text{ nM}$) was identified in a screen to have a higher affinity for the human CRTH2 ($K_i = 4 \text{ nM}$) (see structure in Fig. 1). The affinity values were determined by equilibrium competition analysis using [^3H]PGD₂ and cell membranes expressing recombinant human DP or CRTH2 receptors. Separation of the two enantiomers yielded L-888,291 with affinities (K_i) for DP and CRTH2 of 40 and 48 nM, respectively, and L-888,607 with affinities of 2331 and 0.8 nM, respectively.

Other analogs of L-883,595 shown in Fig. 1 were tested for their affinity for the human CRTH2 receptor. Results obtained from these racemic mixtures provided useful information about the structure-activity relationship around the L-883,595 structure. The first evidence was related to the carbocycle fused with the indole ring at positions 1 and 2 (see the annotated indole structure at the bottom of Fig. 1). The cyclopentyl analog compound A exhibited 3-fold better affinity for the human CRTH2 receptor ($K_i = 80 \text{ nM}$) than the cyclohexyl analog compound B ($K_i = 269 \text{ nM}$). The sulfur atom linking the 4-Cl phenyl and the indole groups on the L-883,595 scaffold seemed to be also very important for potency. An example is the replacement of the sulfur atom of

compound C ($K_i = 6 \text{ nM}$) by a carbon atom as for compound B, which led to a compound with an affinity for the human CRTH2 receptor decreased by 45-fold ($K_i = 269 \text{ nM}$).

Substitution at position 4 of the indole ring did not yield increased affinity compared with the hydrogen atom, which conferred maximal affinity for CRTH2 (L-883,595, $K_i = 3 \text{ nM}$). Examples are the substitution by a bromine atom (compound D, $K_i = 21 \text{ nM}$) or by a small alkyl group such as ethyl (compound E, $K_i = 12 \text{ nM}$) or cyclopropyl (compound F, $K_i = 20 \text{ nM}$). These substitutions resulted in compounds with 4- to 7-fold lower affinity for the human CRTH2 receptor. The loss of affinity was amplified with larger substituents such as the cyclopentyl group (compound G, $K_i = 94 \text{ nM}$). Possibilities of substitution were also limited at position 6 of the indole ring. Replacement of the fluorine atom of L-883,595 at this position by larger atoms or groups such as a nitrile group in compound H led to a less potent compound ($K_i = 104 \text{ nM}$) compared with L-883,595.

L-888,607 Is a Highly Selective Ligand for CRTH2. The affinity of L-888,607 for all the other recombinant human prostanoid receptors was evaluated by equilibrium competition analysis (Table 1). This analysis revealed that L-888,607 displays a relatively high selectivity for CRTH2 with an affinity 363-fold lower for the TP receptor and more than 1000-fold lower for all the other prostanoid receptors. The rank order of affinity is CRTH2 \gg TP $>$ EP3 $>$ DP $>$ EP4 $>$ EP2 $>$ FP $>$ IP $>$ EP1. No significant binding was observed at a concentration up to 10 μM on various chemokine receptors (CCR1, CCR2, CCR4, CCR5, CXCR1, CXCR2, and CXCR3) on the anaphylatoxin receptors (C3aR and C5aR) and on the cyclooxygenases-1 and -2 (data not shown).

L-888,607 Is a Potent Agonist at the Human Recombinant CRTH2 Receptor. In a previous study, we reported that recombinant human CRTH2 is coupled to adenylate cyclase via the pertussis toxin-sensitive inhibitory trimeric

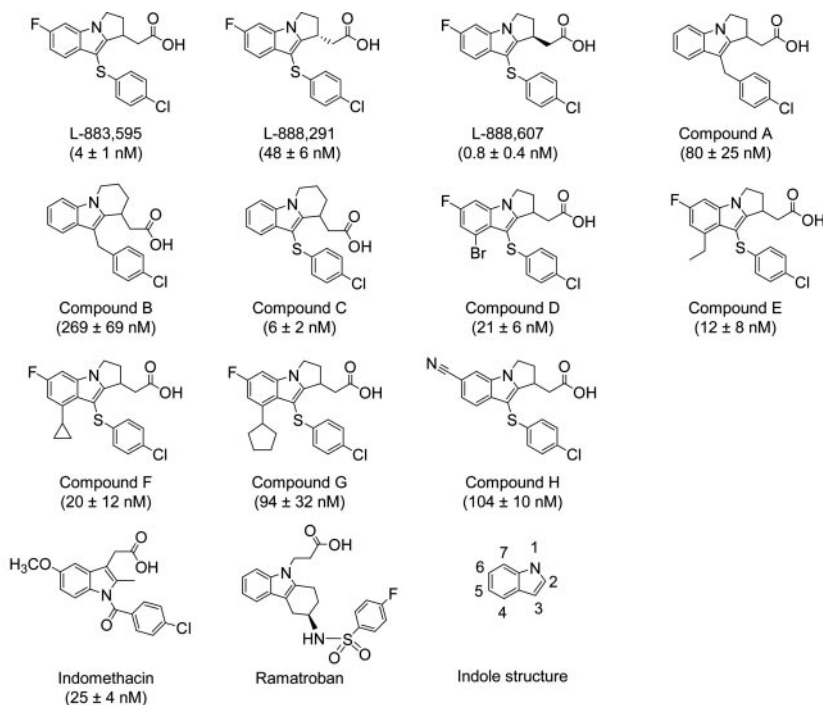


Fig. 1. Chemical structure of indomethacin, ramatroban, the racemic compound L-883,595 and its two enantiomers L-888,607 and L-888,291 as well as racemic analogs of L-883,595.

* values in parenthesis = K_i value at CRTH2 \pm standard deviation; $n \geq 3$

TABLE 1

Affinities of L-888,607, PGD₂, and DK-PGD₂ for the recombinant human prostanoid receptorsRadioligand binding assays were conducted as described under *Materials and Methods*. K_i values were determined as described previously (Sawyer et al., 2002). The results are expressed as mean \pm S.D. with the number of determinations in parentheses.

CRTH2	DP	TP	EP ₁	EP ₂	EP _{3,III}	EP ₄	FP	IP
L-888,607	0.8 \pm 0.4 (5)	283 \pm 52 (7)	>28,671 (7)	8748 \pm 2571 (5)	1260 \pm 211 (5)	4634 \pm 1903 (5)	10,018 \pm 2907 (5)	14,434 \pm 5405 (3)
PGD ₂	1.7 \pm 0.8 (30)	2024 \pm 1091 (19)	5302 \pm 2768 (7)	2390 \pm 1175 (4)	421 \pm 60 (3)	1116 \pm 782 (4)	11.5 \pm 6.5 (4)	>100,000 (8)
DK-PGD ₂	2.5 \pm 0.9 (15)	30,882 \pm 2747 (3)	>95,150 (2)	9059.5 (2)	1268 (2)	3915 (2)	3915 (2)	72,000 (2)
Indomethacin	25.0 \pm 3.6 (4) ^a	10,539 \pm 4329 (3) ^a						

^a Data from Sawyer et al. (2002).

G_{αi/o} protein in HEK293 cells. Activation of CRTH2 on these cells with PGD₂ or its metabolite DK-PGD₂ can reduce forskolin-stimulated cAMP accumulation with a measured potency (EC₅₀) of 1.6 \pm 0.3 and 4.9 \pm 1.1 nM, respectively (Sawyer et al., 2002). Substitution of PGD₂ for L-888,607 in this functional assay revealed that this synthetic compound is a full agonist of the hCRTH2 by inhibiting cAMP production with an EC₅₀ of 0.5 \pm 0.3 nM (Fig. 2). BW245C, a selective DP agonist, did not inhibit cAMP production in this assay at concentrations up to 1 μ M (data not shown).

L-888,607 Triggers Eosinophil Morphological Changes. To validate L-888,607 as an agonist on endogenous levels of CRTH2, we evaluated its effects on isolated human eosinophils. We have previously shown that eosinophils undergo rapid morphological changes when incubated with CRTH2-receptor agonists such as PGD₂ and DK-PGD₂ (Gervais et al., 2001). Using antibodies directed against actin to better visualize morphological changes of the cells we demonstrated that like PGD₂ and DK-PGD₂, L-888,607 is capable of inducing a morphological response in freshly isolated and purified human eosinophils (Fig. 3).

L-888,607 Stimulates Eosinophil Chemotaxis. To further confirm that L-888,607 is an agonist on endogenously expressed CRTH2, we evaluated its capacity to stimulate human eosinophil chemotaxis. It was previously reported that PGD₂ and DK-PGD₂ can optimally stimulate eosinophil chemotaxis at a concentration \geq 100 nM (Hirai et al., 2001; Monneret et al., 2001). We thus placed freshly isolated and purified human eosinophils in the upper compartment of a Transwell chamber and added either DK-PGD₂ or L-888,607 at 100 nM to the bottom chamber. DK-PGD₂ and L-888,607 significantly stimulated the migration of eosinophils to the bottom chamber compared with the vehicle control DMSO (Table 2). The concentration of 100 nM was determined to be optimal in this assay for both DK-PGD₂ and L-888,607 (data not shown). This result thus further confirms the agonistic nature of L-888,607.

Pharmacokinetic Profile of L-888,607 in Mice. L-888,607 was administered to mice either intravenously (i.v.) or orally (p.o.) to determine its pharmacokinetic profile over an 8-h period (Fig. 4). After an intravenous administra-

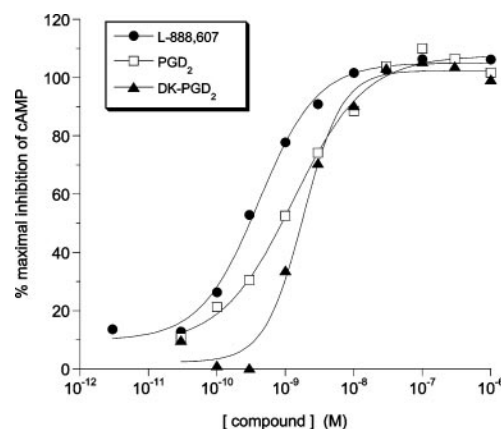


Fig. 2. L-888,607 is a full agonist at the recombinant hCRTH2. L-888,607, PGD₂, and DK-PGD₂ dose-response curve of the inhibition of forskolin-stimulated [i]cAMP levels in HEK293 cells stably expressing the hCRTH2. The potency (EC₅₀) of L-888,607, PGD₂, and DK-PGD₂ in the experiment shown was 0.4, 1.3, and 1.8 nM, respectively. The curves shown are representative of at least four independent experiments where each data point was done in duplicate.

tion of 5 mg/kg compound, blood analysis revealed a peak level (C_{\max}) of 36.1 μM , a half-life ($t_{1/2}$) of 2.9 h, a trough level at 8 h (C_{8h}) of 3.5 μM , and an area under the curve_{0–8h} of 87.1 μM . After oral administration of 20 mg/kg compound, blood analysis revealed a C_{\max} of 31.6 μM , a $t_{1/2}$ of 4 h, a C_{8h} at 8 h of 15.4 μM , an area under the curve_{0–8h} of 166 μM , and a bioavailability of 48%.

Discussion

In this study, we describe the first synthetic compound to selectively bind and activate the PGD_2 receptor CRTH2. We showed that L-888,607 exhibits 1) subnanomolar affinity for the human CRTH2 receptor, 2) high selectivity over all other prostanoid receptors and some other chemokine and anaphylatoxin receptors, 3) agonistic activity on recombinant and endogenously expressed CRTH2 receptor, and 4) relative stability in vivo. L-888,607 thus represents a suitable tool to investigate the in vivo function of CRTH2.

The fact that the synthetic ligands L-888,607 and BW245C can selectively bind to CRTH2 and DP, respectively, indicates that although they share the same natural ligand (PGD_2), their binding pockets are sufficiently different to enable the design of selective ligands. This is consistent with the fact that CRTH2 shares less sequence similarity to DP and other prostanoid receptors than to receptors for leukotrienes and anaphylatoxins.

Another synthetic compound, indomethacin, has previously been reported to be a potent CRTH2 agonist (Hirai et al., 2002). However, the use of indomethacin to study the endogenous function of CRTH2 would be complicated by its well established anti-inflammatory properties through cyclooxygenases inhibition (Barnett et al., 1994). It is noteworthy

that we have demonstrated that L-888,607 does not inhibit cyclooxygenases at a concentration up to 10 μM (data not shown). Nevertheless, comparing the chemical structure of L-888,607 with indomethacin (Fig. 1) reveals interesting chemical properties of synthetic agonists to CRTH2. Both compounds are indole core molecules having an acetic acid side chain. In addition, both the benzoyl group (from indomethacin) and the phenyl sulfide group (from L-888,607) are *para*-substituted by a chlorine atom. Of particular interest is the relative position of the acetic acid side chain compared with the benzoyl group on indomethacin, which occupies the same arrangement in space as the acetic acid side chain and the phenyl sulfide group on L-888,607. This latter similarity could explain, in part, a shared affinity of both compounds for the CRTH2 receptor. It is interesting that the CRTH2 antagonist ramatroban is also an indole core molecule that bears an aliphatic carboxylic acid side chain (Fig. 1; Sugimoto et al., 2003). In addition, the relative position of the acetic acid side chain could be considered as similar to the side chain position of L-888,607 and indomethacin.

When comparing the L-888,291 and L-888,607 stereoisomers, the stereochemistry at the chiral center bearing the acetic acid group seems to play a central role in determining selectivity toward the CRTH2 and DP receptors. The presence of a carboxylic acid is necessary for potency in both cases. Based on L-888,607's affinity for the CRTH2 receptor, it seems that an *S*-configuration at the chiral center allows the compound to acquire a better conformation to fit the CRTH2 receptor rather than the *R* configuration, contrary to the DP receptor, which prefers the *R*-configuration.

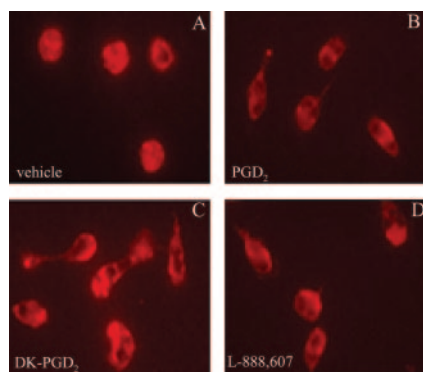


Fig. 3. L-888,607 induces changes in eosinophil morphology. Purified human eosinophils were incubated with vehicle (A) or 100 nM CRTH2 agonists PGD_2 (B), DK- PGD_2 (C), and L-888,607 (D). Cells are stained with an anti-actin antibody coupled to an Alexa-594 fluorescent antibody and visualized using a fluorescence microscope. A representative experiment from three donors tested is shown. Original magnification, 200 \times .

TABLE 2

Chemotactic potential of DK- PGD_2 and L-888,607 on human eosinophils

	Donor 1		Donor 2		Avg. of 6 Donors
	No. Migrating Cells	Fold-Increase vs. DMSO Control	No. Migrating Cells	Fold-Increase vs. DMSO Control	Fold-Increase vs. DMSO Control
DMSO	890		1168		
DK- PGD_2^a	5676	6.4	5371	4.6	5.1 ± 0.7^b
L-888,607 ^a	4274	4.8	5969	5.1	4.0 ± 0.4^b

^a Final concentration of L-888,607 and DK- PGD_2 was 100 nM.

^b Standard deviation.

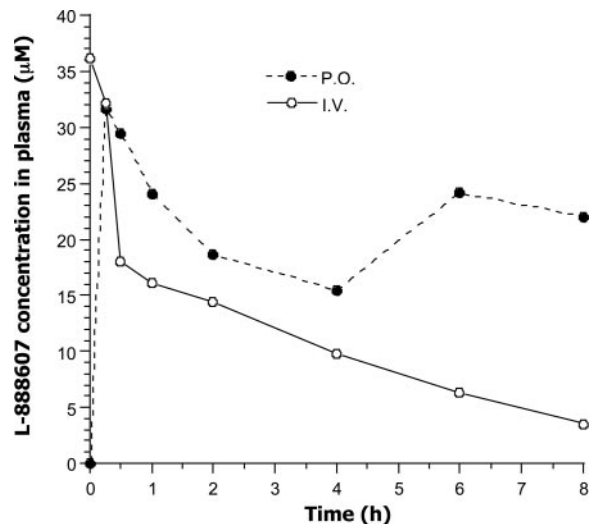


Fig. 4. Pharmacokinetic profile of L-888,607 in mice receiving 5 mg/kg intravenously or 20 mg/kg orally. Each point corresponds to the average obtained from four mice.

Structure-activity relationship based on close analogs of L-888,607 shows the superiority of compounds with a five-membered ring carbocycle fused with the indole ring over the six-membered ring analogs. The different orientations provided to the carboxylic acid group by these two class of compounds might explain their different ability to bind with the CRTH2 receptor. The distance between the 4-Cl phenyl group and the indole ring seems to be important. Replacement of the sulfur atom by a smaller spacer as a carbon atom results in significantly less potent compounds. We also showed that positions 4 and 6 on the indole ring are sensitive to steric effects. Steric hindrance induced by large atom or alkyl groups at these positions results in compounds with weaker affinity for the CRTH2 receptor.

We have shown that the pharmacokinetic parameters of L-888,607 are suitable for in vivo investigations. In mice, exposure levels of L-888,607 when administered once intravenously at 5 mg/kg or orally at 20 mg/kg are well above its affinity at the murine CRTH2 receptor ($IC_{50} = 18.8 \pm 1.7$ nM) for a period of at least 8 h.

In conclusion, we identified L-888,607, a potent, selective, and stable CRTH2 agonist that will prove useful in identifying the role of CRTH2 in vivo, and together with the selective DP agonist BW245C can be used to distinguish whether some biological activities associated with PGD_2 are mediated through CRTH2 or DP.

Acknowledgments

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