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Synthesis and in vitro evaluation of a selective antagonist and the corresponding radioligand for the prostaglandin D_2 receptor CRTH2

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Abstract—Synthesis and preliminary in vitro biological evaluation of a selective high-affinity CRTH2 antagonist is described. The stability of an *N*-benzyl group facilitated synthesis of the corresponding radioligand by tritiation of a brominated precursor. The compound [^{3}H]TRQ11238 represents the first selective CRTH2 antagonist radioligand and exhibited a specific radioactivity of 52 Ci/mmol and a pK_d of 9.0.

It is well established that the arachidonic acid metabolite prostaglandin D_2 (PGD_2) plays a central role as a messenger molecule in inflammatory responses of allergy and asthma. PGD_2 is released in considerable amounts by IgE-activated mast cells upon allergen exposure, and one of its effects is to recruit immune cells to the site of inflammation, which in turn produce the inflammatory response. Two G protein-coupled 7-transmembrane receptors, DP_1 and CRTH2 (or DP_2), have been identified for PGD_2, and current evidence suggests that both receptors play pivotal roles in mediating the effects of PGD_2 in allergic inflammation.[1–3] The more recently identified receptor CRTH2 is responsible for the chemoattractant effect of PGD_2 on eosinophils, basophils, and Th2-cells, supporting the view that this receptor provides the essential link between PGD_2 and various aspects of initiation and perpetuation of allergic inflammation.[4,5] Findings that mutations in the CRTH2 gene segregated with asthma reinforced the conjecture that the receptor might be an excellent drug target.[6] The dual TP/CRTH2 antagonist ramatroban was indeed observed to abrogate inflammatory response in a murine model of contact hypersensitivity, an effect primarily ascribed to inhibition of CRTH2.[7] Later, the selective CRTH2 agonist DK-PGD_2 was found to increase pathology in both allergic asthma and atopic dermatitis models,[8] while two different selective CRTH2 antagonists relieved peribronchial eosinophilia and goblet cell hyperplasia in sensitized mice.[9,10]

In parallel with the growing evidence suggesting a dominant proinflammatory role of CRTH2, the receptor has attracted increasing interest as a drug discovery target for new anti-allergy and anti-asthma therapeutics, and several series of CRTH2 antagonists have recently appeared in the literature.[9,11–13] Many of these originate from the two first non-prostanoid CRTH2 ligands identified, the indole carboxylic acids indomethacin and ramatroban (Fig. 1). Indomethacin is unusual in being a potent CRTH2 agonist, but inhibiting binding of [^{3}H]PGD_2 only at much higher concentrations.[14] We have previously described ramatroban analogs exhibiting highly selective and potent CRTH2 antagonism.[15] In further studies, these closely related compounds were found to exhibit divergent pharmacology, in that the indole-*N*-acetic acid analogs TM30089 and TM30463 behaved as insurmountable antagonists, whereas the longer propionic acid analogs ramatroban and

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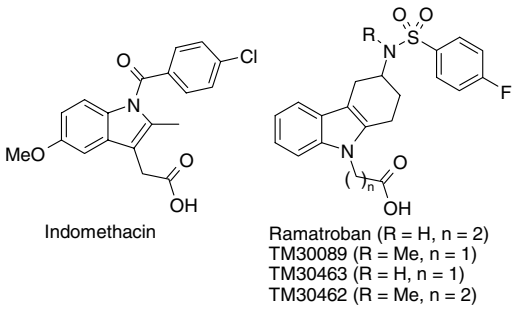
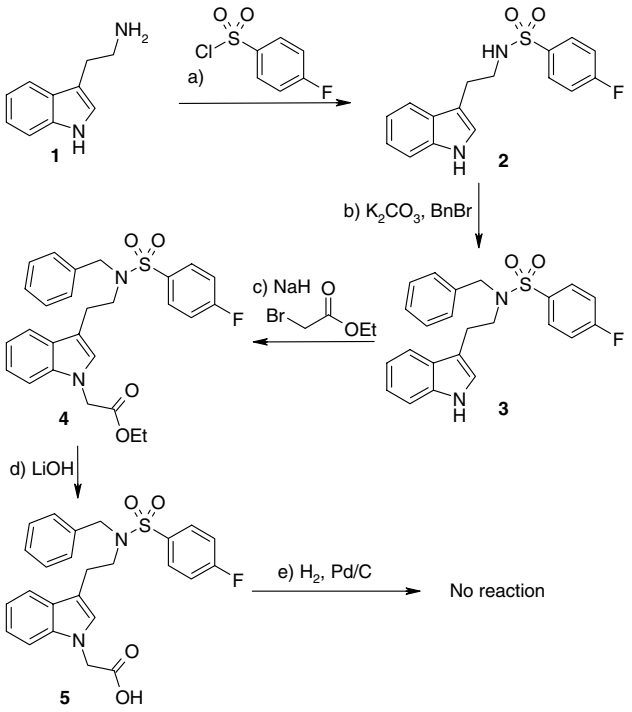


Figure 1.

TM30462 displayed classical competitive antagonism (Fig. 1).[16] Furthermore, we recently identified analogs of indomethacin and ramatroban with divergent effects on binding and function of PGD_2: in radioligand binding assays using [^{3}H]PGD_2 as the tracer, both compounds increased [^{3}H]PGD_2 binding reminiscent of allosteric enhancers. However functional assays revealed both compounds to exclusively inhibit PGD_2-mediated translocation of β-arrestin without interfering with G protein-dependent signaling pathways of the receptor.[17] The molecular mechanisms underlying these observations have not been dissected yet, but a selective antagonist radioligand, which does not discriminate between discrete signaling-competent conformations of CRTH2, could help clarify some of these questions. Although tritiated ramatroban has been described, lacking selectivity and a lower affinity than many of the currently known selective CRTH2 ligands deter somewhat from its usefulness.[18] At present, [^{3}H]PGD_2 is the only CRTH2 radioligand available, thus, providing complementary tools is currently among our prioritized goals.

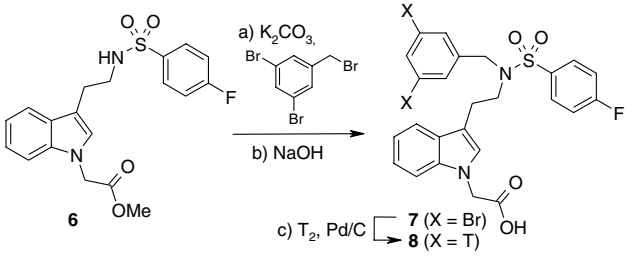
The analogous structural features of indomethacin and ramatroban suggest that they might share binding epitopes despite divergent functional activity, and the structural class obtained by opening the aliphatic ring of the ramatroban analogs may be viewed as bridging toward the structural class of indomethacin (Fig. 1). Being small and synthetically easily accessible, this simplified structural class invited closer investigation. We decided to enter this class by the way of **5**, which was readily synthesized from tryptamine by sulfonylation, followed by benzylation of the sulfonamide, alkylation of the indole nitrogen, and ester hydrolysis (Scheme 1). Compound **5**, which has previously been disclosed in a patent application by others,[19] proved to be a high affinity ($pK_i = 9.2 \pm 0.2$, $n = 8$) CRTH2 antagonist ($pIC_{50} = 8.4 \pm 0.3$, $n = 3$) in assays displacing [^{3}H]PGD_2 binding or inhibiting PGD_2-induced signaling at hCRTH2-transfected cells, respectively. Furthermore and notably, the compound exhibited excellent selectivity (>1000-fold) over relevant receptors such as DP and TP.[20]

Our original plan involved removal of the *N*-benzyl group by hydrogenolysis and subsequent construction of a library of analogs with diverse N-substituents. Complete stability of the substrate to the reaction conditions even after prolonged reaction times made us revert



Scheme 1. Reagents and conditions: (a) 4-$FC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, rt, 4 h, 96%; (b) K_2CO_3, BnBr, acetone, 50 °C, 86%; (c) NaH, $BrCH_2CO_2Et$, DMF, rt, 12 h, 98%; (d) LiOH, H_2O/THF (1:3), 12 h, 89%; (e) H_2 (1 atm), Pd/C, MeOH, rt, 1 week.

to other methods. Nonetheless, realizing the opportunity for easy access to a desired tool compound, we decided to exploit the chemical inertness of the benzyl group in construction of the first selective CRTH2 antagonist radioligand. Synthesis of precursor **8** by the route described in Scheme 1 unexpectedly failed as the 3,5-dibromobenzylated compound decomposed under the conditions used for N-alkylation of the indole. Therefore, the compound was instead synthesized taking advantage of the precursor **6**,[19] and the 3,5-dibromobenzyl moiety was introduced toward the end (Scheme 2). Precursor **7** was radiolabeled by hydrogenation over palladium on carbon under an atmosphere of tritium gas. Purification by reverse phase HPLC provided the compound [^{3}H]TRQ11238 (**8**), exhibiting a specific radioactivity of 52 Ci/mmol and a radiochemical purity of 97.9%.[21] Saturation binding experiments confirmed



Scheme 2. Reagents and conditions: (a) K_2CO_3, 3,5-dibromobenzyl bromide, acetone, 50 °C, 12 h, 80%; (b) NaOH, H_2O/THF/MeOH (1:6:6), rt, 3 h, 98%; (c) T_2, Pd/C, MeOH, rt, 2 h, 89% (yield from test reaction using H_2).

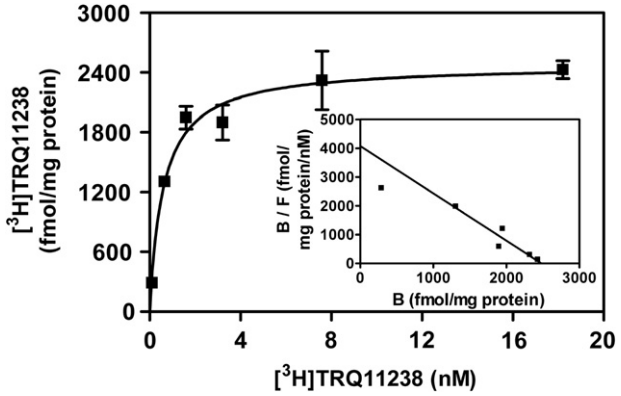


Figure 2. Representative saturation analysis and Scatchard plot (inset) of [^{3}H]TRQ11238 (**8**) binding to cell-membrane preparations of HEK293 cells expressing the human CRTH2 receptor. Data were fitted best to a one-site binding model, and K_d and B_{max} values were determined. All data points are shown as mean values ± SE of one individual experiment representative of four such experiments.

high affinity of **8** for CRTH2, with binding parameters of pK_d = 9.0 ± 0.1 and B_{max} = 2790 ± 360 fmol/mg protein as determined in membranes from stable hCRTH2 transfectants (Fig. 2).[22] No binding was observed in non-transfected cells.

In conclusion, we have described an expedient synthesis of the indole-*N*-acetic acid TRQ11238 (**5**), a high-affinity CRTH2 antagonist with appreciable selectivity over relevant receptors. Furthermore, a route for tritiation of the compound was developed, resulting in the first selective antagonist CRTH2 radioligand. The radioligand [^{3}H]TRQ11238 (**8**) exhibited a pK_d of 9.0 on the human CRTH2 receptor, and we expect this new tool to be of value in the continued discovery and characterization of CRTH2 ligands as well as in studies of the receptor.

Acknowledgment

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20. Affinity and functional activity of the antagonist were determined essentially as described in Refs. 15,16. In brief, antagonist affinity was derived from competition binding analysis, using [^{3}H]PGD_2 as the tracer. Antagonist activity was determined in inositolphosphate-assays (CRTH2 and TP receptors) or in cyclic AMP accumulation assays (DP receptor).
21. Custom synthesis by Amersham Biosciences.
22. For saturation binding experiments, CRTH2-HEK293 cell membranes (15 μg of protein) were incubated with 0.7–70 nM [^{3}H]TRQ11238 (52 Ci/mmol; Amersham Biosciences) in a binding buffer consisting of HBSS (Invitrogen) and 100 mM Hepes (pH 7.4) under continuous gentle shaking at 4 °C for 3 h. The exact concentration of [^{3}H]TRQ11238 used was determined from experiment to experiment. Non-specific binding was defined in the presence of 10 μM TM30089. The receptor bound radioligand was filtered on a Tomtech 96-well Mach III Harvester (Perkin-Elmer Life and Analytical Sciences Wallac, Turku, Finland) using filters presoaked with 0.1% polyethylenimine (Filtermat A; Perkin-Elmer LAS Wallac). Filtration was immediately followed by three rinses with ice-cold 100 mM NaCl. Thereafter, scintillation wax (Meltilex A; Perkin-Elmer LAS Wallac) was melted onto the dried filtermat. The filters were placed in sample bags (Perkin-Elmer LAS Wallac), and filter-bound radioactivity was measured using a Microbeta Trilux-1450 scintillation counter (Perkin-Elmer LAS Wallac). Determinations were made in triplicate in

four-independent experiments. Analysis was performed using Prism 4.02 (GraphPad Software Inc., San Diego, CA). Data sets of saturation binding isotherms were analyzed via non-linear regression applying a hyperbolic, one-site binding model, and individual estimates for total receptor number (B_{max}) and the equilibrium radioligand dissociation constant (K_d) of the radioligand were calculated.