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Novel tricyclic antagonists of the prostaglandin D_2 receptor DP2 with efficacy in a murine model of allergic rhinitis

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ABSTRACT

The synthesis of a series of tricyclic antagonists for the prostaglandin D_2 receptor DP2 (CRTH2) is disclosed. The activities of the compounds were evaluated in a human DP2 binding assay and a human whole blood eosinophil shape change assay. Potential metabolic liabilities of the compounds were addressed through in vitro CYP studies. The lead compound was demonstrated to have efficacy in a mouse model of allergic rhinitis following oral dosing.

Eicosanoids are a group of fatty acid-derived lipids that are important mediators of a variety of physiological processes. They are produced in a biological cascade that begins with the release of arachidonic acid (AA) via phospholipase A_2 from diacylglycerol. Processing of AA via the lipoxygenase pathway results in the production of leukotrienes or alternatively via the cyclooxygenase pathway to produce prostanoids including prostaglandin D_2 (PGD_2).[1] PGD_2 is the major prostanoid released from activated mast cells and recent evidence has demonstrated a role for PGD_2 in a variety of allergic and inflammatory diseases.[2] The biological actions of PGD_2 are mediated by the G protein-coupled receptors (GPCRs) termed DP1 and DP2 (also known as CRTH2). The DP2 receptor is expressed on cells including eosinophils, basophils and Th2 cells and is involved in the migration and activation of these cells. Specifically, the DP2 receptor may have an important role in inflammatory diseases including asthma, COPD, and allergic rhinitis.[3] While there are numerous treatment options for asthma, many of these treat symptoms rather than the underlying inflammatory causes of the disease, creating an unmet medical need.[4]

The emerging role of DP2 in inflammatory diseases has led to significant research aimed at identifying selective DP2 antagonists. A recent review details the structural diversity surrounding characterized DP2 antagonists.[5] Recent disclosures in the patent literature show that a number of companies have active research programs aimed at designing novel DP2 antagonists.[6] While numerous companies are reported to be in clinical development with DP2 antagonists, two companies (Oxagen[7] and Actimis[8]) have published single compound patents and from these we can surmise that compounds **1** and **2** are development candidates (Fig. 1).

Research into DP2 antagonists increased after it was discovered that the known thromboxane receptor (TP) antagonist ramatroban (**3**, sold in Japan as Baynas™ for allergic rhinitis) was also a DP2 antagonist.[9] The tricyclic ramatroban core served as a starting point for the design of many other DP2 antagonists. Researchers at 7TM Pharma found that replacement of the *N*-propionic acid group of ramatroban with an *N*-acetic acid group and N-methylation of the sulfonamide (**4**, TM30089) depleted the affinity for the thromboxane receptor while significantly increasing DP2 binding affinity.[10] Researchers at Athersys have published on isosteric ramatroban analogs as well.[11] Similarly, the tricyclic indole C-linked acetic acid or 'reverse indole' analog of ramatroban (compound **5**) has been disclosed in a recent patent application by Merck.[12]

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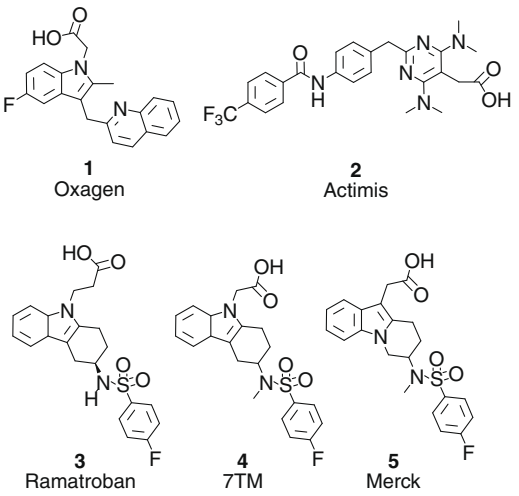


Figure 1. Literature DP2 antagonists.

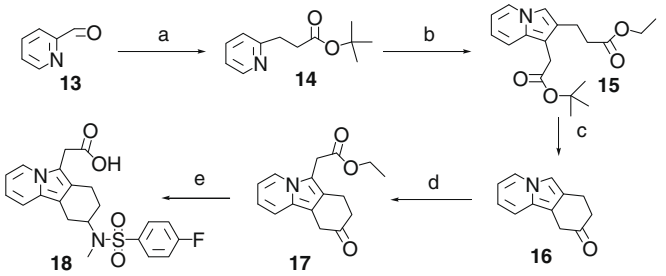
In this Letter we describe the discovery and evaluation of a series of small molecule DP2 antagonists. The effect of replacing the embedded indole nucleus of the tricyclic core with novel N-containing ring systems is detailed and the results are compared to those obtained for the literature compounds **1**–**5**. While these indole-based DP2 antagonists typically display excellent in vitro potency and in vivo pharmacokinetic profiles, 3-alkyl indoles have been shown to have some undesirable metabolic properties. In particular, they have been implicated in the formation of reactive metabolites.[13] Furthermore, researchers at Merck have demonstrated the propensity for structurally related 3-alkylindole based DP1 antagonists to undergo bioactivation and form reactive metabolites.[14] These undesirable effects were mitigated through the installation of electron withdrawing groups on the indole core. Herein, we describe our efforts to prepare novel DP2 antagonists by modulating the reactivity of the indole core within the ramatroban tricyclic series through the use of azaindole, pyrrolopyrazine, and indolizine core replacements.

We first investigated the tricyclic indolizine core as an indole replacement (Scheme 1).[15] The indolizine core was constructed by Tschitschibabin cyclization between 2-picoline (**6**) and bromoketone (**7**) to provide indolizine ester **8**. Reaction with ethyl diazoacetate and copper provided C-3 C–H insertion product **9** together with minor amount of the C-3 regioisomer and corresponding triester. Dieckmann condensation and decarboxylation of the resulting β-ketoester provided ketone **10**, which was converted to **11** through reduction and azide installation, followed by hydrogenation, sulfonylation, and N-methylation. Alkylation of C1 of the indolizine core with ethyl bromoacetate followed by ester hydrolysis provided **12** in which the fused 6-membered ring is attached to C-2 and C-3 of the indolizine nucleus.

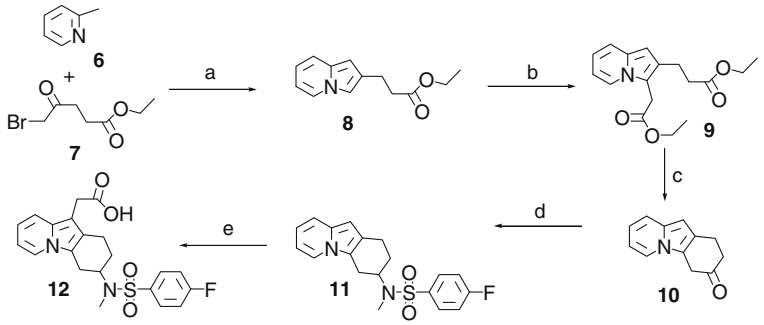
Synthesis of the regioisomeric tricyclic indolizine **18** followed a similar synthetic strategy, starting with conversion of pyridine-2-carboxaldehyde to ester **14** by a Horner–Wadsworth–Emmons reaction followed by a catalytic hydrogenation (Scheme 2).

Following the chemistry outlined in Scheme 1, Tschitschibabin cyclization afforded diester **15**. Dieckmann condensation followed by decarboxylation under acidic conditions provided ketone **16**. Efforts to install the sulfonamide from compound **16** via the reduction/mesylation/azide process in Scheme 1 were unsuccessful due to competing mesylation of the indolizine C3 carbon. Reductive amination under various conditions was also unsuccessful. Installation of the acetic acid group at C3 of the indolizine ring by the action of ethyl bromoacetate and base provided **17**. Sulfonamide installation at this stage proceeded smoothly as in Scheme 1 to provide **18** after ester hydrolysis.

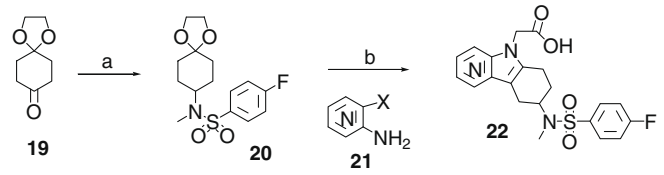
A concise synthetic route was utilized in the preparation of azaindoles **22** (Scheme 3). Ketone **19** was transformed to sulfonamide **20** using standard methods. An intramolecular Heck reaction on the imine generated in situ from condensation of ketal **20** with a 2-haloamino heterocycle **21** provided the corresponding azaindole.[16] N-Alkylation of the crude Heck product followed by HPLC purification of the ester and subsequent ester hydrolysis provided the azaindole *N*-acetic acids **22**. While the yield in the Heck coupling was low, it was acceptable considering the convergence of the synthetic route. Table 1 illustrates examples of azaindoles accessed utilizing this route.



Scheme 2. Synthesis of the C1–C2 fused tricyclic indolizine. Reagents and conditions: (a) *tert*-butyl *P,P*-diethyl phosphonoacetate, THF, NaH; Pd/C, ammonium formate, EtOAc/H_2O, 99%; (b) 1,4-dioxane, $NaHCO_3$, **7**, sealed tube, 140 °C, 40%; (c) KO*t*-Bu, THF, 0 °C, 53%; formic acid, 89%; (d) ethyl bromoacetate, $NaHCO_3$, 1,4-dioxane, 34%; (e) $NaBH_4$, EtOH; MsCl, Et_3N, CH_2Cl_2; NaN_3, DMF; H_2, Pd/C, EtOAc; 4-fluorobenzene sulfonyl chloride, CH_2Cl_2, Et_3N; NaH, MeI, DMF; KOTMS, THF (34% seven steps).

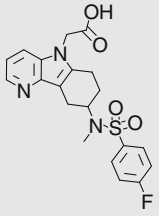
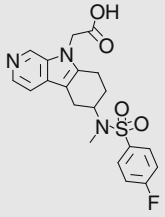
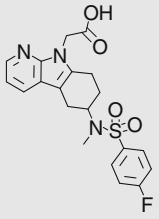
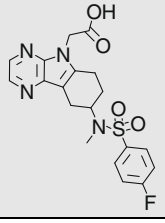


Scheme 1. Synthesis of the C2–C3 fused tricyclic indolizine. Reagents and conditions: (a) 1,4-dioxane, $NaHCO_3$, sealed tube, 140 °C, 73%; (b) ethyl diazoacetate, Cu, toluene, 110 °C, 30%; (c) KO*t*-Bu, THF, −10 °C then NaCl, DMSO, H_2O, 150 °C (66%); (d) $NaBH_4$, MeOH; MsCl, Et_3N, CH_2Cl_2, −40 °C; NaN_3, DMF, 80 °C; H_2 (60 psi), Pd/C, MeOH (82% four steps); 4-fluorobenzene sulfonyl chloride, Et_3N, CH_2Cl_2; NaH, MeI, DMF, 40% (e) ethyl bromoacetate, $NaHCO_3$, MeCN, sealed tube, 150 °C, 57%; NaOH, THF, MeOH, quant.



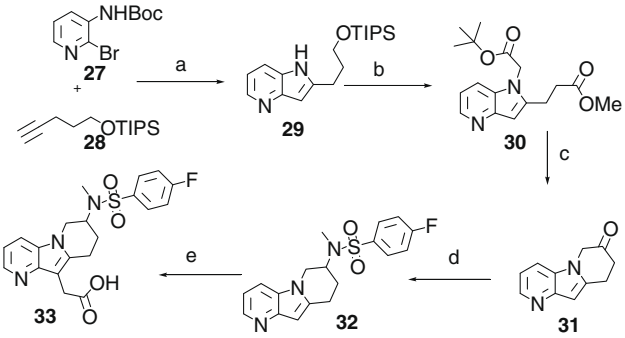
Scheme 3. Synthesis of azaindoles. Reagents and conditions: (a) benzyl amine, $NaBH(OAc)_3$, $MeOH/CH_2Cl_2$; $Pd(OH)_2$, MeOH, H_2 (93% two steps); 4-fluorobenzene sulfonyl chloride, Et_3N, CH_2Cl_2; NaH, MeI, DMF (40% two steps) (b) $Pd(PPh_3)_4$, PPTS, pyridine, 180 °C; LiHMDS, THF, *t*-butyl bromoacetate, 0 °C; NaOH, THF/MeOH (1–5% three steps).

Table 1
Azaindole-based DP2 antagonists

Starting material	Azaindole	Compound #
		**23**
		**24**
		**25**[17]
		**26**

Synthesis of the 'reverse' azaindole **33** is outlined in Scheme 4. Sonagashira coupling between bromopyridine **27** and alkyne **28** followed by cyclization with DBU afforded azaindole **29**.[18] N-Alkylation with *tert*-butyl bromoacetate followed by cleavage of the silyl protecting group and oxidation, afforded diester **30**. Dieckmann condensation and thermal decarboxylation provided ketone **31**. Installation of the amine through reductive amination and sulfonylation, followed by copper-catalyzed C–H insertion and hydrolysis provided reverse azaindole **33**.

Table 2 shows the in vitro activity of the compounds in a DP2 membrane binding assay using ^{3}H-PGD2 as the radioligand. The



Scheme 4. Synthesis of reverse azaindole **23**. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 83%; DBU, $MeOH/H_2O$, 74% (b) *tert*-butyl bromoacetate, NaH, DMF, TBAF, THF; PCC, MeOH/DMF (26% three steps) (c) KO*t*-Bu, THF, −10 °C to rt; silica gel, toluene, reflux, (57% two steps) (d) $MeNH_2$, $NaCNBH_3$, EtOH; 4-fluorobenzene sulfonyl chloride, Et_3N, CH_2Cl_2, 52% (e) ethyl diazoacetate, Cu, toluene, 110 °C; LiOH, THF, MeOH, 6%.

Table 2
Human DP2 binding with and without human serum albumin and human whole blood eosinophil shape change data for compounds **1–5**, **12**, **18**, **23–26**, and **33**

Compound	hDP2 binding[a] IC_{50} (nM)	hDP2 binding[a] + HSA IC_{50} (nM)	hESC[a] IC_{50} (nM)
1 (Oxagen)	6	44	261
2 (Actimis)	42	744	744
3 (Ramatroban)	311	754	1210
4 (7TM)	1	4	1
5 (Merck)	6	20	7
12	18	46	6
18	94[b]	61[b]	16
23	6	11	1
24	860	940	452
25	457	1337	3100
26	96	127	140
33	24,900	51,000	41,600

[a] Values are means of at least three experiments.
[b] Value from a single experiment. hESC = human whole blood eosinophil shape change assay.

DP2 binding assay was also carried out in the presence of 0.2% human serum albumin (HSA) to evaluate the protein shift associated with these compounds. Additionally, a whole blood eosinophil shape change assay (hESC) was developed. Incubation of purified eosinophils or blood with PGD_2 activates the DP2 receptor and its downstream intracellular signaling pathways. Activation of these intracellular signaling pathways results in eosinophil degranulation and changes in eosinophil morphology which can be analyzed by flow cytometry and is evident by a change in forward light scatter.[19] These assays demonstrate the intrinsic potency of the compounds as well as the potency under conditions which are more therapeutically relevant. Additionally, the hESC assay could serve as means of obtaining a pharmacodynamic readout during in vivo pharmacology studies or human clinical trials.

As the data in Table 2 shows, compounds **4** and **5** are potent in the DP2 binding assay, and show a low protein shift in the presence of HSA. Furthermore, these compounds were found to be quite potent in the hESC assay. Compound **1** was potent in the binding assay without and with HSA, but its potency was shifted in the hESC assay, possibly indicating a potential for lower in vivo efficacy. Compound **2** was intrinsically potent, but shifted to lower potency in the presence of HSA or human blood. Indolizine compounds **12** and **18** were potent in the binding assay and were even more potent in the functional hESC assay than their activity in the binding assay would predict. Of the tricyclic azaindole compounds

exemplified, compound **23** displayed the best overall profile with excellent potency in all of the in vitro assays. Significantly lower potency was observed in the other azaindole isomers **24** and **25** as well as with the pyrrolopyrazine **26** and 'reverse' azaindole compound **33**. It is important to note that with the exception of compounds **1**, **2**, and **3**, all of the compounds were prepared as racemic mixtures.

Because the molecules within the prostaglandin family are structurally similar, there is some degree of cross-reactivity of the ligands with the various prostanoid receptors. Not surprisingly, there is also a potential for the small molecule inhibitors to show cross-reactivity to the prostaglandin pathway receptors and enzymes. The set of compounds were counterscreened for their potential to bind to selected prostanoid receptors, including the other PGD_2 receptor DP1, the human thromboxane receptor (hTP) and the human prostacyclin receptor (hIP) (Table 3). Some of the compounds showed significant binding to the DP1 receptor (compounds **1**, **4**, **5**, **12**, and **25**) but considering the DP2 receptor affinity, were still highly selective for the DP2 receptor. However, the observed DP1 binding results could lead to pharmacologically significant DP1 inhibition depending on the drug concentrations achieved in vivo.[20] With the exception of ramatroban, the compounds were selective against the TP receptor and none showed significant activity against the IP receptor.

Table 3
DP1, TP, and IP counterscreen data for compounds **1**–**5**, **12**, **18**, **23**–**26**, and **33**

Compound	hDP1 binding[a] IC_{50} (μM)	hTP binding[a] IC_{50} (μM)	hIP binding[a] IC_{50} (μM)
1 (Oxagen)	7.3	>100	57.9
2 (Actimis)	25.6	62.6	>100
3 (Ramatroban)	33.4	0.014	>100
4 (7TM)	2.2	>100	>100
5 (Merck)	1.0	>100	>100
12	4.4	>100	>100
18	31[b]	nt	>100
23	>100	>100	>100
24	>100	>100	>100
25	9.0	>100	>100
26	37.8	>100	>100
33	41.7	>100	>100

[a] Values are means of three experiments (nt = not tested).
[b] Value from a single experiment. hDP1 binding performed using ^{3}H-PGD_2 and human platelet membranes. hTP binding performed using human platelet membranes and ^{3}H-SQ-29,548. hIP binding perfomed using hIP/293 membranes and ^{3}H-iloprost.

Table 4
Inhibition and Induction of human CYP isoforms for compounds **1**–**5**, **12**, **18**, **23**–**26**, and **33**

Compound	CYP3A4 inhibition[a] IC_{50} (μM)	CYP 2C9 inhibition[a] IC_{50} (μM)	CYP2D6 inhibition[a] IC_{50} (μM)	CYP3A4 induction[b] (% rifampicin)
1 (Oxagen)	>30	>30	>30	58.8%
2 (Actimis)	>30	>30	>30	0.4%
3 (Ramatroban)	>30	15	>30	2.9%
4 (7TM)	>30	18	>30	30.8%
5 (Merck)	7	5	>30	0.7%
12	>30	17	>30	0.9%
18	11	10	>30	nt
23	>30	>30	>30	2.8%
24	>30	>30	>30	0.4%
25	>30	>30	>30	2.8%
26	>30	15	>30	0.9%
33	>30	>30	>30	0.9%

[a] Values are means of two experiments (nt = not tested).
[b] Values are from a single experiment.

The cytochrome P450 (CYP) enzymes play a crucial role in the metabolism of drugs. Drugs which inhibit or induce CYP enzymes can result in drug/drug interactions or affect the therapeutic concentrations of the drug during the course of treatment.[21] It is critical to assess potential CYP liabilities of drug candidates early in the discovery process to minimize costly failures during clinical development. We analyzed the set of compounds for CYP inhibition[22] versus the common CYP isoforms and also for their potential for CYP3A4 induction (Table 4).[23] The CYP induction assay involves incubation of the test compounds at a concentration of 10 μM with cultured cryopreserved human hepatocytes for 2 days, then determining CYP3A4 activity of the cells using the specific substrate midazolam. Induction effects are compared to the positive control rifampicin. Typically compounds showing 40% or more of the rifampicin effect are considered to be significant CYP3A4 inducers. Significant levels of CYP3A4 induction versus rifampicin were detected in tricyclic indole **4** and indole **1**. It is unclear what structural aspects of these compounds give rise to the CYP3A4 induction observed. In general, these compounds showed little CYP inhibition, with the exception of reverse indole **5** and indolizine **18**, both of which showed modest CYP3A4 and CYP2D6 inhibition. Since compounds **4**, **5**, and **18** are racemates, the contribution of the individual enantiomers is unknown.

The formation of reactive metabolites has been implicated in the mechanism of idiosyncratic drug reactions.[24] We have utilized a qualitative assay that involves incubating compounds with human liver microsomes in the presence of β-NADPH and glutathione in an attempt to provide evidence for the potential to form reactive metabolites and to further assess the viability of our new DP2 antagonists as potential drug candidates.[25] The compounds in this paper were tested in this assay, and only compound **3** (ramatroban) produced an observable GSH adduct.

Compound **23** has a superior in vitro profile when compared to literature compounds that are likely under clinical development (compounds **1** and **2**). We therefore selected compound **23** for in vivo evaluation in a model of allergic disease. While there is a rationale for DP2 antagonists to be efficacious in a variety of allergic conditions, we chose to examine the activity of our novel DP2 antagonist in a mouse model of allergic rhinitis. Allergic rhinitis can be modeled in mice by first sensitizing animals to ovalbumin (OVA) by intraperitoneal injection and subsequently performing a nasal challenge with OVA.[26] The symptoms that develop in mice mimic those observed in human allergic rhinitis including nasal itch, sneezing and nasal congestion.

In this assay, female BALB/c mice were primed by intraperitoneal OVA on days 0 and 7 (10 μg in Alum). Then beginning on day 21, the mice were dosed orally with compound **23** (10 mg/kg, as the sodium salt) or vehicle (water, 10 ml/kg) once per day for 3 days. One hour after each dose, mice were challenged with OVA by intranasal administration (200 μg in saline). This procedure produced a progressive and significant increase in the number of sneezes and nasal rubs recorded in 8 min sessions immediately following the OVA challenge. Plasma drug concentrations from a satellite group receiving the same oral dose of Compound **23** revealed a C_{max} of 770 nM at 1 h and a C_{min} of 120 nM at 8 h. Compound **23** significantly rhinitis symptoms in the sensitized mice (Fig. 2).

In summary, we have synthesized a number of novel indole core replacements within the tricyclic series of DP2 antagonists. This work led to the discovery of the 4-azaindole core and compound **23**, containing a 4-azaindole core, as a potent, selective DP2 antagonist. Compound **23** showed no inhibition of the three major CYP isoforms and it did not induce CYP3A4 in cryopreserved human hepatocytes. Compound **23** was demonstrated to have efficacy in a mouse model of allergic rhinitis following oral dosing. This DP2 antagonist compares favorably with literature compounds which are likely under clinical development. Based on the excellent

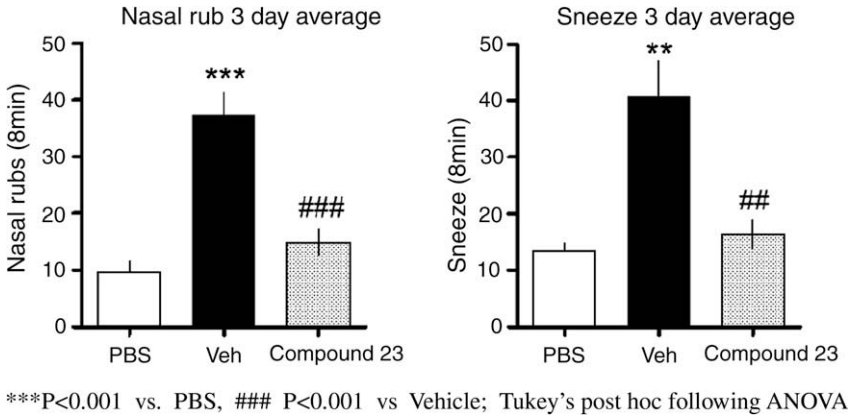


Figure 2. Mouse allergic rhinitis data for compound **23**.

in vitro and in vivo profile, compound **23** is a promising lead compound that warrants further pre-clinical development.

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