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Discovery and optimization of a biphenylacetic acid series of prostaglandin D_2 receptor DP2 antagonists with efficacy in a murine model of allergic rhinitis

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ABSTRACT

Biphenylacetic acid (**5**) was identified through a library screen as an inhibitor of the prostaglandin D_2 receptor DP2 (CRTH2). Optimization for potency and pharmacokinetic properties led to a series of selective CRTH2 antagonists. Compounds demonstrated potency in a human DP2 binding assay and a human whole blood eosinophil shape change assay, as well as good oral bioavailability in rat and dog, and efficacy in a mouse model of allergic rhinitis following oral dosing.

Prostaglandin D_2 (PGD_2), a product of the arachidonic acid cascade, is the major cyclooxygenase product formed and released from activated mast cells, and is known to be involved in allergic inflammatory response.[1] The biological actions of PGD_2 are mediated by two G-protein coupled receptors (GPCRs) termed DP1 and DP2 (also known as CRTH2). While DP1 is expressed on airway epithelium, smooth muscle, and platelets, DP2 is expressed on eosinophils, basophils, and Th2 cells. It has been shown that the PGD_2-mediated activation and migration of these cells proceeds selectively through the DP2 receptor. Therefore, the DP2 receptor may have an important role in inflammatory diseases including asthma, COPD, and allergic rhinitis.[2] Towards that end, there have been many recent publications detailing the discovery of small molecule DP2 antagonists,[3,4] as well as reports of their efficacy in pre-clinical rodent models of various allergic diseases.[4e,5]

We have previously described a novel tricyclic series of DP2 antagonists which were shown to have efficacy in a murine model of allergic rhinitis.[4e] In this Letter, we describe the discovery and exploration of a new series of small molecule DP2 antagonists. Biphenylacetic acid **5**, identified through a screen of existing compounds, inhibited the binding of ^{3}H-PGD_2 to DP2 receptors on cell membranes with an IC_{50} of 0.182 μM, and thus became a lead for optimization. The molecule was divided into three sections for a preliminary survey of the structure–activity relationship (see structure, Table 1). All compounds were screened in a DP2 binding assay[6] both in the presence and absence of 0.2% human serum albumin (HSA) to evaluate intrinsic potency and ascertain the degree of protein shift associated with the compounds.

Compound **5** was synthesized according to Scheme 1, beginning with the commercially available benzyl bromide **1** and bromophenylacetic acid **3**. (4*S*,5*R*)-4-Methyl-5-phenyl-oxazolidin-2-one was alkylated with benzyl bromide **1** using sodium hydride as base. Bromophenylacetic acid **3** was first converted to the ethyl ester then treated with bis(pinacolato)diboron in the presence of tetrakis(triphenylphosphine)palladium to generate aryl boronate **4**. Palladium-catalyzed Suzuki coupling with bromide **2** furnished the biphenyl core, which was hydrolyzed to provide compound **5** (Table 1). Compounds **6–14** were synthesized in an analogous fashion.

Initial SAR studies began by exploring the substitution pattern of the carboxylic acid function on the A ring (Table 1, compounds **5–11**). The des-methoxy analog **6** was synthesized to use as a benchmark in determining the correct chain length and placement of the acid relative to the biphenyl ring. In the *meta* position, phenylacetic acid proved to be the optimal linker length, as contracting the chain to the benzoic acid **7** or extending to the phenyl-propionic acid **8** led to decreased binding activity. Placing the acid in

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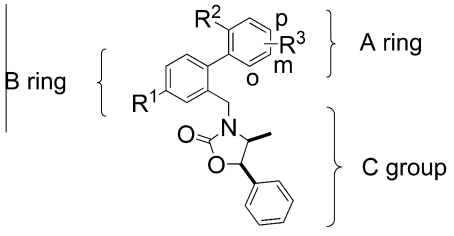
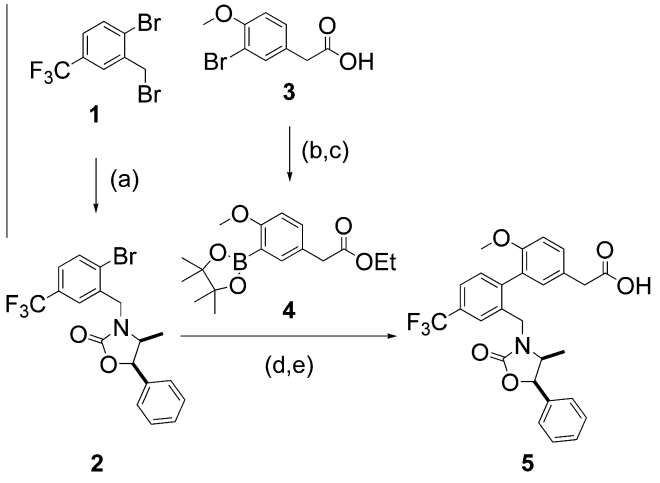
Table 1
Exploration of phenyl acetic acid moiety and B ring substitution



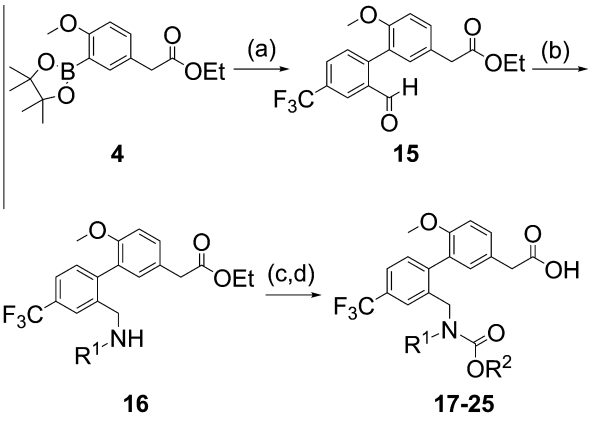
5-14

Compd	R^1	R^2	R^3	hDP2 binding IC_{50}[a] (μM)	hDP2 binding + 0.2% HSA IC_{50}[a] (μM)
5	CF_3	OMe	*m*-CH_2CO_2H	0.18	4.5
6	CF_3	H	*m*-CH_2CO_2H	0.34	3.0
7	CF_3	H	*m*-CO_2H	27	91
8	CF_3	H	*m*-$CH_2CH_2CO_2H$	9.8	75
9	CF_3	H	*o*-CH_2CO_2H	41	62
10	CF_3	H	*o*-$CH_2CH_2CO_2H$	11	38
11	F	H	*p*-CH_2CO_2H	0.36	1.8
12	F	OMe	*m*-CH_2CO_2H	0.36	1.3
13	H	OMe	*m*-CH_2CO_2H	0.88	2.0
14	OMe	OMe	*m*-CH_2CO_2H	1.3	6.1

[a] Values are the mean of at least three experiments.



Scheme 1. Representative synthesis for compounds **5–14**. Reagents and conditions: (a) oxazolidinone, NaH, DMF, 0 °C, 85–95%; (b) $SOCl_2$, EtOH; (c) bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, 1,4-dioxane, 80 °C, 80% two steps; (d) K_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 85 °C, 60–80%; (e) 1 M NaOH, EtOH, THF, 70–80%.



Scheme 2. Generic synthesis for compounds **17–25**. Reagents and conditions: (a) 2-bromo-5-(trifluoromethyl)benzaldehyde, K_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 85 °C, 75%; (b) RNH_2, $NaBH_3CN$, HOAc, MeOH; (c) ClCOOR, NEt_3, CH_2Cl_2; (d) 1 M NaOH, EtOH, THF (40% three steps).

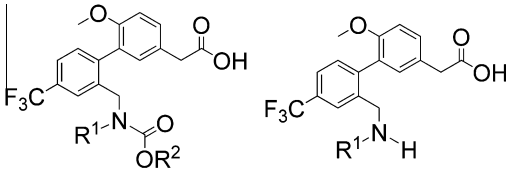
the *ortho* position decreased potency regardless of chain length (**9**, **10**). Compound **11** showed that *para*-phenylacetic acid was comparable to *meta*-phenylacetic acid.

The effect of substituents in the B ring was then explored while maintaining the *meta*-phenylacetic acid in the A ring and reincorporating the *ortho*-methoxy group (Table 1, entries **12**–**14**). Electron-deficient groups were preferred, as the increased potency of trifluoromethyl **5** ~ fluoro **12** > hydrogen **13** > methoxy **14** tracked with the electron withdrawing nature of the substituent. Changes to the acid placement in the A ring and B ring substituent resulted in no improvement in binding activity and minimal decrease in protein shift. The focus was therefore shifted to the C group.

Since the oxazolidinone is a cyclic carbamate, the effect of opening the oxazolidinone into its two parts, a carbamate and an alkyl group, was explored. Compounds were synthesized as shown in Scheme 2. Suzuki coupling between aryl boronate **4** and 2-bromo-5-(trifluoromethyl)benzaldehyde provided aldehyde **15,** which was then subjected to reductive amination with the appropriate amine to furnish the benzyl amines **16**. Acylation and ester hydrolysis afforded compounds **17–25** (Table 2). In general, opening the oxazolidinone at the C5–O1 bond provided highly potent compounds with low serum shifts. Shortening the R^1 chain length from phenethyl **17** to benzyl **18** gave a twofold increase in potency in the presence of HSA. While the secondary carbamate **19** showed only a modest reduction in potency, deletion of the carboxyl group, revealing a basic amine in **26** was not tolerated. Reversing the substituents such that the smaller alkyl group was attached directly to the nitrogen and the phenyl chain was part of the carbamate also gave potent compounds (**20**–**24**). There was some tolerance for the size of the R^1 alkyl group, as methyl, ethyl, cyclopropyl, and cyclobutyl (**20**–**23**) were approximately equipotent across all assays. However, cyclopentyl **24** showed a decrease in both binding and functional activity. Finally, compound **25** demonstrated that the C group phenyl ring was not necessary to maintain good binding potency with a low protein shift.

Lastly, the phenyl ring of the carbamate of compound **21** was elaborated. The halogenated compounds were synthesized as shown in Scheme 3. Commercially available alcohols **28** were treated with phosgene to generate chloroformates **29**, which were isolated by removal of solvent and reacted immediately with the

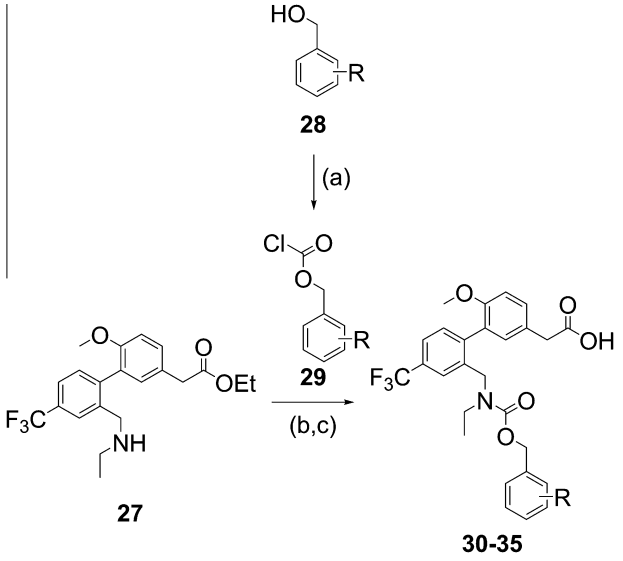
Table 2
Benzylamine substitution



17-25 **26**

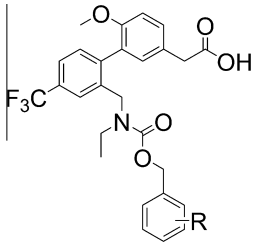
Compd	R^1	R^2	hDP2 binding IC_{50}[a] (nM)	hDP2 binding + 0.2% HSA IC_{50}[a] (nM)	hESC IC_{50}[a] (nM)
17	CH_2CH_2Ph	Me	24	62	34
18	CH_2Ph	Me	8	31	22
19	H	CH_2Ph	45	238	709
20	Me	CH_2Ph	4	18	9
21	Et	CH_2Ph	4	8	5
22	cyPropyl	CH_2Ph	4	18	10
23	cyButyl	CH_2Ph	4	19	14
24	cyPentyl	CH_2Ph	11	53	53
25	Et	Me	9	16	8
26	CH_2CH_2Ph	—	3.4 μM	11 μM	nt

hESC = human whole blood eosinophil shape change assay.
[a] Values are the mean of at least three experiments.



Scheme 3. Representative synthesis for compounds **30**–**35**. Reagents and conditions: (a) Phosgene, CH_2Cl_2; (b) iPr_2NEt, CH_2Cl_2; (c) 1 M NaOH, EtOH, THF, 40% yield.

Table 3
Carbamate substitution



30-35

Compd	R	hDP2 binding IC_{50}[a] (nM)	hDP2 binding + 0.2% HSA IC_{50}[a] (nM)	hESC IC_{50}[a] (nM)
30	2-Cl	3	11	7
31	3-Cl	2	10	7
32	4-Cl	3	7	6
33	3,5-Di-Cl	4	29	14
34	4-F	2	8	4
35	3,5-Di-F	3	11	5

[a] Values are the mean of at least three experiments.

ethylamine intermediate **27** to furnish the substituted benzyl carbamates. Hydrolysis to the acid provided compounds **30**–**35** (Table 3). In general, single halogens were well tolerated (**30–32** and **34**). The 3,5-difluoro derivative **35** was comparable to the single halogen compounds, while the 3,5-dichloro derivative **33** was threefold less potent in the presence of HSA.

In order to assess functional activity, compounds were tested in a human whole blood eosinophil shape change (hESC) assay.[7] When incubated with purified eosinophils or blood, PGD_2 activates the DP2 receptor and its downstream intracellular signaling pathways, which results in eosinophil degranulation and changes in eosinophil morphology. These effects can be analyzed by flow cytometry due to a change in forward light scatter. As the data in Tables 2 and 3 shows, compounds **20**–**22**, **25**, **30**–**32**, and **34**–**35** were found to be quite potent (⩽10 nM) in the hESC assay. Since the hESC assay is performed in whole blood, the conditions of this assay are more physiologically relevant than the binding assays. More importantly, this assay could potentially be used to provide a pharmacological readout during in vivo pharmacology studies or human clinical trials.

Due to the structural similarity of ligands in the arachidonic acid pathway, there exists the possibility for cross-reactivity of ligands with the various prostanoid receptors. Since small molecule inhibitors also have the potential for cross-reactivity, a subset of compounds was counterscreened against a select group of prostanoid receptors, including the other PGD_2 receptor DP1, the human thromboxane receptor (hTP), and the human prostacyclin receptor (hIP) (Table 4). The compounds tested were highly selective for the DP2 receptor. This subset was also profiled against the common cytochrome P450 (CYP) isoforms to assess the potential for drug/drug interactions[8] as well as the ability to inhibit the hERG channel utilizing a patch clamp assay.[9] As the data in Table 5 show, these compounds showed no CYP inhibitory concerns or activity at the hERG channel.

The pharmacokinetic properties of the most potent compounds in the series (**25**, **30–32**, **34–35**) were evaluated in rat (sodium salt, PO 10 mpk in 0.5% Methocel). Compounds showed good exposure

Table 4
DP1, TP, IP counterscreen

Compd	hDP1 binding IC_{50}[a] (μM)	hTP binding IC_{50}[a] (μM)	hIP binding IC_{50} (μM)
5	31.3	63.7[b]	nt
18	12.6	62.4	nt
21	4.0[b]	23.0	56.2[c]
25	36.4	67.4	nt
32	4.0	11.3	33.0[c]

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 performed using hIP/293 membranes and ^{3}H-iloprost.
[a] Values are the mean of two experiments.
[b] Values are the mean of at least three experiments.
[c] Value from a single experiment (nt = not tested).

Table 5
Inhibition of human CYP isoforms and hERG

Compd	CYP3A4 inhibition IC_{50}[a] (μM)	CYP2C9 inhibition IC_{50}[a] (μM)	CYP2D6 inhibition IC_{50}[a] (μM)	hERG[a]
5	>30	>30	>30	nt
18	>30	>30	>30	1.2% at 10 μM
21	18	18	>30	IC_{50} >30 μM
25	>30	>30	>30	5.9% at 10 μM[b]
32	21	18	>30	22% at 10 μM[b]

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

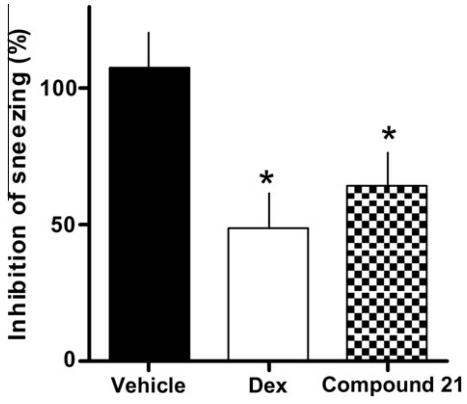


Figure 1. Mouse allergic rhinitis data for compound **21** (10 mg/kg) versus dexamethasone (10 mg/kg) and vehicle. Bars represent means ± SEM of $n = 4–10$ mice per group. $*P < 0.05$ versus vehicle, Dunnett's post hoc comparisons following ANOVA. Average plasma concentration of compound **21** at 2 h post-dose was 1 μM.

following oral dosing. Compound **21** was tested further in rat (sodium salt, IV 2 mpk in water) and dog (sodium salt, IV 2 mpk in saline, PO 5 mpk in 0.5% Methocel). In both species, the compound displayed a long half-life (8.7 h and 7.4 h, respectively), with oral bioavailability of 77% in rat and 89% in dog.

Given the desirable PK profile observed, compound **21** was then evaluated in an in vivo model of allergic disease. Allergic rhinitis can be modeled in mice by first sensitizing animals to ovalbumin (OVA) by intraperitoneal injection and subsequently performing nasal challenge with OVA.[10] The mice then develop symptoms which are similar to those observed in human allergic rhinitis, including nasal itch, sneezing, and nasal congestion. The effect of drug treatment prior to challenge can be quantified by counting the frequency of these behaviors and comparing to untreated animals.

In this assay female BALB/c mice were primed by intraperitoneal OVA on days 0 and 7 (10 μg in Alum). On days 21 through 32 mice received daily intranasal OVA (200 μg in saline) challenge to induce an allergic response consisting of sneezing and nasal rubs. Compound **21** (10 mg/kg), dexamethasone (10 mg/kg) or water vehicle was administered orally 1 h prior to OVA challenge on days 31 and 32 only. The frequency of sneezing was recorded during an 8 min session immediately following OVA challenge on day 32. Compound **21** significantly reduced sneezing symptoms in the sensitized mice with an effect similar to that of dexamethasone (Fig. 1).

In summary, we have discovered a new series of small molecule DP2-selective antagonists. The biphenylacetic acid series has been optimized to provide compounds such as **21** that are highly potent in both binding and functional assays, and show good pharmacokinetic properties in rat and dog. Compound **21** was efficacious in a murine model of allergic rhinitis following oral dosing. Further investigation of compound **21** and others in this series is ongoing.

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6. The DP2 radioligand binding assay was performed on membranes from 293 cells stably expressing human DP2. To measure binding, [^{3}H]-PGD_2 was incubated together with 293(hDP2) membranes in the presence of increasing concentrations of compounds. Membranes were harvested and washed using a Brandel Harvester and the amount of [^{3}H]-PGD_2 that remained bound to the cells was measured on a TopCount. The concentration of compounds required to achieve a 50% inhibition of [^{3}H]-PGD_2 binding (the IC_{50}) was determined. The binding assay was carried out both in the absence and presence of 0.2% human serum albumin (HSA) to evaluate the protein shift associated with the compounds.
7. Human blood was drawn into EDTA tubes and used within 2 h of draw. 100 μl aliquots of fresh blood were incubated for 15 min at 37 °C plus or minus test compound in 50% DMSO/water. PGD_2 (50 nM final concentration) or vehicle was added from a 1 μM stock in PBS and incubations were continued for 5 min at 37 °C. The reactions were placed on ice and 250 μl of ice-cold 1:4 diluted Cytofix (BD Biosciences) in PBS immediately added. The reactions were transferred to FACS tubes and lysed with ammonium chloride lysing solution at room temperature for 15 min. Tubes were centrifuged and the cells washed

once with 3 ml cold PBS before re-suspension in 200 μl of ice-cold 1:4 diluted Cytofix in PBS. Eosinophil shape change was analyzed on a FACScalibur by analyzing forward light scatter of the autofluorescent cells.
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