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Optimization of phenylacetic acid derivatives for CRTH2 and DP selective antagonism

Yingcai Wang, Zice Fu, Michael Schmitt, Xuemei Wang, Wang Shen, Erika Rickel, Tod Martin, Alison Budelsky, Derek Marshall, Tassie Collins, H. Lucy Tang, Julio C. Medina, Jiwen (Jim) Liu *

Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA

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ABSTRACT

We have previously reported that optimization of a series of phenylacetic acid derivatives led to the discovery of CRTH2 and DP dual antagonists, such as AMG 009 and AMG 853. During the optimization process, we discovered that minor structural modifications also afforded potent and selective CRTH2 or DP antagonists. Here we report the structure–activity relationship that led to the discovery of selective CRTH2 antagonists such as **2** and **17**, and selective DP antagonists, such as **4** and **5**.

CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells, also known as DP_2) and DP (prostanoid D receptor, also known as DP_1) are both G-protein coupled receptors. Their endogenous ligand is prostaglandin D_2 (PGD_2), which plays a key role in mediating allergic reactions seen in allergic diseases.[1,2] Stimulation of these two GPCRs promotes a number of biological effects associated with the development and maintenance of allergic responses. Numerous studies using DP and CRTH2 antagonists, combined with genetic analysis, support the view that these receptors play a pivotal role in mediating allergic diseases.[3–5] Therefore, there has been great interest in the discovery and development of DP and CRTH2 antagonists, especially for CRTH2 antagonists for the treatment of asthma and other allergic diseases.[6–21]

We have previously reported the optimization of phenylacetic acid derivatives that led to the discovery of AMG 009 and AMG 853 (Fig. 1), two potent CRTH2 and DP dual antagonists.[22,23] Here we report that the same optimization also identified potent selective antagonists of CRTH2 and DP.

The synthesis of compounds in this publication is shown in Schemes 1–3. Compounds **1**–**4** and **8**–**11** were prepared using the same route as previously reported (Scheme 1).[22,23]

Compound **5** was prepared in a similar manner to the one in Scheme 1 with an additional fluorination step (Scheme 2). Difluorination of the α-oxophenylacetate to form the α,α-difluorophenylacetate was achieved using DAST (step iii of b, Scheme 2).[24] Compounds **6** and **7** were synthesized from AMG 009 using reported procedures.[25,26]

Compounds **14**–**19** were synthesized in five steps (Scheme 3). 2-Fluoro-5-nitrobenzoic acid was treated with 3-hydroxy-4-methoxyphenylacetic acid ethyl ester in the presence of potassium carbonate to give the bisaryl ether, whose nitro group was reduced using hydrogenation. The aniline was then converted into the corresponding propionyl amide. The benzoic acid was also converted into various amides through reaction with amines in step d. The ethyl ester was finally hydrolyzed to afford the phenylacetic acids.

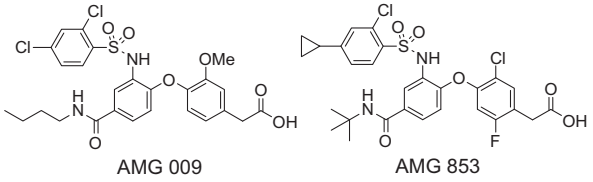

Figure 1.

* Corresponding author.
E-mail address: jiwenl@amgen.com (J. Liu).

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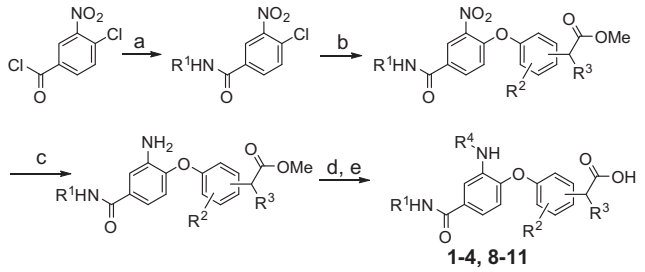
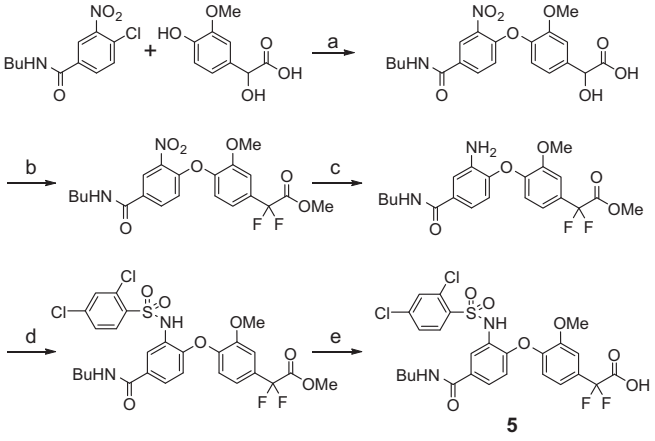
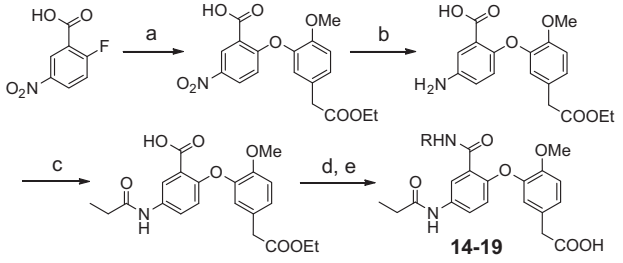


Scheme 1. Reagents and conditions: (a) Ethylamine or *n*-butylamine, triethylamine, DCM, rt, 4 h, ~90%; (b) phenylacetic acids methyl ester, cesium carbonate, DMSO, 70 °C, 6 h, ~85%; (c) H_2, Pd/C, EtOH, rt, 1 h, 100% or $SnCl_2$, EtOAc, 60 °C, 4 h, 80%; (d) For sulfonamides **1**–**4** and **8**, sulfonyl chlorides, pyridine, rt, 24 h, 70%; for amines **9** and **10**, aldehydes, $NaBH(OAc)_3$, DCE, rt, 3 h, 80%; for urea **11**, isocyanate, triethylamine, EtOAc, rt, 12 h, 80%; (e) LiOH, MeOH/THF/H_2O, 23 °C, 2 h, ~80%.



Scheme 2. Reagents and conditions: (a) Cs_2CO_3, DMSO, 60 °C, 3 days, 68%; (b) (i) $TMSCHN_2$, MeOH/benzene, 23 °C, 0.5 h; (ii) Dess–Martin periodinane, DCM, 23 °C, 14 h; (iii) DAST, DCE, 90 °C, 4 h, 55%, three steps (Ref.[23]); (c) H_2, Pd/C, EtOH/EtOAc, 23 °C, 48 h, 88%; (d) 2,4-dichlorobenzenesulfonyl chloride, 2,6-lutidine, 40 °C, 20 h, 67%; (e) LiOH, MeOH/THF/H_2O, 23 °C, 2 h, 47%.



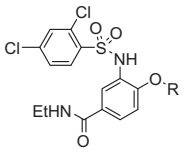
Scheme 3. Reagents and conditions: (a) 3-Hydroxy-4-methoxyphenylacetic acid ethyl ester, K_2CO_3, DMSO, 80 °C, 8 h, 70%; (b) H_2, Pd/C, EtOH, rt, 1 h, 100%; (c) propionyl chloride, triethylamine, DCM, rt, 3 h, 90%; (d) Amines, EDC, HOBt, triethylamine, DCM, rt, 1 day, 80%; (e) LiOH, MeOH/THF/H_2O, 23 °C, 2 h, ~80%.

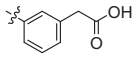
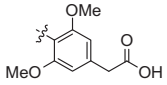
Modification of the phenylacetic acid moiety affected the selectivity toward CRTH2 and DP. As previously reported, *meta*-phenylacetic acid derivatives, such as **1**, are CRTH2 selective (Table 1) and the *para*-phenylacetic acid derivatives can either be CRTH2 and DP dual antagonist (AMG 009) or CRTH2 selective antagonist (**2**), depending on the substitutions on the phenyl ring of the phenylacetic acid.[22]

DP selective antagonists were generated through modifying the acetic acid moiety (Table 2). Dialkylation at the α-position of the acetic acid moiety provided molecules, exemplified by **3** and **4**, with increased selectivity for DP over CRTH2. The α,α-difluorophenylacetate (**5**) has improved affinity for DP and significantly reduced activity on CRTH2 compared to AMG 009. Bioisosteric replacements of the carboxylic acid also afforded DP selective antagonists. The tetrazole (**6**) and acyl sulfonamide (**7**) maintained similar activity on the DP receptor as compared to the corresponding carboxylic acid (AMG 009). However, their affinity for the CRTH2 receptor was greatly reduced.

CRTH2 selective antagonists were also obtained by modifying the sulfonamide moiety. Several replacements of the sulfonamide moiety were evaluated and a few representative compounds are listed in Tables 3 and 4. The data in these tables indicates that replacement of the sulfonamide moiety leads to loss of DP activity. With the exception of sulfone **12**, none of the compounds in Table 3

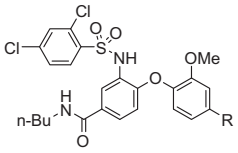
Table 1
CRTH2 selective antagonists



Compd	R	CRTH2 IC_{50}[a] in buffer (μM)	DP IC_{50}[a] in buffer (μM)
1		0.016	>10
2		0.002	16.0

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA. See Ref. 27 for assay protocol. Values are means of three experiments, standard deviation is ± 30%.

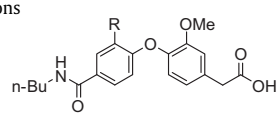
Table 2
Modification of the acetic acid moiety

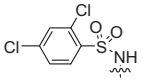
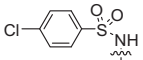
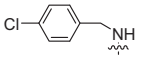
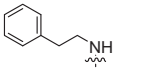
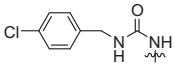
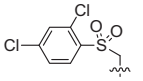
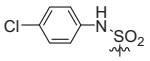


Compd	R	CRTH2 IC_{50}[a] in buffer (μM)	DP IC_{50}[a] in buffer (μM)
AMG 009		0.003	0.012
3		>10	0.91
4		>10	0.013
5		0.42	0.003
6		0.35	0.007
7		0.33	0.006

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA. See Ref. 27 for assay protocol. Values are means of three experiments, standard deviation is ± 30%.

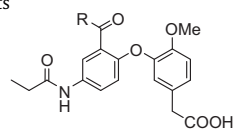
Table 3
Sulfonamide modifications

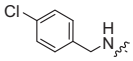
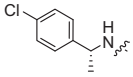
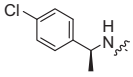
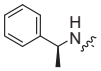
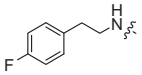
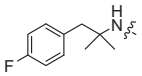


Compd	R	CRTH2 IC_{50}[a] in buffer (μM)	DP IC_{50}[a] in buffer (μM)
AMG 009		0.003	0.012
8		0.004	0.013
9		0.23	>10
10		0.11	>10
11[b]		0.20	>10
12[c]		0.005	0.57
13		0.076	>10

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA. See Ref. 27 for assay protocol. Values are means of three experiments, standard deviation is ± 30%.
[b] The compound has an ethyl amide, instead of a *n*-butyl amide.
[c] The compound has a cyclobutyl amide, instead of a *n*-butyl amide.

Table 4
CRTH2 selective antagonists

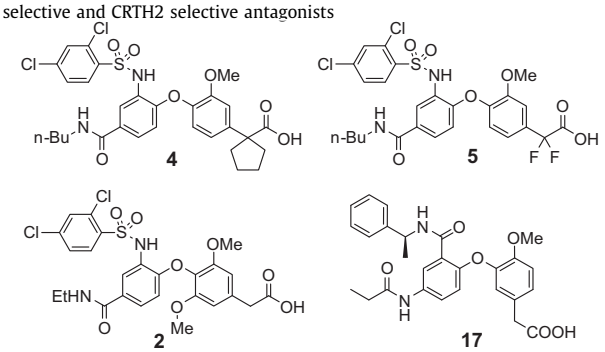


Compd	R	CRTH2 IC_{50}[a] in buffer (μM)	CRTH2 IC_{50}[a] in plasma (μM)
14		0.007	0.065
15		0.014	0.26
16		0.002	0.015
17		0.001	0.006
18		0.002	0.013
19		0.002	0.015

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA. See Ref. 27 for assay protocol. Values are means of three experiments, standard deviation is ± 30%.

had IC_{50} less than 10 μM in the DP binding assay. Compound **12** is a noticeable molecule, because it was able to maintain activity on the CRTH2 receptor, comparable to AMG 009. Also noticeable is reversed sulfonamide **13**, which displayed good CRTH2 activity, but was weaker than the corresponding sulfonamide (**8**).

Table 5
DP selective and CRTH2 selective antagonists



Compd	CRTH2 IC_{50}[a] in buffer (μM)	CRTH2 IC_{50}[a] in plasma (μM)	DP IC_{50}[a] in buffer (μM)	DP IC_{50}[a] in plamsa (μM)
AMG 009	0.003	0.021	0.012	0.28
4	>10	ND	0.012	0.46
5	0.42	ND	0.003	0.21
2	0.002	0.016	16.0	ND
17	0.001	0.006	>10	ND

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptor expressed on 293 cells. Assay run in buffer containing 0.5% BSA or in 50% human plasma. See Ref. 27 for assay protocol. Values are means of three experiments, standard deviation is ± 30%.

In order to obtain compounds with increased activity on CRTH2 and selectivity over DP, we decided to combine the features that favor affinity to the CRTH2 receptor. Therefore, we combined modifications to the sulfonamide moiety with the *meta*-phenylacetic acid group which, as shown in Table 1, confers selectivity for CRTH2. We previously reported that a reverse amide, such as the propionamide group in **14**–**19** (Table 4), afforded compounds with similar or improved CRTH2 activity when compared to those with the corresponding butyl amide.[22] As shown in Table 4, the amide replacements of the sulfonamide moiety afforded potent CRTH2 selective antagonists, such as compounds **16**–**19**. All of the compounds in Table 4 had IC_{50} greater than 10 μM in the DP binding assay.

Two CRTH2 selective antagonists (**2** and **17**) and two DP selective antagonists (**4** and **5**) listed in Table 5 were also potent in the presence of plasma, as indicated by their activity in the plasma binding assays.[27]

The functional activity of DP selective antagonist **4** and **5** was evaluated in a cAMP assay using human whole blood. Compounds **4** and **5** inhibited PGD_2 induced cAMP response mediated by DP in platelets in 80% human whole blood with a K_b of 160 nM and 38 nM, respectively, which compares favorably to AMG 009 (AMG 009 K_b = 170 nM).[28] Functional activity for the CRTH2 selective antagonists **2** and **17** was evaluated using an eosinophil shape change assay. Compounds **2** and **17** inhibited human eosinophil shape change mediated by PGD_2 through the CRTH2 receptors with K_b of 0.26 and 0.13 nM, respectively.[29] AMG 009 had a K_b of 0.09 nM in the same assay.

In summary, we found that modifications to the phenylacetic acid moiety affected the selectivity toward CRTH2 and DP. *Meta*-phenylacetic acid derivatives, such as **1**, are CRTH2 selective, while *para*-phenylacetic acid derivatives can either display selectivity for CRTH2 (**2**) or afford dual CRTH2 and DP antagonists (AMG 009), depending on the substitutions on the phenyl ring of the phenylacetic acid. Also, it was found that modifications to the carboxylic acid or in its vicinity favored the DP receptor over CRTH2, such

as in **3**, **4**, **6** and **7**. On the other hand, replacing the sulfonamide moiety with an amide group, such as in compounds **16–19**, afforded potent CRTH2 selective antagonists. In short, these phenylacetic acid derivatives are flexible enough to provide CRTH2 and DP dual antagonists and selective antagonists of CRTH2 and DP.

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27. The CRTH2 or DP radioligand binding assay was performed on 293 cells stably expressing human CRTH2or DP, respectively. To measure binding, [^{3}H]-PGD_2 was incubated together with 293(hCRTH2) cells in the presence of increasing concentrations of compounds. After washing, the amount of [^{3}H]-PGD_2 that remained bound to the cells was measured by scintillation counting and the concentration of compounds required to achieve a 50% inhibition of [^{3}H]-PGD2 binding (the IC_{50}) was determined. The binding buffer contains either 0.5% BSA (buffer binding) or 50% human plasma (plasma binding).
28. Whole blood was collected from healthy human volunteers into ACD Vacutainer tubes (VWR). IBMX (3-Isobutyl-1-methylxanthine, Sigma) was added to whole blood at a final concentration of 1 mM. 200 μL/well of IBMX treated blood was incubated in the presence of a small molecule DP antagonist in a 96 well plate at 37 °C for 30 min. 20 μL of the antagonist incubated blood was then aliquoted into a 96 well plate containing PGD_2 (Cayman Chemicals) at various concentrations. The plate was incubated at 37 °C for 60 min. After the PGD_2 stimulation, the blood was lysed with 55 μL/well of Tropix Lysis buffer (ABI) and subsequently assayed for cAMP levels using the cAMP-Screen Direct® Immunoassay System (ABI). The PGD_2 stimulated cAMP curves were fit Genedata Screener Condoseo (Genedata AG) using a Hill fit model. A Schild plot is the constructed and K_b is calculated.
29. Human erythrocytes and granulocytes were enriched from normal donor peripheral blood by Isolymph (Gallard–Schlesinger Industries, Plainview, NY) gradient centrifugation. The erythrocytes were removed using ACK lysing buffer (Gibco, Carlsbad, CA). The mixed granulocyte population was pre-incubated with vehicle (0.05% DMSO) or antagonists for 10 min at room temperature prior to stimulation with PGD_2 (600–0.003 nM at 1:3 dilution) (Cayman Chemical Co, Ann Arbor, MI) for 10 min at 37 °C. The cells were fixed using 1% final paraformaldehyde (Alpha Aesar, Ward Hill, MA) and were analyzed on a FACS caliber (BD Biosciences, San Jose, CA) flow cytometer. Leukocytes were gated on using forward/side scatter parameters. The FL2 positive cells (eosinophils) were then gated and their geometric mean of the forward scatter was calculated. The geometric means were graphed using GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA) and IC_{50} values were calculated. The K_b values were calculated using the equation A/(R-1) where A is the concentration of the inhibitor used. The value $R = X/Y$ where X is the IC_{50} value of PGD_2 in the presence of the inhibitor and Y is the IC_{50} value of PGD_2 alone.