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LETTER

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Discovery of AMG 853, a CRTH2 and DP Dual Antagonist

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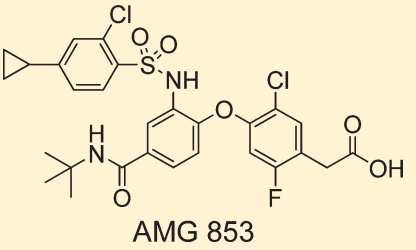
Supporting Information

ABSTRACT: Prostaglandin D_2 (PGD_2) plays a key role in mediating allergic reactions seen in asthma, allergic rhinitis, and atopic dermatitis. PGD_2 exerts its activity through two G protein-coupled receptors (GPCRs), prostanoid D receptor (DP or DP_1), and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2 or DP_2). We report the optimization of a series of phenylacetic acid derivatives in an effort to improve the dual activity of AMG 009 against DP and CRTH2. These efforts led to the discovery of AMG 853 (2-(4-(4-(*tert*-butylcarbamoyl)-2-(2-chloro-4-cyclopropylphenyl sulfonamido)phenoxy)-5-chloro-2-fluorophenyl)acetic acid), which is being evaluated in human clinical trials for asthma.



AMG 853

KEYWORDS: AMG 853, CRTH2 receptor, DP receptor, prostaglandin D2, antagonist, asthma

Prostaglandin D_2 (PGD_2) is produced by mast cells in high concentrations during allergic responses and plays a key role in mediating allergic reactions seen in asthma, allergic rhinitis, atopic dermatitis, and allergic conjunctivitis.[1,2] PGD_2 exerts its activity through two G protein-coupled receptors (GPCRs), DP (prostanoid D receptor, DP_1) and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells, DP_2). These two GPCRs act in concert to promote a number of biological effects associated with the development and maintenance of the 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.[3−6] Therefore, there has been significant interest in the development of selective DP and CRTH2 antagonists.[7−23] We have pursued the thesis that blockade of both receptors may prove more beneficial in alleviating allergic diseases triggered by PGD_2 than inhibiting either one separately; therefore, we have been interested in identifying potent CRTH2/DP dual inhibitors. We previously reported on the discovery of AMG 009 (**1**; Figure 1) as a potent CRTH2 and DP dual antagonist.[23] In this article, we report our continued optimization efforts, which led to the discovery of AMG 853 (**2**), which possesses increased DP and CRTH2 potency as compared to **1**.

Compounds **1**−**25** were synthesized according to the chemistry depicted in Scheme 1. 4-Chloro-3-nitrobenzoyl chloride was reacted with amines to form the corresponding amides. Displacement of the chlorine adjacent to the nitro group with 4-hydroxyphenylacetic acids gave the bis-aryl ethers in good yields. Reduction of the nitro group followed by protection of the carboxylic acids as methyl esters provided the aniline methyl esters. The final two steps were carried out in one pot.

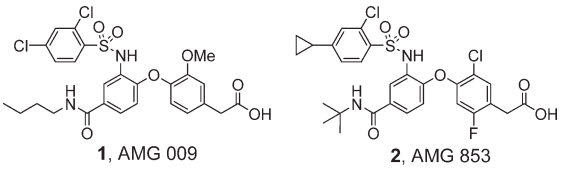


Figure 1. AMG 009 and AMG 853.

The anilines were converted to the benzene sulfonamides by treating with sulfonyl chlorides, and then, hydrolysis of the methyl esters using sodium hydroxide afforded the final compounds (**1**−**25**).

Compound **1** had high affinity toward the CRTH2 receptor, even in the presence of human plasma. It inhibited the binding of ^{3}H-PGD_2 to the CRTH2 receptors on HEK-293 cells with an IC_{50} of 0.021 μM in the presence of human plasma. It also inhibited the binding of ^{3}H-PGD_2 to the DP receptors with a moderate IC_{50} of 0.28 μM in the presence of plasma. Therefore, we set out to find compounds with improved activity on DP while maintaining the excellent activity on CRTH2 displayed by **1**. In addition, in vitro metabolism studies with **1** identified the methoxy, amide, and sulfonamide phenyl groups as potential metabolic sites. Thus, we focused our efforts on modifying these regions.

Compound **1** was one of the most potent dual CRTH2 and DP inhibitors identified from this series when it was selected for preclinical studies. Potency comparisons were mainly based on IC_{50} values in the presence of 50% human plasma,

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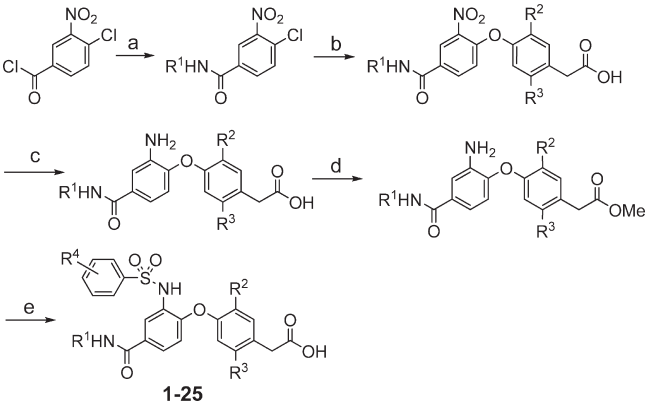
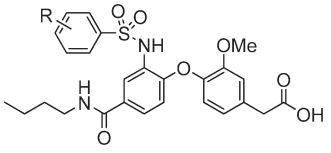
Scheme 1[a]



[a] Reagents and conditions: (a) Amines, triethylamine, THF, 0 °C, 12 h, ~90%. (b) Phenylacetic acids, cesium carbonate, DMSO, 70 °C, 9 h, ~85%. (c) H_2, Pd/C, NaOH, water, room temperature, 3 h, 100%; or $SnCl_2$, EtOAc, 60 °C, 4 h, 80%. (d) H_2SO_4, MeOH, 60 °C, 5 h, 100%. (e) Sulfonyl chlorides, 2,6-lutidine, THF, 60 °C, 12 h; then water, NaOH, room temperature, 2 h, ~70% two steps in one pot.

Table 1. Sulfonamide Modifications



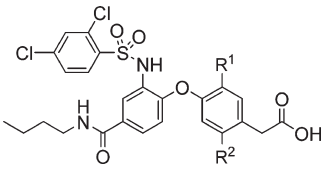
		IC$_{50}$[a] in buffer/plasma (μM)	
compd	R	CRTH2	DP
1	2,4-Cl	0.003/0.021	0.012/0.28
3	3,4-Cl	0.005/0.023	0.021/0.12
4	2,5-Cl	0.008/0.069	0.050/3.64
5	3,5-Cl	0.68/4.38	0.93/>10
6	2,3-Cl	0.032/0.30	0.52/2.34
7	2,6-Cl	0.012/1.18	>10/ND
8	2-Cl-4-CF_3	0.004/0.047	0.021/0.19
9	4-Cl	0.004/0.10	0.013/ND

[a] Displacement of ^{3}H-lPGD$_2$ from the CRTH2 receptor expressed on HEK 293 cells. Assay run in buffer containing 0.5% bovine serum albumin. See ref 24 for assay protocol. Values are means of three experiments, and the standard deviation is ±30%.

which correlated well with the functional activity in human whole blood. During earlier optimization efforts, it was found that single changes to the structure of **1** failed to yield significant improvements in potency. For example, a variety of chloro-substituted benzene sulfonamides were studied (Table 1). Two compounds (**3** and **8**) had similar potency as compared to **1**; all other compounds suffered a loss in potency.

Substitution of the phenyl ring of the phenylacetic acid moiety was also investigated (Table 2). A few substituents (methyl in **10** and difluoro in **14**) provided similar CRTH2 and DP potencies as the methoxy group in **1**. However, other substitutions, such as mono halogen (**11** and **12**) and cyano (**13**), afforded weaker potencies, especially in the presence of human plasma.

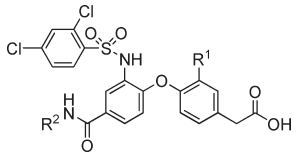
Table 2. Phenylacetic Acid Modifications



			IC$_{50}$[a] in buffer/plasma (μM)	
compd	R^1	R^2	CRTH2	DP
1	OMe	H	0.003/0.021	0.012/0.28
10	Me	H	0.003/0.026	0.006/0.23
11	Cl	H	0.006/0.090	0.008/1.33
12	F	H	0.007/0.11	0.030/0.44
13	CN	H	0.018/0.21	0.31/ND
14	F	F	0.004/0.032	0.007/0.60

[a] Displacement of ^{3}H-PGD$_2$ from the CRTH2 or DP receptor expressed on HEK 293 cells. Assay run in buffer containing 0.5% bovine serum albumin or in 50% human plasma. See ref 24 for assay protocol. Values are means of three experiments, and the standard deviation is ±30%.

Table 3. Combination of Amide and Phenylacetic Acid Modifications



			IC$_{50}$[a] in buffer/plasma (μM)	
compd	R1	R2	CRTH2	DP
1	OMe	*n*-Bu	0.003/0.021	0.012/0.28
15	OMe	*c*-Pr	0.004/0.028	0.041/1.03
11	Cl	*n*-Bu	0.006/0.090	0.008/1.33
16	Cl	*c*-Pr	0.007/0.025	0.014/0.22

[a] Displacement of ^{3}H-PGD$_2$ from the CRTH2 or DP receptor expressed on HEK 293 cells. Assay run in buffer containing 0.5% bovine serum albumin or in 50% human plasma. See ref 24 for assay protocol. Values are means of three experiments, and the standard deviation is ±30%.

Furthermore, a previous study around the amide demonstrated that the *n*-butyl amide as in **1** was one of the most potent amides.[23] A structure−activity relationship study also showed that the bis-aryl ether linker was preferred. It could be replaced by a methylene linker, and the potencies were maintained. All other linker modifications, such as sulfide, sulfoxide, sulfone, and amine linkers, afforded compounds exhibiting weaker potencies (data not shown).

When more than one change was made to the structure of **1**, and interesting combination effects were seen. For example, substitution of the *n*-butyl amide by a cyclopropyl amide in combination with replacement of the methoxy moiety by

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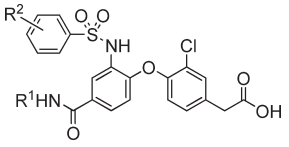
Table 4. Combination of Sulfonamide and Amide Modifications



compd	R^1	R^2	IC_{50}[a] in buffer/plasma (μM) CRTH2	DP
17	*c*-Bu	4-Cl	0.006/0.012	0.016/0.085
18	*t*-Bu	4-Cl	0.009/0.015	0.046/0.45
19	*c*-Bu	2,4-Cl	0.005/0.019	0.009/0.21
20	*t*-Bu	2,4-Cl	0.005/0.012	0.015/0.23

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptor expressed on HEK 293 cells. Assay run in buffer containing 0.5% bovine serum albumin or in 50% human plasma. See ref 24 for assay protocol. Values are means of three experiments, and the standard deviation is ±30%.

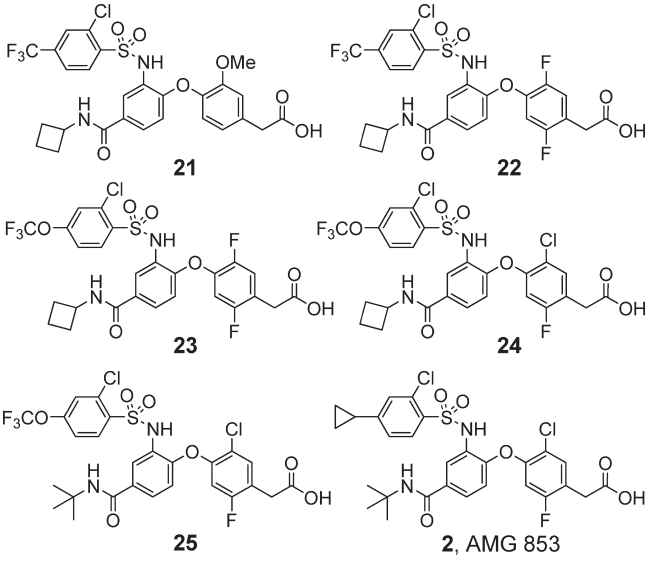
chlorine afforded a compound (**16**) with similar potency to **1** in the displacement assays (Table 3). On the other hand, substitution of the *n*-butyl amide moiety of **1** by a cyclopropyl amide group provided a compound (**15**) with decreased DP potency (Table 3). Likewise, replacement of the methoxy group in the phenylacetic acid moiety of **1** by a chlorine atom also resulted in a compound (**11**) that was less potent than **1** in the presence of plasma. This suggested that the combination of modifications at different parts of the molecule needed to be taken into consideration when optimizing this series for potency in the presence of plasma.

Table 4 further illustrates the effect that combination of different groups had on this series. The *t*-butyl amide functional group in combination with the 4-chlorobenzenesulfonamide moiety afforded a molecule (**18**) with decreased activity on the DP receptor, as compared to the analogous cyclo-butyl amide derivative (**17**). However, compound **20**, which contained a *t*-butyl amide group in combination with the 2,4-dichlorobenzenesulfonamide moiety, had similar affinity for the DP receptor as the corresponding cyclo-butyl amide (**19**) in the presence of plasma.

On the basis of the observations exemplified in Tables 3 and 4, we explored multiple combinations of preferred groups in the phenylacetic acid, amide, and sulfonamide regions. The combination produced dual CRTH2 and DP inhibitors with significantly improved DP potency as compared to **1**. Table 5 shows a few examples of the potent dual antagonists. Compound **2** was among the potent dual antagonists discovered.

Key compounds were also evaluated in CRTH2 and DP human whole blood functional assays. Consistent with the displacement assay data, **2** was also significantly more potent than **1** in the human whole blood functional assays. Compound **2** inhibited the PGD_2-induced down-modulation of CRTH2 on CD16 negative granulocytes (eosinophils) in human whole blood with a K_b of 0.2 nM, while **1** had a K_b of 1 nM.[25] Compound **2** also inhibited PGD_2-induced cAMP response in platelets in 80% human whole blood with a K_b of 4.7 nM, which was significantly improved, as compared to the K_b of 148 nM of **1**.[26] In addition, **2** demonstrated similar antagonist activity in an aequorin assay

Table 5. Potent CRTH2 and DP Dual Antagonists



compd	CRTH2 IC_{50}[a] (μM) in buffer	in plasma	DP IC_{50}[a] (μM) in buffer	in plasma
1	0.003	0.021	0.012	0.28
21	0.004	0.013	0.013	0.045
22	0.013	0.041	0.009	0.022
23	0.025	0.053	0.020	0.042
24	0.005	0.008	0.010	0.019
25	0.009	0.019	0.014	0.038
2	0.003	0.008	0.004	0.035

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptor expressed on HEK 293 cells. Assay run in buffer containing 0.5% bovine serum albumin or in 50% human plasma. See ref 24 for assay protocol. Values are means of three experiments, and the standard deviation is ±30%.

using CRTH2-transfected HEK 293 cells and an eosinophil shape change assay, as compared to the CRTH2 receptor down-modulation human whole blood assay.[25]

The significant improvement of DP potency of **2** over **1** was also demonstrated in vivo in a guinea pig model of PGD_2-induced airway constriction (Figure 2).[27] In this model, airway resistance (Penh, enhanced pause) was measured in response to PGD_2 challenge. The efficacy seen in this model was most likely due to the effect on the DP receptors, because CRTH2 selective antagonist had no effect in the model.[23] The in vitro guinea pig DP potency was evaluated in guinea pig whole blood cAMP assay. Compound **2** had a K_b of 5 nM, while the K_b of **1** was 82 nM.

The pharmacokinetic properties of **2** were evaluated in several species (Table 6) and were similar to those of **1**.[23] Compound **2** had low to moderate clearance across species, excellent oral absorption, and low potential of inducing drug−drug interactions as indicated by CYP inhibition and induction tests.

In summary, further optimization of a series of phenylacetic acid derivatives led to the discovery of **2** (AMG 853), which displayed significantly improved potency, as compared to **1** (AMG 009), in CRTH2 and DP displacement and functional

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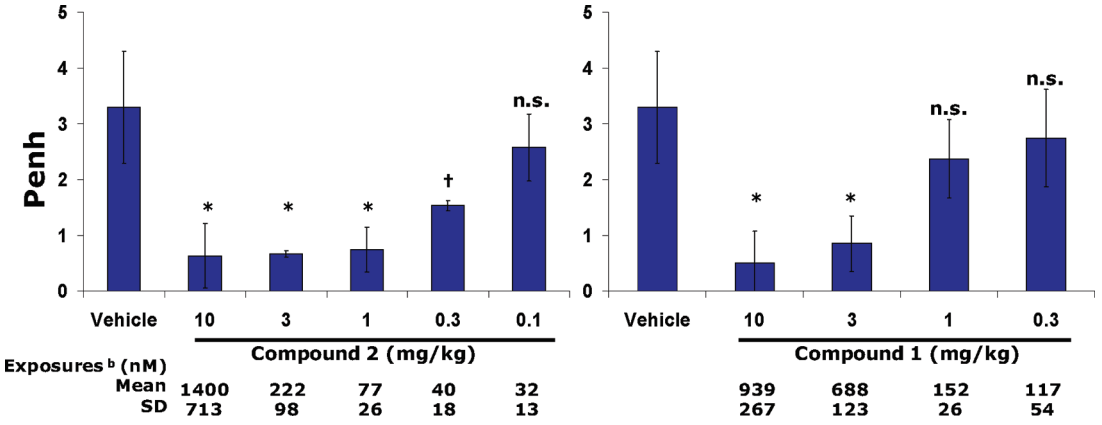

Figure 2. Evaluation of **2** and **1** in the guinea pig model of PGD_2-induced airway constriction. Compounds **2** and **1** were dosed sc 4 h prior to aerosolized PGD2 exposure (0.6 mg/mL); $n = 4$ animals per group. [b]Plasma exposure at 4 h after dosing; $^*p < 0.02$, $^\dagger p < 0.05$, and n.s., not significant relative to vehicle treatment.

Table 6. Pharmacokinetic Properties of 2

species	clearance (L/h/kg)[a]	MRT (h)	Vdss (L/kg)	oral bioavailability[b] (%)
rat	0.19	3.9	0.71	87
dog	0.9	3.0	2.72	100
cyno[c]	0.96	1.0	0.97	79

[a] iv dose at 0.5 mg/kg. [b] Oral dose at 2 mg/kg. [c] Cynomolgus monkey.

assays, including assays performed in human whole blood, and in vivo. Compound **2** was selected for evaluation in human clinical trials for asthma.

■ ASSOCIATED CONTENT

Supporting Information. Detailed synthetic experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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(24) The CRTH2 or DP radioligand binding assay was performed on HEK-293 cells stably expressing human CRTH2 or DP. To measure binding, [^{3}H]-PGD_2 was incubated together with 293 (hCRTH2 or hDP) cells in the presence of increasing concentrations of compounds. After teh cells were washed, 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).

(25) Human whole blood was drawn into acid-citrate-dextrose (ACD) anticoagulated tubes, treated with compounds or DMSO, and then stimulated with a dose response of PGD_2. Fluorochrome conjugated antibodies were used to label CRTH2 positive granulocytes, and CRTH2 receptor internalization was monitored by flow cytometry. The K_b was determined using the Schild equation.

(26) Human whole blood was drawn into ACD vacutainer tubes, treated with compounds or DMSO, and then stimulated with a dose response of PGD_2. Cells were lysed, and cAMP was measured using a competitive ELISA. Comparison of the dose response to PGD_2 in samples containing DMSO only and samples containing compounds was used in determining K_b using the Schild equation.

(27) Hamelman, E.; Schwarze, J.; Takeda, K.; Oshiba, A.; Larsen, G. L.; Irvin, G.; Gelfand, G. W. *Am. J. Respir. Crit. Care Med.* **1997**, *156*, 766–775.