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Discovery of novel and potent CRTH2 antagonists

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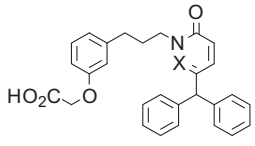
ABSTRACT

High throughput screening of our chemical library for CRTH2 antagonists provided a lead compound **1a**. Initial optimization of the lead led to the discovery of a novel, potent and orally bioavailable CRTH2 antagonist **17**.

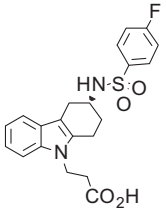
Mast cells, which play important roles in allergic diseases, are activated by immunoglobulin E (IgE). Activated mast cells produce a variety of inflammation mediators, of which prostaglandin D2 (PGD_2) is representative.[1] It has been reported that antigen-challenge-induced PGD_2 production is promoted in the airway of asthmatic patients[2] and that overexpression of PGD synthase enhances airway eosinophil infiltration and Th2 cytokine production in an asthma model.[3] These reports indicate that PGD_2 is closely related to the pathogenesis of allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis.

Although PGD_2 was initially considered to elicit its biological actions through a classical PGD_2 receptor (DP1), later findings suggested that several PGD_2-mediated actions of eosinophils arise via DP2,[4,5] which is also known as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). CRTH2 is expressed on inflammatory cells, such as Th2 cells,[6] eosinophils and basophils, and induces the chemotaxis of these cells.[7] CRTH2 also plays important roles in cytokine release by Th2 cells[8] and in the degranulation of eosinophils.[9] CRTH2 antagonists are therefore expected to be useful as anti-inflammatory agents in the treatment of patients with allergic diseases.[10]

High throughput screening (HTS) of our chemical library for CRTH2 antagonists identified benzhydryl pyridone compound **1a** as a hit (Fig. 1), which inhibited the binding of ^{3}H-labeled PGD_2 to human and guinea pig CRTH2 receptors on HEK293 cells with IC_{50} values of 42 and 256 nM, respectively.[11] Moreover, **1a** proved to be selective over DP1[12] (IC_{50} > 1000 nM to human DP1) and displayed oral absorption in guinea pigs, with oral dosing of **1a** at 10 mg/kg leading to a maximum plasma concentration (C_{max}) of



1a: X = CH
b: X = N



Ramatroban

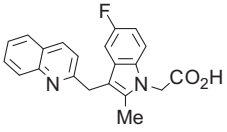


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Figure 1. Chemical structures of HTS hit **1a**, **1b** and known CRTH2 antagonists.

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0.84 μg/mL and area under the blood concentration-time curve (AUC) of 5.95 μg·h/mL.[13] In addition, a similar pyridazinone compound **1b** was discovered from further HTS. The pyridazinone **1b** also showed CRTH2 inhibitory activity and moderate oral absorption, that is, dosing of **1b** (10 mg/kg) to guinea pigs showed a similar C_{max} (0.73 μg/mL) to **1a** and 2.5-fold decrease in AUC (2.40 μg·h/mL). Although a number of CRTH2 antagonists have been reported to date, including Ramatroban[14] and indole acetic acids exemplified with compound **2**[15] (Fig. 1), pyridone or pyridazinone scaffolds like **1a** or **1b** are not known as CRTH2 antagonists and **1a** was accordingly selected as a first lead compound. Given that it was discovered by HTS, no information on the structure–activity relationships (SAR) for this compound was available. We therefore attempted to optimize compound **1a** in order to obtain SAR and to improve its activity and pharmacokinetic properties. In this paper, we describe our initial optimization efforts and discovery of novel analogs with higher potency for CRTH2.

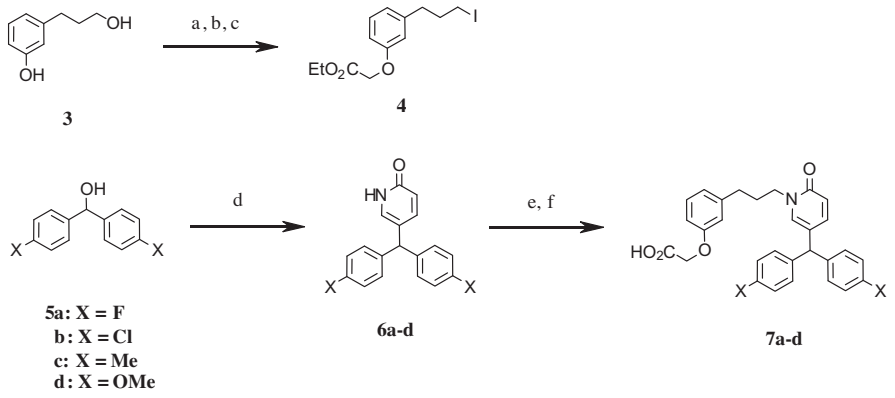
While compound **1a** had moderate inhibitory activity against human CRTH2 receptor, its activity against guinea pig CRTH2 was relatively weak at only one-sixth that in humans. The common use of a guinea pig hyperresponsiveness model to examine anti-asthmatic activity in vivo mandated that we enhance inhibitory activity against not only human but also guinea pig CRTH2.

The synthetic routes to 4,4′-substituted benzhydryl derivatives are shown in Scheme 1. 3-(3-Hydroxypropyl)phenol **3** was alkylated with ethyl bromoacetate in the presence of potassium carbonate in acetonitrile. The resulting alcohol was converted to the corresponding mesylate, followed by displacement with sodium iodide in acetone to afford the alkyl iodide **4**. *p*-Substituted diphenylmethanols **5** were converted to diphenylmethylpyridones **6** in the presence of sulfuric acid at 180–250 °C. Pyridones **6** were alkylated with the alkyl iodide **4** in the presence of lithium hydride in *N*,*N*-dimethylformamide, followed by hydrolysis to give **7a**–**d**.

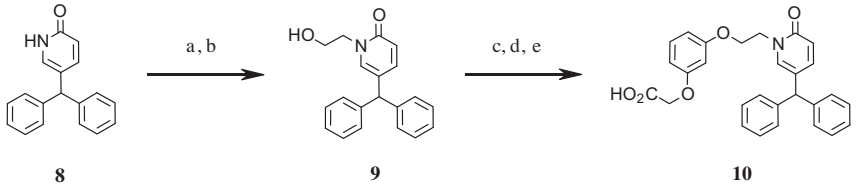
The ether-linked compound **10** was synthesized by the routes shown in Scheme 2. A nitrogen atom of pyridone **8** was alkylated with 2-bromoethyl acetate and the resulting acetate was cleaved with sodium hydroxide to afford alcohol **9**. The alcohol was mesylated with methanesulfonyl chloride, followed by alkylation and hydrolysis of the ester to give **10**.

The benzamide derivatives **13a–b** were synthesized as shown in Scheme 3. 3,3-Diphenylpropylamine **11** was alkylated with the iodide **4** to give the secondary amine **12**. Amine **12** was acylated with benzoyl chloride or 4-methoxybenzoyl chloride, followed by hydrolysis of the ester group to afford benzamides **13a–b**.

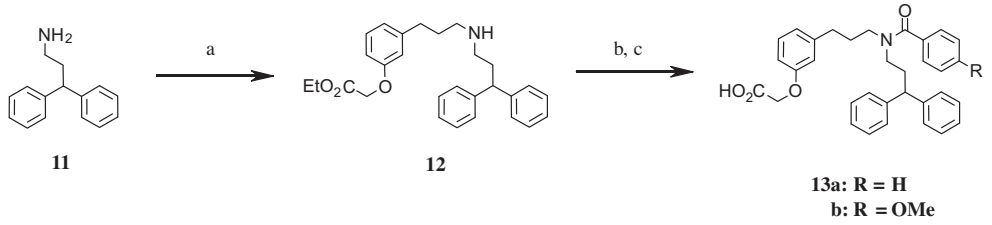
The synthetic routes to convert the acetic acid moiety are outlined in Scheme 4. 3-(3-Hydroxypropyl)phenol **3** was converted



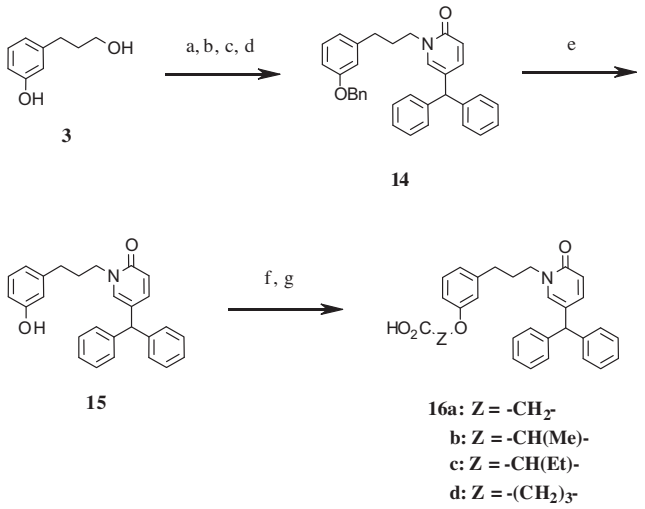
Scheme 1. Reagents and conditions: (a) ethyl bromoacetate, K_2CO_3, MeCN, rt, 24 h; (b) methanesulfonyl chloride, Et_3N, CH_2Cl_2, rt, 1 h; (c) NaI, acetone, rt, 18 h; (d) 2-pyridone, H_2SO_4, 180–250 °C, 2 h; (e) **4**, LiH, DMF, rt, overnight; (f) NaOH aq, MeOH, rt, 1 h.



Scheme 2. Reagents and conditions: (a) 2-bromoethyl acetate, NaH, DMF, rt, 24 h; (b) NaOH aq, EtOH, 5 °C, 1 h; (c) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 5 °C, 2 h; (d) ethyl (3-hydroxyphenoxy)acetate, K_2CO_3, NaI, DMF, 80 °C, 2 h; (e) NaOH aq, EtOH, rt, 2 h.



Scheme 3. Reagents and conditions: (a) **4**, DMF, rt, 8 h; (b) BzCl or 4-MeO–BzCl, Py, CH_2Cl_2, rt, 8 h; (c) NaOH aq, MeOH, rt, 5 h.



Scheme 4. Reagents and conditions: (a) BnBr, K_2CO_3, MeCN, 60 °C, 2 h; (b) MsCl, Et_3N, CH_2Cl_2, rt, 30 min; (c) NaI, acetone, rt, 13 h; (d) **8**, LiH, DMF, 50 °C, 3 h; (e) TFA, 1,2,3,4,5-pentamethylbenzene, rt, 12 h; (f) Br–Z–CO_2Et, K_2CO_3, MeCN, 60 °C, 14 h; (g) NaOH aq, MeOH, rt, 1 h.

to pyridone **14** in four steps, followed by removal of the benzyl group with trifluoroacetic acid in the presence of 1,2,3,4,5-pentamethylbenzene[16] to give phenol **15**. The hydroxyl group of **15** was alkylated with ethoxycarbonyl bromoalkane, followed by hydrolysis of ethyl ester to afford **16a–d**. Compound **17** was prepared by similar procedure to that of compound **7**.

The CRTH2 inhibitory activities of the synthesized compounds are listed in Tables 1 and 2. At first we introduced halogen or other substituents at the 4,4′-position of phenyl rings in the benzhydryl moiety in order to obtain SAR and to improve the metabolic stability at this moiety (**7a–d**). It is well-known that introduction of halogen atom at the *para*-position of the phenyl ring can protect from metabolism.[17] All four *p*-substituted analogs **7a–d** displayed 4–7-fold more potent activity against human CRTH2 than **1a**, but these modifications did not contribute to enhancing activity against guinea pig CRTH2. Specifically, the fluoro (**7a**) and methoxy (**7d**) derivatives were comparable to **1a**, but introduction of the chloro (**7b**) and methyl (**7c**) groups led to a threefold or more loss. These data suggest that only the hydrogen bond acceptor is tolerable to guinea pig CRTH2 whereas a variety of substituents may be acceptable to the human receptor, and accordingly that the introduction of substituents at this position might enhance activity against human CRTH2 but would not improve the species difference between humans and guinea pigs.

To facilitate synthesis and block the metabolically labile benzyl position, we converted the methylene moiety of **1a** into oxygen (**10**). However, **10** showed 2–3-fold less potent inhibitory activity both to human and guinea pig CRTH2. We did not perform further optimization with this linker.

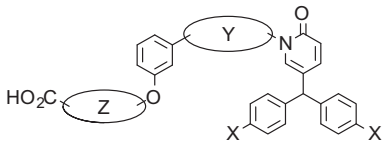
In consideration of planarity around the nitrogen atom, we converted the pyridone scaffold of **1a** to benzamides. The benzamides **13a** and **13b** showed highly potent (nM order) activity with IC_{50} values to human CRTH2 of 9.7 and 5.5 nM, respectively. In addition, these benzamides also displayed a greater than 10-fold improvement in affinity for guinea pig CRTH2. These data suggest that the lipophilicity of the scaffold may cause the enhancement of the CRTH2 inhibitory activity.

Finally, we converted the terminal acetic acid moiety to explore the capability (**16a–d**). Introduction of only one methyl group to the α-position of the carboxyl group enhanced CRTH2 inhibitory activity (**16a**), whereas the activity was drastically diminished in the case of the ethyl (**16b**) or dimethyl (**16c**) groups. Meanwhile, elongation of the methylene chain contributed to the enhancement of activity (**16d**). These data suggest that bulky substituents around the acid moiety are not acceptable. Although the CRTH2 inhibitory activity of **16a** and **16d** are comparable, **16d** is favorable because it is free from the problem of chirality.

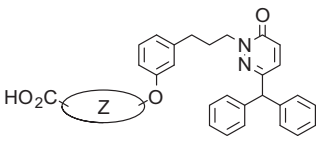
Given that **16d** showed the most potent activity among the pyridone series so far, we accordingly modified the pyridone scaffold of **16d** into pyridazinone **17**. Although the inhibitory activity of **17** against human CRTH2 was comparable to that of **16d**, **17** showed twofold more potent activity in guinea pigs than **16d**.

Compounds **13b**, **16d** and **17**, with high potency compared to **1a** and **1b,** were subjected to pharmacokinetic experiments in guinea pigs as advanced candidates (Table 3). The resulting most potent antagonist **13b** showed poor oral availability. We consider that the poor PK profile of **13b** was due to greater metabolic lability to in vitro clearance in liver microsomes in guinea pigs (766 mL/

Table 1
The CRTH2 inhibitory activities of the leads **1a**, **1b** and their derivatives



1a, 7, 10, 16

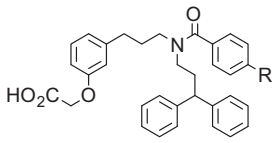


1b, 17

Compound	X	Y	Z	Human CRTH2 IC_{50}[a] (nM)	Guinea pig CRTH2 IC_{50}[a] (nM)
1a	H	$-(CH_2)_3-$	$-CH_2-$	42	256
1b			$-CH_2-$	18	147
7a	F	$-(CH_2)_3-$	$-CH_2-$	11	359
7b	Cl	$-(CH_2)_3-$	$-CH_2-$	9.2	>1000
7c	Me	$-(CH_2)_3-$	$-CH_2-$	6.2	772
7d	OMe	$-(CH_2)_3-$	$-CH_2-$	5.8	211
10	H	$-O(CH_2)_2-$	$-CH_2-$	99	797
16a	H	$-(CH_2)_3-$	$-CH(Me)-$	17	128
16b	H	$-(CH_2)_3-$	$-CH(Et)-$	502	990
16c	H	$-(CH_2)_3-$	$-C(Me)_2-$	NT[b]	>1000
16d	H	$-(CH_2)_3-$	$-(CH_2)_3-$	11	99
17			$-(CH_2)_3-$	13	52

[a] See Ref. 11 for assay protocol. All values are mean of at least two experiments.
[b] Not tested.

Table 2
The CRTH2 inhibitory activities of the benzamide derivatives **13a–b**



13a-b

Compound	R	Human CRTH2 IC_{50}[a] (nM)	Guinea pig CRTH2 IC_{50}[a] (nM)
13a	H	9.7	18
13b	OMe	5.5	17

[a] See Ref. 11 for assay protocol. All values are mean of at least two experiments.

Table 3
Pharmacokinetic parameters of CRTH2 antagonists after p.o. administration to guinea pigs (10 mg/kg)

Compound	Pharmacokinetic parameters in guinea pigs[a]	
	C_{max} (μg/mL)	AUC (μg·h/mL)
1a	0.84	5.95
1b	0.73	2.40
13b	0.04	0.27
16d	0.50	1.02
17	2.86	4.85

[a] See Ref. 13 for assay protocol. All values are mean of $n = 3$ measurements.

min/kg) than **1a** (120 mL/min/kg). The chain elongated compound **16d** showed poorer oral availability than the corresponding lead **1a**. In contrast, pyridazinone **17** displayed excellent oral availability, and dosing (10 mg/kg) to guinea pigs led to an approximately threefold increase in C_{max} (2.86 μg/mL) and comparable AUC (4.85 μg·h/mL) to **1a**.

Evaluating the inhibitory activity of **17** against human DP1 proved that this compound was a selective CRTH2 antagonist (IC_{50} = 5200 nM to human DP1). We next evaluated the in vivo anti-asthmatic activity of compounds **1a** and **17** in a guinea pig model, and found that both showed in vivo efficacy orally in a guinea pig model of airway hyperresponsiveness,[18] with ED_{50} values of 4.7 and 0.05 mg/kg u.i.d., respectively. The in vivo efficacy of **17** was thus drastically improved than that of **1a**.

In summary, we discovered a novel and selective CRTH2 antagonist **1a** from HTS of our chemical library. Initial optimization based on **1a** resulted in the discovery of the novel, potent and orally bioavailable CRTH2 antagonist **17**. We achieved not only a improvement in in vitro CRTH2 antagonistic activity against both human and guinea pig but also a drastic improvement in in vivo efficacy compared to that of **1a**. Further optimization of this series aimed at improving activities and pharmacokinetic properties will be reported later.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.079.

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11. The human or guinea pig CRTH2 stable transfectants were generated by transfecting the pcDNA3.1 expression vector containing human or guinea pig CRTH2 gene into HEK293 cells (purchased from ATCC). They were suspended in binding assay buffer (10 mM BES, 1 mM EDTA, 10 mM $MnCl_2$, pH 7.0) and disrupted by strong mixing using the injection needle. Cell suspension (10 μg protein/well for human CRTH2 assay or 20 μg protein/well for guinea pig CRTH2 assay) and [^{3}H]PGD_2 were mixed in a 96-well round bottom polypropylene plate (Nunc) and incubated for 2 h at 4 °C in the absence or the presence of increasing concentrations of the tested compounds. After incubation, the cell suspension was transferred to a glass-filter plate (GF/B, Perkin–Elmer). Scintillant was added to the filtration plate, and radioactivity remaining on the filter was measured with a scintillation counter, TopCount (Packard Bioscience). Nonspecific binding was determined in the presence of 10 μM DK-PGD_2. The concentration of compounds causing a 50% decrease in the binding of [^{3}H]PGD_2 to the receptor was calculated as [IC_{50}]value.
12. The human DP1 stable transfectants were generated by transfecting the pcDNA3.1 expression vector containing human DP1 gene into HEK293 cells (purchased from ATCC). They were suspended in binding assay buffer (10 mM BES, 1 mM EDTA, 10 mM $MnCl_2$, pH 7.0) and disrupted by strong mixing using the injection needle. Cell suspension (10 μg protein/well for human DP1 assay) and [^{3}H]PGD_2 were mixed in a 96-well round bottom polypropylene plate (Nunc) and incubated for 2 h at 4 °C in the absence or the presence of increasing concentrations of the tested compounds. After incubation, the cell suspension was transferred to a glass-filter plate (GF/B, Perkin–Elmer). Scintillant was added to the filtration plate, and radioactivity remaining on the filter was measured with a scintillation counter, TopCount (Packard Bioscience). Nonspecific binding was determined in the presence of 10 μM PGD_2. The concentration of compounds causing a 50% decrease in the binding of [^{3}H]PGD_2 to the receptor was calculated as [IC_{50}]value.
13. Male Hartley guinea pigs (6 weeks) were purchased from Japan SLC, Inc., (Shizuoka, Japan). Guinea Pigs were fasted overnight prior to dosing. Dosing solution were prepared as a 10 mg/mL suspending solution in 0.5% MC (methylcellulose, COSMO BIO, Tokyo, Japan) in water. Guinea Pigs received 10 mg/kg dose by oral administration. Samples were analyzed in Astellas Analysis & Pharmacokinetics Research Labs.
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18. Male Hartley guinea pigs were actively sensitized to ovalbumin (OVA) by i.p. injection of 20 mg in 0.9% saline at day 0, and of 1 mg at day 2. Animals were exposed for 10 min on days 14–21 to an aerosol of 0.5% OVA solution in 0.9% saline or saline alone generated from a nebulizer. All animals were treated with compound by po administration 60 min prior to the aerosol, and with 1 mg/kg pyrilamine by ip injection 30 min prior to the aerosol. On day 22, animals were anesthetized with urethane (1.5 g/kg). The trachea was cannulated and the animal was mechanically ventilated (60 strokes/minutes; 1 mL/100 g body weight) with a small animal respirator. Pulmonary inflation pressure (PIP) was monitored with a pressure transducer. Doses of methacholine (0–14 mg/kg) were administered sequentially at 3-minute intervals to each animal via the right jugular vein. The area under the peak inflation pressure versus methacholine dose curve (AUC) was calculated for each animal. The doses of compounds causing 50% inhibition of the increase in the AUC were calculated as [ED_{50}] values.