Isoquinoline Derivatives as Potent, Selective, and Orally Active CRTH2 Antagonists

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Synthesis and structure–activity relationship of a novel series of isoquinoline CRTH2 antagonists bearing a methylene linker between the isoquinoline and benzamide moieties were described. Optimization focusing on the substituents of the benzamide portion in the right hand part of the molecule led to the identification of TASP0412098 (9l), which is a potent, selective CRTH2 antagonist (binding affinity: IC_{50}=2.1 nM, functional activity: IC_{50}=12 nM). Compound 9l, which was orally bioavailable in mice and guinea pigs, showed *in vivo* efficacy after oral administration in a bronchial asthma model of guinea pigs.

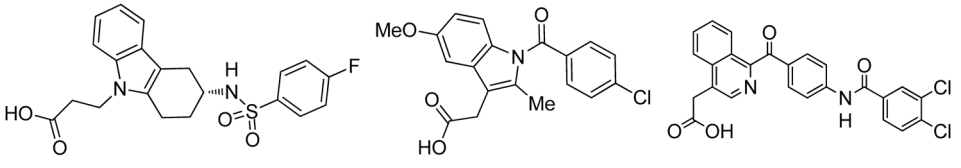
Key words CRTH2 antagonist; allergic diseases; isoquinoline; prostaglandin D2

The chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTH2) was reported in 2001 as a G protein-coupled receptor for prostaglandin D2 (PGD2). CRTH2 is expressed on eosinophils, basophils, and Th2 cells, and plays an important role in allergic diseases, driving the immunoglobulin E (IgE) response, eosinophilia, and the release of proinflammatory cytokines.[1–3] Activation of CRTH2 promotes the release of histamine from basophils and degranulation of eosinophiles.[4–6] Thus, a CRTH2 antagonist might be beneficial for a variety of inflammatory diseases.[7,8] Research designed to identify selective CRTH2 antagonists dramatically increased after the discovery that the thromboxane receptor antagonist, ramatroban, which is approved for the treatment of allergic rhinitis, and the anti-inflammatory drug, indomethacin, were found to be CRTH2 antagonists. A large number of structure–activity relationship (SAR) information around the indoleacetic acids have been disclosed.

In our previous paper, we reported that the lead generation process and initial SAR study of a novel isoquinoline chemotype of CRTH2 antagonists, led to the identification of compound **1** (binding affinity: IC_{50}=19 nM, functional activity: IC_{50}=13 nM) as a potent and selective CRTH2 antagonist.[9,10] In our continuous research focused on identifying an alternative lead class, we took particular note of the carbonyl linker of **1**, because the modification of this linker would impact the physicochemical properties of **1**. We designed novel CRTH2 antagonists bearing a methylene linker in place of the carbonyl linker, expecting to change the biological profile of this chemotype by changing its physicochemical properties. In the present study, we described the synthesis and SAR of isoquinoline CRTH2 antagonists containing a methylene linker, and the preliminary pharmacokinetic and pharmacological profiles of the potent, selective, and orally active CRTH2 antagonist **9l**, which is the most potent compound of this class.

Chemistry Preparation of isoquinoline derivatives **9a**–**9u** is outlined in Chart 1. The ylide **3**, derived from commercially available benzyl bromide **2**, was treated with methyl 1-chloroisoquinoline-4-carboxylate in the presence of sodium hexamethyldisilazane, followed by hydrolysis, to give **4** in 58% yield.[11] The carboxylic acid **5**, prepared by the basic hydrolysis of **4**, was converted into the corresponding acid chloride, which was treated with trimethylsilyldiazomethane to afford the diazoketone intermediate. Wolff rearrangement of the diazoketone intermediate followed by methylation of the resulting carboxylic acid moiety with trimethylsilyldiazomethane provided **6** in 35% yield from **5**.[12] Deprotection of the *t*-butoxycarbonyl group of **6** under an acidic condition (trifluoroacetic acid, TFA), and subsequent acylation of the resulting acid chloride of **7** with appropriate amines gave **8a**, **8l**, **8o**, and **8s**. Compound **8b**–**8k**, **8m**, **8n**, **8p**–**8v**, **8t**, and **8u** were obtained by coupling the acid **7** with appropriate amines using a standard protocol (WSC·HCl, HOBT·H_2O). Finally, hydrolysis of the ester moieties of **8a**–**8u** afforded the target isoquinoline derivatives **9a**–**9u**.

Compounds **9v**, **9w**, dl-**9x**, and **9y**, in which the acetic acid moiety at the 4-position of the isoquinoline core is modified,



Ramatroban Indomethacin **1**

Fig. 1. Ligands for the CRTH2 Receptor

The authors declare no conflict of interest.

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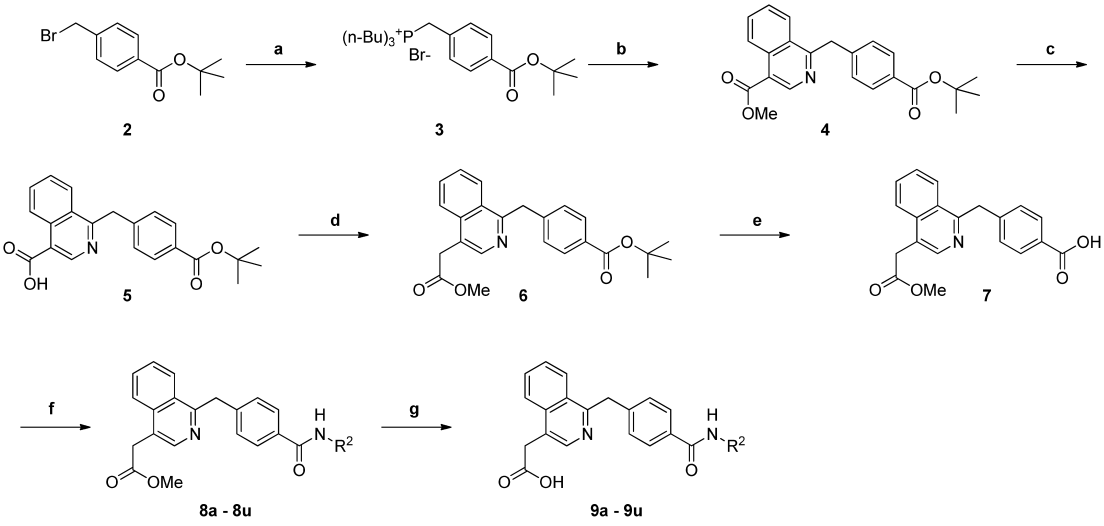
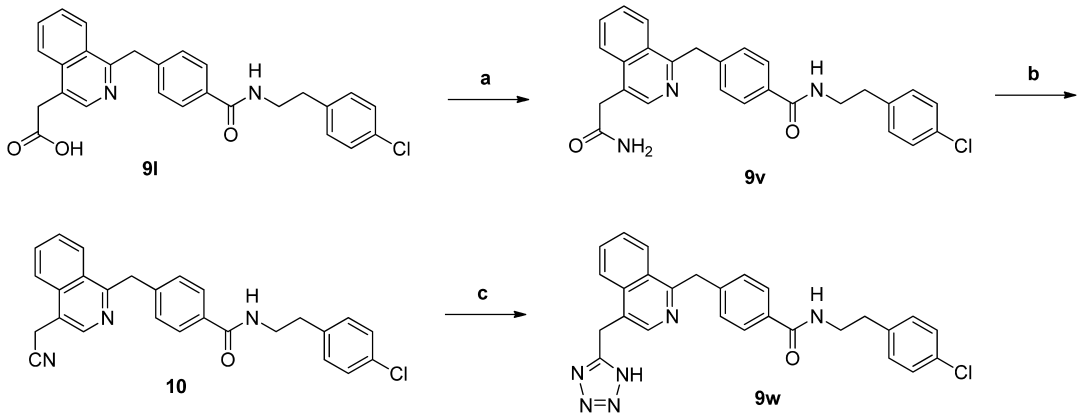


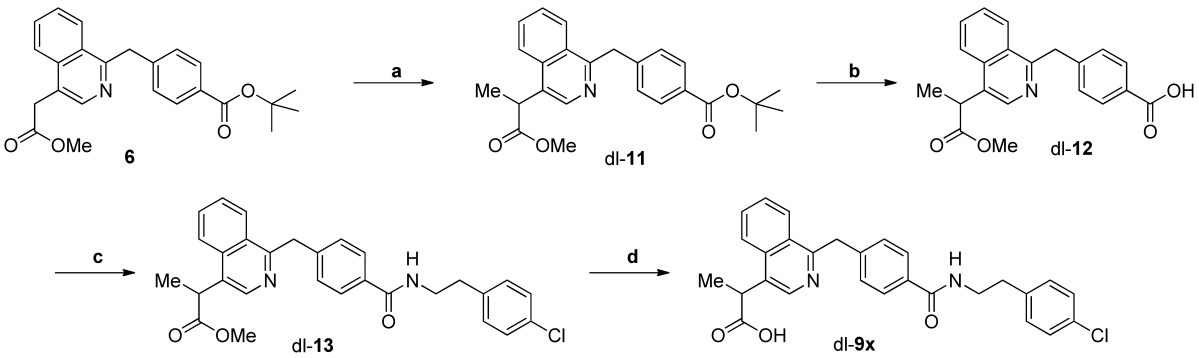
(a) *n*-Bu_3P, toluene, 60°C, 93%; (b) methyl 1-chloroisoquinoline-4-carboxylate, NaHMDS, THF, −30–50°C, then Na_2CO_3 aq., 60°C, 58%; (c) 1 N NaOH aq., THF, 50°C, quant; (d) $(COCl)_2$, $CHCl_3$, rt, $TMSCHN_2$, THF/MeCN, rt, silver acetone, H_2O/dioxane, 60°C then $TMSCHN_2$, MeOH, rt, 35% (4 steps); (e) TFA, $CHCl_3$, 0°C-rt, quant; (f) for **8a**, **8l**, **8o**, **8s**, $(COCl)_2$, DMF, $CHCl_3$ then R^2NH_2, pyridine, 0°C-rt, 23–93%, for **8b**–**k**, **8m**, **8n**, **8p**–**v**, **8t**, **8u**, R^2NH_2, WSC·HCl, HOBT·H_2O, Et_3N, $CHCl_3$, rt, 36–80%; (g) 1 N NaOH aq., THF, 0°C, 19–90%.

Chart 1



(a) CDI then NH_3 aq., THF, rt, 96%; (b) $POCl_3$, DMF, rt, 74%; (c) NaN_3, Et_3N–HCl, 14%.

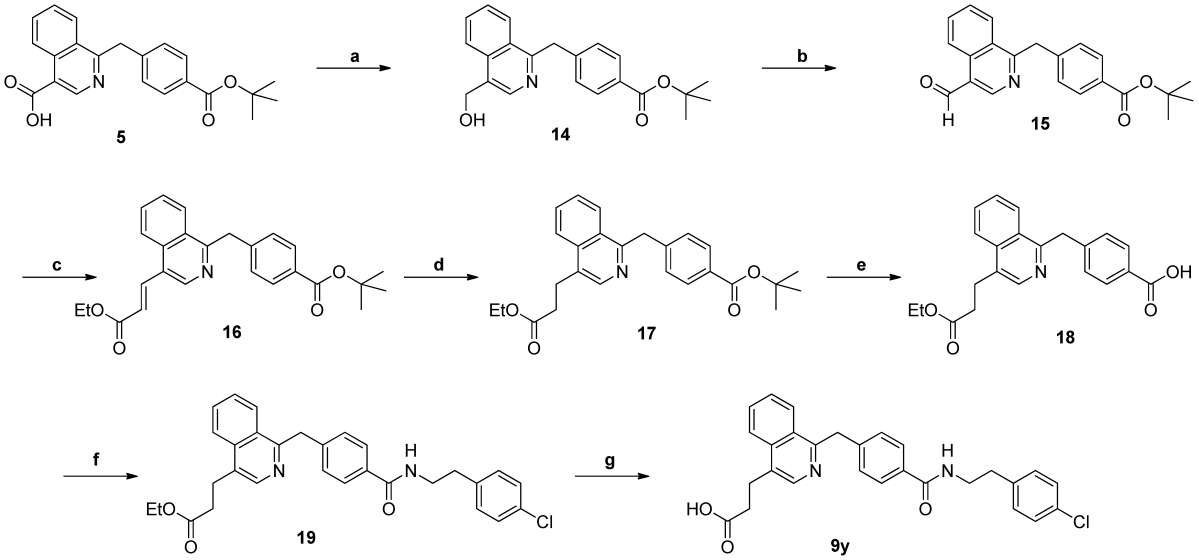
Chart 2



(a) *t*-BuOK, MeI, THF, 0°C, 61%; (b) TFA, $CHCl_3$, 0°C-rt; (c) 2-(4-chlorophenyl)ethane amine, WSC·HCl, HOBT·H_2O, Et_3N, $CHCl_3$, rt, 58% (2 steps); (d) 1 N NaOH aq., THF, 0°C, 71%.

Chart 3

were synthesized as shown in Charts 2, 3, and 4. Carbamoylation of compound **9l** afforded compound **9v** in 96% yield. Following the reaction of **9v** with phosphorus oxychloride, cyclization to the tetrazole was achieved using sodium azide to give compound **9w**. Methylation of **6** using *t*-BuOK and iodomethane produced dl-**11** in 61% yield. Deprotection of the *t*-butyl ester moiety using trifluoroacetic acid (TFA), followed by the condensation of dl-**12** with 2-(4-chlorophenyl)ethanamine



(a) BH_3-THF, THF, 0°C-rt, 39%; (b) Dess–Martin periodinane, $CHCl_3$, 0°C, 85%; (c) ethyl 2-(dithoxyphosphoryl)acetate, NaH, 0°C, 82%; (d) $NaBH_4$, $CuCl_2$, MeOH, rt, 0°C, 60%; (e) TFA, $CHCl_3$, 0°C-rt, quant; (f) 2-(4-chlorophenyl)ethaneamine, WSC·HCl, HOBT·H_2O, THF, rt, 82%; (g) 1 n NaOH aq., THF, 0°C-rt, 83%.

Chart 4

(WSC·HCl, HOBT·H_2O) gave dl-**13** in 58% yield. Finally, hydrolysis of the ester moiety afforded the target derivative dl-**9x**. Compound **9y** was synthesized from the intermediate **5**, which was reduced by borane–tetrahydrofuran complex to give alcohol **14**. Subsequent oxidation with Dess–Martin periodinane, followed by olefination of the aldehyde, afforded **16**. Compound **16** was reduced by sodium borohydride to give **17**, which was converted to **9y** using the following conventional method: (1) deprotection of the *t*-butoxycarbonyl group of **17** using TFA; (2) condensation reaction with 2-(4-chlorophenyl)-ethanamine (WSC·HCl, HOBT·H_2O); and (3) hydrolysis of the ethyl ester moiety.

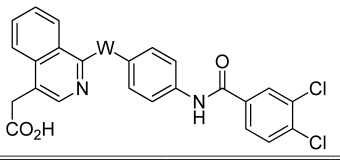
Results and Discussion

Compounds were tested in a radioligand binding assay using ^{3}H-PGD_2 and human CRTH2 stably transfected in CHO cells. Their functional activities were assessed in PGD_2 driven Ca^{2+} flux assay in KB8 cells expressing human CRTH2.

Initially, the binding affinity and the functional activity of the methylene linker compound **9a** were compared with those of the original carbonyl linker compound **1** (Table 1). These results suggested that both compounds were potent CRTH2 antagonists. And compound **1** and compound **9a** shows different physicochemical properties. For example, with regard to the aqueous solubility (20 mM phosphate buffer), **9a** showed much lower solubility than **1** (**9a**: 1.2 mg/mL, **1**: 540 mg/mL). In addition, comparison of the log*P* values at pH 6.8 revealed that **9a** is more hydrophobic than **1** (**9a**: 3.1, **1**: 2.7). These data suggested that the change of this linker part would have an impact on the physicochemical properties as we had expected.

With these data in hand, we conducted the SAR study of the methylene linker compounds, focusing on the effect of the R^2 substituent of the benzamide moiety on the CRTH2 binding affinity and functional activity (Table 2). When the methyl group in **9b**, which showed moderate potency (IC_{50}=360 nM) in the binding assay, was replaced with a cycloalkylethyl moiety such as a cyclopropylethyl (**9c**), cyclohexylethyl (**9d**),

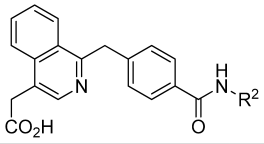
Table 1. *In Vitro* Data of Isoquinoline Derivatives with Linker Modifications



Compound	W	IC_{50} (nM)[a]	
		Binding	Ca^{2+}
1	CO	19	13
9a	CH_2	8.3	15

a) Mean values from at two or more independent experiments. IC_{50} values were determined from full ten-point, half-log concentration–response curves.

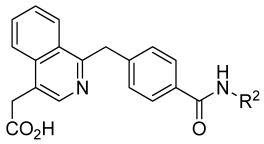
Table 2. *In Vitro* Data of Isoquinoline Derivatives with R^2 Substitution Modifications



Compound	R^2	IC_{50} (nM)[a]	
		Binding	Ca^{2+}
9b	Methyl	360	>1000
9c	Cyclopropylethyl	16	141
9d	Cyclohexylethyl	3.7	29
9e	Adamantylethyl	14	6.6
9f	2-(Tetrahydro-2*H*-pyran-4-yl)ethyl	35	>1000
9g	2-(Morpholin-4-yl)ethyl	>1000	>1000
9h	Phenethyl	4.6	110
9i	2-(Pyridin=4-yl)ethyl	320	>1000

a) Mean values from at two or more independent experiments. IC_{50} values were determined from full ten-point, half-log conentration–response curves.

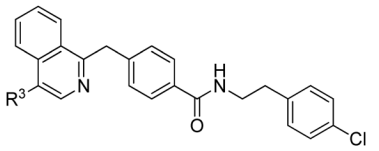
Table 3. *In Vitro* Data of Isoquinoline Derivatives with R^2 Substitution Modifications



Compound	R^2	IC_{50} (nM)[a]	
		Binding	Ca^{2+}
9h	Phenethyl	4.6	110
9j	2-Cl-phenythyl	2.4	28
9k	3-Cl-phenethyl	3.8	21
9l	4-Cl-phenethyl	2.1	12
9m	4-Me-Phenethyl	4.8	17
9n	4-NMe_2-phenethyl	10	210
9o	4-OMe-phenethyl	19	52
9p	4-OH-phenethyl	9.0	510
9q	4-SO_2Me-phenethyl	180	>1000
9r	4-CO_2H-phenethyl	900	>1000
9s	4-Cl-benzyl	27	400
9t	3-(4-Cl-phenyl)propyl	6.5	42
9u	2-(4-Cl-phenoxy)ethyl	7.7	250

a) Mean values from at two or more independent experiments. IC_{50} values were determined from full ten-point, half-log concentration–response curves.

Table 4. *In Vitro* Data of Isoquinoline Derivatives with R^3 Substitution Modifications



Compound	R^3	IC_{50} (nM)[a]	
		Binding	Ca^{2+}
9l		2.1	12
9v		>1000	>1000
9w		590	>1000
dl-**9x**		34	790
9y		28	54

a) Mean values from two or more independent experiments. IC_{50} values were determined from full ten-point, half-log concentration–response curves.

or adamantylethyl (**9e**), these moieties contributed greatly to enhancing not only the binding affinity but also the functional activity. The 2-(tetrahydro-2*H*-pyran-4-yl)ethyl group (**9f**) showed only moderate binding affinity (IC_{50}=35 nM), while the 2-(morpholin-4-yl)ethyl group (**9g**) resulted in the complete loss of potency. These data suggested that the R^2 group does not accept hydrophilic substituents. Interestingly, the phenethyl (**9h**) group led to greatly enhanced binding affinity, (IC_{50}=4.6 nM), although the functional activity was not sufficient (IC_{50}=110 nM).

Further SAR study on **9h** was conducted to examine the effect of substituents of the terminal phenyl ring on the CRTH2 binding and functional activities (Table 3). Incorporation of a chlorine atom into the 2-, 3-, or 4-position on the phenyl group led to an increase not only in the binding potency (**9j**: IC_{50}=2.4 nM, **9k**: IC_{50}=3.8 nM, **9l**: IC_{50}=2.1 nM) but also in the functional assay (**9j**: IC_{50}=28 nM, **9k**: IC_{50}=21 nM, **9l**: IC_{50}=12 nM). With regard to the substituent at the 4-position, methyl (**9m**), dimethylamino (**9n**), methoxy (**9o**), and hydroxyl (**9p**) groups were found to be tolerated in the binding affinity (**9m**: IC_{50}=4.8 nM, **9n**: IC_{50}=10 nM, **9o**: IC_{50}=19 nM, **9p**: IC_{50}=9.0 nM), while dimethylamino (**9n**) and hydroxyl (**9p**) resulted in a decrease in the functional activity compared to **9h**. Installation of a methylsulfonyl (**9q**) or a carboxylic acid (**9r**) at this position resulted in a significant loss in both the binding affinity and functional activity. Regarding the linker part between the amide and the terminal 4-chlorophenyl group of **9l**, replacement of the ethylene linker with a methylene (**9s**), propylene (**9t**), or oxyethylene (**9u**) linker led to slight losses in both the binding affinity and functional activity, compared to those of **9l**. These data suggest that a two-carbon linker is the most favorable.

Following this, we examined the rough SAR of the carboxylic acid moiety, which is commonly shared among the representative CRTH2 antagonists (Table 4). Replacement of the carboxylic acid with its isosteres, such as a carbamoyl and tetrazole revealed that the carbamoyl led to the loss of binding activity (**9v**: IC_{50}=>1000 nM, **9w**: IC_{50}=590 nM). Methylation of the methylene moiety next to the carboxylic acid of **9l** resulted in a 17-fold drop in the binding affinity compared with the original compound. And the carboxymethyl moiety in **9l** was replaced with the corresponding carboxyethyl group, the binding affinity of the resulting compound **9y** was decreased by a factor of 13, although both compounds were tolerable (dl-**9x**: IC_{50}=34 nM, **9y**: IC_{50}=28 nM). These data suggest that the carboxylic acid moiety is essential for CRTH2 activity and the binding space, where the acid moiety of the antagonists interacts with the CRTH2, is limited.

Table 5. Pharmacokinetic Parameters of **1** and **9l** Following Oral Administration to Preclinical Species

Compound	**1**	**9l**	
Species	Mouse	Mouse	Guinea pig
Dose (mg/kg)	25	10	30
C_{max} (nm)	1600	2200	540
T_{max} (h)	0.50	0.50	0.75
$T_{1/2}$ (h)	1.7	2.0	6.5
AUC_{0-24h} (ng h/mL)	510	1400	1500

Thus, compound **9l** was selected as one of the most potent antagonists of this chemotype. The preliminary physicochemical study indicated that **9l** had moderate aqueous solubility (14 μg/mL in 20 mM phosphate buffer at pH 6.8) and its log*P* value (pH 6.8) was 2.4. The selectivity for the binding of **9l** to CRTH2 over not only DP1, TP, EP1, FP, and IP (prostanoid receptors), but also BLT1, cysLT1, and cysLT2 (leukotriene receptors) was tested (IC_{50}: >10 μM),[13] revealing that this compound is a highly selective CRTH2 antagonist. Furthermore,

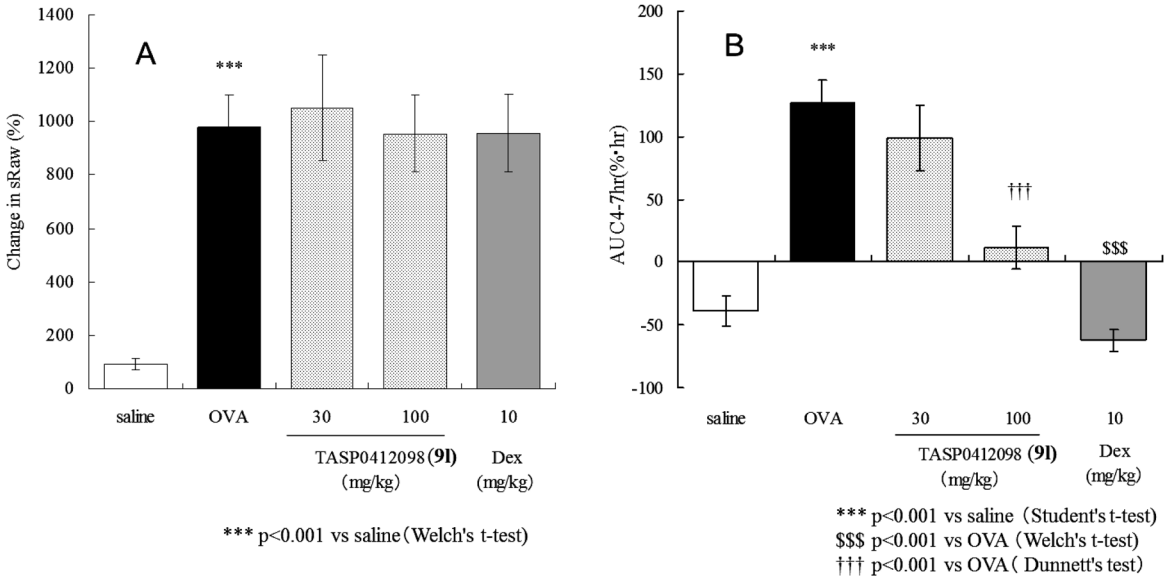


Fig. 2. Effect of TASP0412098 (**9l**) on Antigen-Induced 1AR (A) and LAR (B) in Guinea Pigs

sRaw was measured before, 1 min, 2, 4, 5, 6 and 7 h after OVA challenge. The results are expressed as IAR (1 min after the challenge) (a) and the LAR (*AUC* of 4–7 h after the challenge; AUC_{4-7h}) (B). Values represent means±S.E.M. (n=17–20). Dex, dexamethasone.

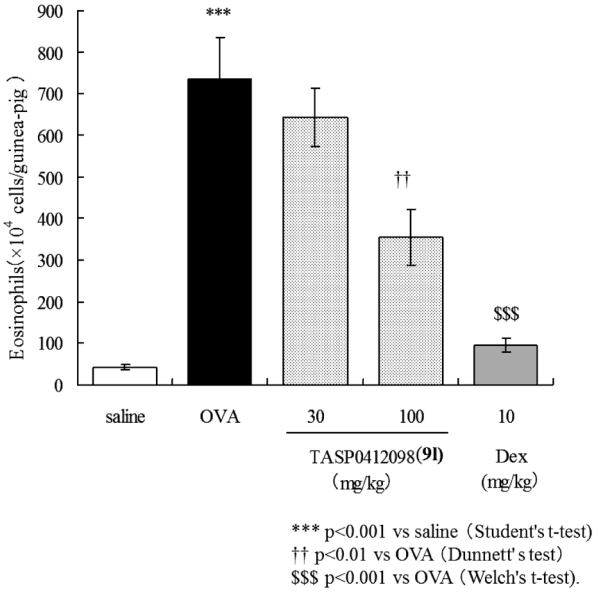


Fig. 3. Effect of TASP0412098 (**9l**) on Antigen-Induced Increase in Eosinophil Counts in the BALF of Guinea-Pig

Values represent means±S.E.M. (n=17–20). Dex, dexamethasone.

9l was effective in chemotaxis assay (IC_{50}=23 nM). A mouse pharmacokinetic (PK) study of **1** and **9l** indicated that **9l** had a longer half-life and higher exposure than **1**. In addition, a guinea pig PK study suggested that **9l** gave acceptable plasma exposure for an *in vivo* pharmacological study (Table 5).

Given the favorable *in vitro* profile of **9l** and acceptable PK profile, compound **9l** was assessed in the ovalbumin (OVA)-induced asthmatic model. Inhalational OVA challenge led to a two-phase airway response at 1 min [immediate airway response (IAR)] and at 4–7 h [late airway response (LAR)] after the OVA challenge in OVA-sensitized guinea pigs, compared with the airway response obtained from OVA-sensitized and saline-challenged guinea pigs. Inhibitory effects on IAR were not observed at any dose of **9l** (Fig. 2A), while the remarkable inhibitory effects on LAR were observed at 100 mg/kg of **9l** (Fig. 2B). The discrepancy between the *in vitro* functional activity (IC_{50}: 12 nM) and *in vivo* effects (100 mg/kg, oral administration) may be due to the high protein binding ratio of **9l** (>99.4% in humans). More specifically the free fraction of **9l** in the plasma at 30 mg/kg (C_{max}: 540 nM) might not rise to the level needed for eliciting the *in vivo* effects. At 24 h after OVA challenge, a significant increase in the total number of cells and the number of macrophages, eosinophils, and neutrophils was observed in the bronchoalveolar lavage fluid (BALF). Compound **9l** significantly inhibited the migration of eosinophils into the BALF in a dose-dependent manner (30, 100 mg/kg), and significant inhibitory effects were observed after oral administration at 100 mg/kg (Fig. 3). These data suggest that CRTH2 has an important role in bronchial asthma, and compound **9l** may be useful as therapeutic drug for bronchial asthma.

Conclusion

In our continuing research focused on identifying an alternative lead class, novel CRTH2 antagonists bearing a methylene linker in place of the carbonyl linker were designed and synthesized. These compounds were expected to change the biological profile of this class. As a result of SAR studies of the methylene linker isoquinoline CRTH2 antagonists, we identified the potent, selective, and orally active CRTH2 antagonist **9l**. A pharmacological study revealed that **9l** showed anti-asthma effect in the bronchial asthma model using guinea pigs at 100 mg/kg, suggesting that CRTH2 has an important role in bronchial asthma. Based on these results, compound **9l** (TASP0412098) is a promising lead compound that warrants further preclinical development.

Experimental

Chemistry All starting materials and reagents were commercial products that were used without further purification. The reaction progresses were usually monitored by TLC using Merck silica gel 60 F_{254} plates or Fuji Silysia chroma-

torex NH plates. Column chromatography was performed using silica gel Wako Pure Chemical Industries, Ltd. C-200 and NH-silica gel Fuji Silysia chromatorex DM1020. Melting points were determined on a Mettler FP-61 or a Yanaco MP-500D melting point apparatus and were uncorrected. ^{1}HNMR spectra were recorded on a Varian Gemini-600 instrument at 600 MHz. Chemical shifts were reported in parts per million as δ units relative to tetramethylsilane as an internal reference. Multiplicity was defined as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet), or brs (broad singlet). Mass spectra (MS) were recorded on a Shimadzu LCMS-2010EV mass spectrometer with electrospray ionization (ESI)/atmospheric pressure chemical ionization (APCI) dual source. Elemental analyses were performed on a Yanaco MT-6 elemental analyzer, and the results were within ±0.4% of the calculated values.

Tributyl-[[4-[(2-methylpropan-2-yl)oxycarbonyl]phenyl]-methyl]phosphanium Bromide (3) To a solution of *tert*-butyl 4-(bromomethyl)benzoate (22.1 g, 81.6 mmol) in toluene (440 mL) was added tri-*n*-butyl phosphine (30.5 mL, 121 mmol), and the mixture was stirred at 60°C for 70 min. The reaction mixture was evaporated and the residue was added *n*-hexane. The mixture was stirred and the resulting precipitate was collected by filtration, and dried to yield **3** (36.0 g, 93%) as a colorless powder: ^{1}H-NMR ($CDCl_3$) δ: 0.93–0.98 (m, 9H), 1.44–1.52 (m, 12H), 1.60 (s, 9H), 2.37–2.46 (m, 6H), 4.34–4.40 (m, 2H), 7.50–8.02 (m, 4H); MS (ESI/APCI Dual) *m/z* 393 $(M+H)^+$.

Methyl 1-[[4-[(2-Methylpropan-2-yl)oxycarbonyl]phenyl]-methyl]isoquinoline-4-carboxylate (4) A 1.9 M of NaHMDS in THF (2.60 mL, 2.60 mmol) was added to a solution of **3** (33.8 g, 71.4 mmol) and methyl 1-chlorosioquinoline-4-carboxylate (11.3 g, 60.0 mmol) in THF (235 mL) at −30°C, and the mixture was stirred for 15 min and at room temperature for 45 min. After stirring 50°C for 75 min, to the mixture was added a sodium carbonate solution (10.8 g in 120 mL) and the mixture was stirred at 60°C for 2 h. The reaction was acidified (pH=5) by adding 1 N HCl, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 10–30% EtOAc/hexane to give **4** (11.1 g, 58%) as orange oil: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (s, 9H), 4.02 (s, 3H), 4.76 (s, 2H), 7.27–8.98 (m, 8H), 9.14 (s, 1H); MS (ESI/APCI Dual) *m/z* 378 $(M+H)^+$.

1-[[4-[(2-Methylpropan-2-yl)oxycarbonyl]phenyl]methyl]-isoquinoline-4-carboxylic Acid (5) To a solution of **4** (11.8 g, 29.5 mmol) in THF (150 mL) was added 1 N NaOH (150 mL), and the mixture was stirred at 50°C for 2.5 h. The mixture was acidified with 1 N HCl, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated to give **5** (11.1 g, quant) as a pale orange powder: ^{1}H-NMR ($CDCl_3$) δ: 1.50 (s, 9H), 4.76 (s, 2H), 7.38–8.99 (m, 9H); MS (ESI/APCI Dual) *m/z* 364 $(M+H)^+$.

***tert*-Butyl 4-[[4-(2-methoxy-2-oxoethyl)isoquinolin-1-yl]-methyl]benzoate (6)** To a suspension of **5** (11.1 g, 29.5 mmol) in $CHCl_3$ (265 mL) was added oxalyl chloride (5.1 mL, 59.0 mmol) and DMF (1 drop) at 0°C, and the mixture was stirred for 70 min. After evaporation of the volatile in the reaction mixture, the residue was dissolved in a mixture of THF (133 mL) and CH_3CN (133 mL). To this mixture was added a 2.0 M of $TMSCHN_2$ in CH_2Cl_2 (29.5 mL, 59.0 mmol) at 0°C, and the resulting mixture was stirred for 3 h. Then the reaction mixture was concentrated under reduced pressure. To a solution of the residue in a mixture of 1,4-dioxane (133 mL) and H_2O (133 mL) was added silver acetate (2.95 g, 17.6 mmol), and the mixture was stirred at 60°C for 45 min. After cooling, the mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was dissolved in MeOH (265 mL), and a 2.0 M of $TMSCHN_2$ in CH_2Cl_2 (44.2 mL, 88.4 mmol) was added to this mixture. After being stirred for 20 min, the reaction was quenched by adding a few drops of AcOH. The mixture was diluted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60N) eluting with 10–40% EtOAc/hexane to give **6** (4.07 g, 35% from **5**) as brown oil: ^{1}H-NMR ($CDCl_3$) δ: 1.50 (s, 9H), 4.76 (s, 2H), 7.38–8.99 (m, 9H); MS (ESI/APCI Dual) *m/z* 392 $(M+H)^+$.

4-[[4-(2-Methoxy-2-oxoethyl)isoquinolin-1-yl]methyl]benzoic Acid (7) To a solution of **6** (4.07 g, 10.4 mmol) in $CHCl_3$ (40 mL) was added TFA (20 mL) at 0°C, and the mixture was stirred at room temperature for 3.5 h. The reaction mixture was basified with 1 N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give **7** (3.42 g, 98%) as a pale brown powder. This compound was used for the next reaction without further purification: ^{1}H-NMR ($CDCl_3$) δ: 3.62 (s, 3H), 4.13 (s, 2H), 4.71 (s, 2H), 7.39–8.36 (m, 8H), 8.37 (s, 1H), 12.79 (brs, 1H); MS (ESI/APCI Dual) *m/z* 336 $(M+H)^+$.

Methyl 2-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]acetate (8l) To a suspension of **7** (2.69 g, 8.02 mmol) in $CHCl_3$ (40 mL) was added oxalyl chloride (1.07 mL, 12.0 mmol) and DMF (3 drop) at 0°C, and the mixture was stirred for 1 h. After evaporation of the volatile in the reaction mixture, the residue was dissolved in $CHCl_3$ (40 mL). To this mixture was added 2-(4-chlorophenyl)-ethylamine (1.68 mL, 12.0 mmol) and pyridine (0.973 mL, 12.0 mmol) at 0°C, and the resulting mixture was stirred for 1 h. Then the reaction was quenched by adding a saturated ammonium chloride solution, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (NH) eluting with 10–100% EtOAc/hexane to give **8l** (1.31 g, 35%) as a pale yellow powder: ^{1}H-NMR ($CDCl_3$) δ: 2.87 (t, J=6.9 Hz, 2H), 3.61–3.67 (m, 2H), 3.70 (s, 3H), 4.01 (s, 2H), 4.68 (s, 2H), 6.04 (t, J=5.3 Hz, 1H), 7.11–8.13 (m, 12H), 8.40 (s, 1H); MS (ESI/APCI Dual) *m/z* 473 $(M+H)^+$.

2-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]acetic Acid (9l) To a solution of **8l** (1.31 g, 2.77 mmol) in THF (55 mL) was added 1 N NaOH (14 mL) at 0°C, and the mixture was stirred for 2.5 h at room temperature. The mixture was acidified with diluted hydrochloric acid and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 0–15% $CHCl_3$/MeOH. The resulting powder was added ethanol (250 mL) and the mixture was stirred at room temperature for 17 h. Then the resulting precipitate was collected by filtration, and dried to yield **9l** (944 mg,

74%) as a pale yellow powder: mp 184.0–185.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.79 (t, J=7.3 Hz, 2H), 3.39–3.46 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 7.20–8.43 (m, 14H), 12.59 (brs, 1H); MS (ESI/APCI Dual) m/z 459 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{23}ClN_2O_3 \cdot 0.6H_2O$: C, 69.04; H, 5.19; N, 5.96. Found: C, 69.01; H, 5.17; N, 5.97.

Compound **9a**–**9u** were obtained according to the procedure of **9l**.

2-[1-[[4-[(3,4-Dichlorobenzoyl)amino]phenyl]methyl]-isoquinolin-4-yl]acetic Acid (9a) Colorless powder; mp 145.0–152.0°C; ^{1}H-NMR (DMSO-d_6) δ: 4.00 (s, 2H), 4.60 (s, 2H), 7.24–7.32 (m, 2H), 7.58–7.67 (m, 3H), 7.73–7.83 (m, 2H), 7.87–7.92 (m, 1H), 7.94–8.00 (m, 1H), 8.14–8.19 (m, 1H), 8.29–8.37 (m, 2H), 10.31 (brs, 1H), 12.59 (brs, 1H); MS (ESI/APCI Dual) m/z 465 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{18}Cl_2N_2O_3 \cdot 1.0H_2O$: C, 62.12; H, 4.17; N, 5.80. Found: C, 61.17; H, 4.12; N, 5.67.

2-[1-[[4-(Methylcarbamoyl)phenyl]methyl]isoquinolin-4-yl]acetic Acid (9b) Pale yellow powder; mp 219.0–221.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.73 (d, J=4.6 Hz, 3H), 4.00 (s, 2H), 4.67 (s, 2H), 7.32–8.38 (m, 10H), 12.55 (brs, 1H); MS (ESI/APCI Dual) m/z 335 $(M+H)^+$; high resolution (HR)-MS m/z Calcd for $C_{20}H_{18}N_2O_3$ $(M+Na)^+$, 357.1210. Found: 357.1199.

2-[1-[[4-(2-Cyclopropylethylcarbamoyl)phenyl]methyl]-isoquinolin-4-yl]acetic Acid (9c) Pale yellow powder; mp 188.5–189.0°C; ^{1}H-NMR (DMSO-d_6) δ: 0.00–0.06 (m, 2H), 0.34–0.42 (m, 2H), 0.64–0.74 (m, 1H), 1.33–1.44 (m, 2H), 3.23–3.32 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 7.32–8.40 (m, 10H), 12.57 (brs, 1H); MS (ESI/APCI Dual) m/z 389 $(M+H)^+$; *Anal.* Calcd for $C_{24}H_{24}N_2O_3 \cdot 0.3H_2O$: C, 73.19; H, 6.30; N, 7.11. Found: C, 73.03; H, 6.13; N, 6.98.

2-[1-[[4-(2-Cyclohexylethylcarbamoyl)phenyl]methyl]isoquinolin-4-yl]acetic Acid (9d) Pale yellow amorphous; mp 176.5.0–177.0°C; ^{1}H-NMR (DMSO-d_6) δ: 0.82–1.72 (m, 13H), 3.20–3.26 (m, 2H), 4.01 (s, 2H), 4.67 (s, 2H), 7.34–8.39 (m, 10H), 12.52 (brs, 1H); MS (ESI/APCI Dual) m/z 431 $(M+H)^+$; HR-MS m/z Calcd for $C_{27}H_{30}N_2O_3$ $(M+H)^+$, 431.2329. Found: 431.2309.

2-[1-[[4-(2-Cyclohexylethylcarbamoyl)phenyl]methyl]-isoquinolin-4-yl]acetic Acid (9e) Pale yellow powder; mp 140.0–142.0°C; ^{1}H-NMR (DMSO-d_6) δ: 1.24–1.70 (m, 14H), 1.87–1.95 (m, 3H), 3.18–3.26 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 7.32–8.37 (m, 10H), 12.57 (brs, 1H); MS (ESI/APCI Dual) m/z 483 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{34}N_2O_3$: C, 77.15; H, 7.10; N, 5.80. Found: C, 76.17; H, 7.04; N, 5.72.

2-[1-[[4-[2-(Oxan-4-yl)ethylcarbamoyl]phenyl]methyl]-isoquinolin-4-yl]acetic Acid (9f) Pale yellow powder; mp 197.5–198.5°C; ^{1}H-NMR (DMSO-d_6) δ: 1.07–1.61 (m, 7H), 3.20–3.28 (m, 4H), 3.77–3.83 (m, 2H), 4.01 (s, 2H), 4.67 (s, 2H), 7.35–8.37 (m, 10H), 12.57 (brs, 1H); MS (ESI/APCI Dual) m/z 433 $(M+H)^+$; *Anal.* Calcd for $C_{26}H_{28}N_2O_4 \cdot 0.5H_2O$: C, 70.73; H, 6.62; N, 6.34. Found: C, 70.61; H, 6.47; N, 6.13.

2-[1-[[4-(2-Morpholin-4-ylethylcarbamoyl)phenyl]methyl]isoquinolin-4-yl]acetic Acid (9g) Colorless powder; mp 130.0–131.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.36–2.43 (m, 6H), 2.60–2.62 (m, 2H), 3.54 (s, 4H), 3.95 (brs, 2H), 4.67 (s, 2H), 7.32–8.36 (m, 10H), 12.55 (brs, 1H); MS (ESI/APCI Dual) m/z 434 $(M+H)^+$; HR-MS m/z Calcd for $C_{25}H_{27}N_3O_4$ $(M+H)^+$, 434.2074. Found: 434.2065.

2-[1-[[4-(2-Phenylethylcarbamoyl)phenyl]methyl]isoquinolin-4-yl]acetic Acid (9h) Pale yellow powder; mp 197.0–199.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.80 (t, J=7.3 Hz, 2H), 3.41–3.46 (m, 2H), 4.02 (s, 2H), 4.68 (s, 2H), 7.16–8.45 (m, 15H), 12.52 (brs, 1H); MS (ESI/APCI Dual) m/z 425 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{24}N_2O_3 \cdot 0.5H_2O$: C, 74.81; H, 5.81; N, 6.46. Found: C, 75.10; H, 5.80; N, 6.36.

2-[1-[[4-(2-Pyridin-4-ylethylcarbamoyl)phenyl]methyl]isoquinolin-4-yl]acetic Acid (9i) Colorless powder; ^{1}H-NMR (DMSO-d_6) δ: 2.80–2.86 (m, 2H), 3.45–3.51 (m, 2H), 3.69 (brs, 2H), 4.63 (s, 2H), 7.19–8.48 (m, 14H); MS (ESI/APCI Dual) m/z 426 $(M+H)^+$; HR-MS m/z Calcd for $C_{26}H_{23}N_3O_3$ $(M+H)^+$, 426.1812. Found: 426.1782.

2-[1-[[4-[2-(2-Chlorophenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]acetic Acid (9j) Pale yellow powder; mp 181.0–183.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.91–2.97 (m, 2H), 3.44–3.50 (m, 2H), 4.01 (s, 2H), 4.67 (s, 2H), 7.21–8.49 (m, 14H), 12.52 (brs, 1H); MS (ESI/APCI Dual) m/z 459 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{23}ClN_2O_3 \cdot 0.5H_2O$: C, 69.30; H, 5.33; N, 5.81. Found: C, 69.15; H, 5.33; N, 5.81.

2-[1-[[4-[2-(3-Chlorophenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]acetic Acid (9k) Pale yellow powder; mp 121.0–123.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.82 (t, J=7.1 Hz, 2H), 3.42–3.48 (m, 2H), 4.01 (s, 2H), 4.67 (s, 2H), 7.14–8.45 (m, 14H), 12.54 (brs, 1H); MS (ESI/APCI Dual) m/z 459 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{23}ClN_2O_3 \cdot 0.4H_2O$: C, 69.57; H, 5.15; N, 6.01. Found: C, 69.66; H, 5.27; N, 5.81.

2-[1-[[4-[2-(4-Methylphenyl)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]acetic Acid (9m) Pale yellow powder; mp 121.0–124.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.24 (s, 3H), 2.75 (t, J=7.6 Hz, 2H), 3.36–3.43 (m, 2H), 4.19 (s, 2H), 4.90 (s, 2H), 7.05–8.58 (m, 14H); MS (ESI/APCI Dual) m/z 439 $(M+H)^+$; HR-MS m/z Calcd for $C_{28}H_{26}N_2O_3$ $(M+H)^+$, 439.2016. Found: 439.1974.

2-[1-[[4-[2-[4-(Dimethylamino)phenyl]ethylcarbamoyl]-phenyl]methyl]isoquinolin-4-yl]acetic Acid (9n) Pale yellow powder; mp 187.0–188.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.67 (t, J=7.6 Hz, 2H), 2.83 (s, 6H), 3.32–3.38 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 6.62–8.40 (m, 14H), 12.57 (brs, 1H); MS (ESI/APCI Dual) m/z 468 $(M+H)^+$; *Anal.* Calcd for $C_{29}H_{29}N_3O_3 \cdot 0.5H_2O$: C, 73.09; H, 6.34; N, 8.82. Found: C, 73.00; H, 6.19; N, 8.76.

2-[1-[[4-[2-(4-Methoxyphenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]acetic Acid (9o) Colorless powder; mp 172.0–173.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.70–2.76 (m, 2H), 3.37–3.42 (m, 2H), 3.70 (s, 3H), 4.01 (s, 2H), 4.67 (s, 2H), 6.79–8.42 (m, 14H), 12.53 (brs, 1H); MS (ESI/APCI Dual) m/z 477 $(M+H)^+$; *Anal.* Calcd for $C_{28}H_{26}N_2O_4 \cdot 0.4H_2O$: C, 72.84; H, 5.85; N, 6.07. Found: C, 72.88; H, 5.67; N, 6.01.

2-[1-[[4-[2-(4-Hydroxyphenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]acetic Acid (9p) Pale yellow powder; mp 145.0–150.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.67 (t, J=7.6 Hz, 2H), 3.34–3.39 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 6.63–8.41 (m, 14H), 9.15 (brs, 1H), 12.54 (brs, 1H); MS (ESI/APCI Dual) m/z 441 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{24}N_2O_4 \cdot 0.8H_2O \cdot 0.1Et_2O$: C, 71.18; H, 5.80; N, 6.06. Found: C, 71.54; H, 5.86; N, 5.69.

2-[1-[[4-[2-(4-Methylsulphonylphenyl)ethylcarbamoyl]-phenyl]methyl]isoquinolin-4-yl]acetic Acid (9q) Pale yellow powder; mp 121.5–122.5°C; ^{1}H-NMR (DMSO-d_6) δ: 2.89–2.95 (m, 2H), 3.17 (s, 3H), 3.45–3.51 (m, 2H), 4.00 (s, 2H), 4.67 (s, 2H), 7.35–8.47 (m, 14H), 12.56 (brs, 1H); MS (ESI/APCI Dual) m/z 503 $(M+H)^+$; *Anal.* Calcd for

$C_{28}H_{26}N_2O_5S \cdot 1.2H_2O$: C, 64.16; H, 5.46; N, 5.34. Found: C, 64.17; H, 5.33; N, 5.21.

4-[2-[[4-[[4-(Carboxymethyl)isoquinolin-1-yl]methyl]benzoyl]amino]ethyl]benzoic Acid (9r) Pale yellow powder; mp 198.0–200.5°C; ^{1}H-NMR (DMSO-d_6) δ: 2.84 (t, J=7.1 Hz, 2H), 3.41–3.46 (m, 2H), 3.98 (s, 2H), 4.64 (s, 2H), 7.28–8.43 (m, 14H), 12.62 (brs, 1H); MS (ESI/APCI Dual) *m/z* 469 (M+H)$^+$; *Anal.* Calcd for $C_{28}H_{24}N_2O_5 \cdot 0.8H_2O$: C, 69.64; H, 5.34; N, 5.80. Found: C, 69.71; H, 5.29; N, 5.71.

2-[1-[[4-[(4-Chlorophenyl)methylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]acetic Acid (9s) Colorless powder; mp 187.5–188.5°C; ^{1}H-NMR (DMSO-d_6) δ: 3.98 (s, 2H), 4.37–4.41 (m, 2H), 4.65 (s, 2H), 7.24–8.94 (m, 14H), 12.33 (brs, 1H); MS (ESI/APCI Dual) *m/z* 445 (M+H)$^+$; HR-MS *m/z* Calcd for $C_{26}H_{21}ClN_2O_3$ (M+Na)$^+$, 467.1133. Found: 467.1125.

2-[1-[[4-[3-(4-Chlorophenyl)propylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]acetic Acid (9t) Pale yellow powder; mp 124.0–127.0°C; ^{1}H-NMR (DMSO-d_6) δ: 1.74–1.80 (m, 2H), 2.56–2.61 (m, 2H), 3.19–3.25 (m, 2H), 4.01 (s, 2H), 4.67 (s, 2H), 7.21–8.37 (m, 14H), 12.54 (brs, 1H); MS (ESI/APCI Dual) *m/z* 473 (M+H)$^+$; *Anal.* Calcd for $C_{28}H_{25}ClN_2O_3 \cdot 0.2H_2O$: C, 70.57; H, 5.37; N, 5.88. Found: C, 70.41; H, 5.43; N, 5.78.

2-[1-[[4-[2-(4-Chlorophenoxy)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]acetic Acid (9u) Pale yellow powder; mp 116.0–118.0°C; ^{1}H-NMR (DMSO-d_6) δ: 3.52–3.57 (m, 2H), 3.97 (s, 2H), 4.03 (t, J=6.0 Hz, 2H), 4.64 (s, 2H), 6.90–8.56 (m, 14H), 12.54 (brs, 1H); MS (ESI/APCI Dual) *m/z* 475 (M+H)$^+$; *Anal.* Calcd for $C_{27}H_{23}ClN_2O_4 \cdot 0.4H_2O$: C, 67.26; H, 4.98; N, 5.81. Found: C, 67.26; H, 5.04; N, 5.71.

4-[[4-(2-Amino-2-oxoethyl)isoquinolin-1-yl]methyl]-N-[2-(4-chlorophenyl)ethyl]benzamide (9v) To a suspension of **9l** (400 mg, 0.872 mmol) in THF (9 mL) was added carbonyldiimidazole (293 mg, 1.74 mmol), and the mixture was stirred for 2 h at room temperature. Then the mixture was added 28% ammonia solution (0.45 mL, 6.75 mmol) and stirred for 1 h. The resulting precipitate was collected by filtration, and dried to yield **9v** (384 mg, 96%) as a colorless powder: mp 265.0–267.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.77–2.82 (m, 2H), 3.40–3.45 (m, 2H), 3.81 (s, 2H), 4.66 (s, 2H), 7.00–8.43 (m, 16H); MS (ESI/APCI Dual) *m/z* 480 (M+H)$^+$; *Anal.* Calcd for $C_{27}H_{24}ClN_3O_2 \cdot 0.4H_2O$: C, 69.72; H, 5.37; N, 9.03. Found: C, 69.41; H, 5.42; N, 9.33.

***N*-[2-(4-Chlorophenyl)ethyl]-4-[[4-(cyanomethyl)isoquinolin-1-yl]methyl]benzamide (10)** To a suspension of **9v** (362 mg, 0.790 mmol) in DMF (8 mL) was added phosphoryl chloride (0.088 mL, 0.949 mmol), and the mixture was stirred for 1 h at room temperature. The mixture was poured into ice water, extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60N) eluting with 2–10% $CHCl_3$/MeOH to give **10** (256 mg, 74%) as a pale yellow powder: ^{1}H-NMR (DMSO-d_6) δ: 2.80 (t, J=7.3 Hz, 2H), 3.40–3.47 (m, 2H), 4.45 (s, 2H), 4.70 (s, 2H), 7.20–8.44 (m, 13H), 8.49 (s, 1H); MS (ESI/APCI Dual) *m/z* 462 (M+Na)$^+$.

***N*-[2-(4-Chlorophenyl)ethyl]-4-[[4-(1*H*-tetrazol-5-ylmethyl)isoquinolin-1-yl]methyl]benzamide (9w)** To a suspension of **10** (20 mg, 0.045 mmol) in toluene (0.45 mL) was added sodium azide (9 mg, 0.136 mmol) and triethylamine hydrochloride (19 mg, 0.136 mmol), and the mixture was refluxed for 72 h. After cooling to room temperature, the mixture was diluted with $CHCl_3$ and MeOH, washed with H_2O, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60N) eluting with 10–20% $CHCl_3$/MeOH to give triethylamine salt of **9w**. The salt was dissolved with $CHCl_3$ and MeOH, and the solution was washed with 0.5 N HCl aq and H_2O, dried over $MgSO_4$ and concentrated under reduced pressure to give **9w** (3.1 mg, 14%) as a colorless powder: mp 237.0–239.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.77–2.82 (m, 2H), 3.40–3.46 (m, 2H), 4.69 (s, 2H), 4.68 (s, 2H), 7.21–8.45 (m, 14H); MS (ESI/APCI Dual) *m/z* 483 (M+H)$^+$; HR-MS *m/z* Calcd for $C_{27}H_{23}ClN_6O$ (M+H)$^+$, 483.1695. Found: 483.1694.

***tert*-Butyl 4-[[4-(1-Methoxy-1-oxopropan-2-yl)isoquinolin-1-yl]methyl]benzoate (dl-11)** To a solution of **6** (500 mg, 1.28 mmol) in THF (5 mL) was added potassium *tert*-butoxide (150 mg, 1.34 mmol) at −30°C and stirred for 5 min. Then the mixture was added iodomethane (0.318 mL, 5.10 mmol) and stirred for 3 h. The reaction was quenched by adding a saturated ammonium chloride solution, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 10–20% EtOAc/hexane to give dl-**11** (319 mg, 61%) as a yellow oil: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (s, 9H), 1.70 (d, J=7.0 Hz, 3H), 3.67 (s, 3H), 4.36 (q, J=7.0 Hz, 1H), 4.70 (s, 2H), 7.30–8.14 (m, 8H), 8.45 (s, 1H); MS (ESI/APCI Dual) *m/z* 406 (M+H)$^+$.

4-[[4-(1-Methoxy-1-oxopropan-2-yl)isoquinolin-1-yl]methyl]benzoic Acid (dl-12) To a solution of dl-**11** (319 mg, 0.787 mmol) in $CHCl_3$ (3 mL) was added TFA (1.5 mL) at 0°C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was basified with 1 N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give dl-**12** (254 mg) as a pale yellow powder. This compound was used for the next reaction without further purification: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (s, 9H), 1.70 (d, J=7.0 Hz, 3H), 3.67 (s, 3H), 4.36 (q, J=7.0 Hz, 1H), 4.70 (s, 2H), 7.30–8.14 (m, 8H), 8.45 (s, 1H); MS (ESI/APCI Dual) *m/z* 350 (M+H)$^+$.

Methyl 2-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]propanoate (dl-13) A mixture of dl-**12** (254 mg), 2-(4-chlorophenyl)amine (0.132 mL, 0.994 mmol), WSC (266 mg, 0.1.18 mmol), HOBT (181 mg, 1.18 mmol), and triethylamine (0.331 mL, 2.36) in $CHCl_3$ (5 mL) was stirred at room temperature for 12 h. The reaction was quenched by adding a saturated ammonium chloride solution, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 10–50% EtOAc/hexane to give dl-**13** (289 mg, 58%) as a pale yellow amorphous: ^{1}H-NMR ($CDCl_3$) δ: 1.70 (d, J=7.2 Hz, 3H), 2.87 (t, J=6.9 Hz, 2H), 3.63–3.66 (m, 2H), 3.67 (s, 3H), 4.36 (q, J=7.2 Hz, 1H), 4.68 (s, 2H), 6.03 (brs, 1H), 7.12–8.14 (m, 12H), 8.45 (s, 1H); MS (ESI/APCI Dual) *m/z* 487 (M+H)$^+$.

2-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]propanoic Acid (dl-9x) To a solution of dl-**13** (110 mg, 0.226 mmol) in THF (1.5 mL) was added 1 N NaOH (1 mL), and the mixture was stirred for 17 h at room temperature. The mixture was acidified with 1 N HCl aq. and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) elut-

ing with 30–75% EtOAc/hexane to give dl-**9x** (76 mg, 71%) as a colorless powder: mp 189.0–190.0°C; ^{1}H-NMR (DMSO-d_6) δ: 1.55 (d, J=7.3 Hz, 3H), 2.77–2.83 (m, 2H), 3.40–3.46 (m, 2H), 4.39 (q, J=7.3 Hz, 1H), 4.67 (s, 2H), 7.21–8.44 (m, 14H), 12.49 (brs, 1H); *Anal.* Calcd for $C_{28}H_{25}ClN_2O_3 \cdot 0.4H_2O$: C, 70.04; H, 5.42; N, 5.83. Found: C, 69.98; H, 5.48; N, 5.78.

***tert*-Butyl 4-[[4-(Hydroxymethyl)isoquinolin-1-yl]methyl]-benzoate (14)** To a solution of **5** (600 mg, 1.65 mmol) in THF (17 mL) was added BH_3-THF (3.30 mL, 3.30 mmol) at 0°C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was added MeOH at 0°C and evaporated. The residue was purified by silica gel column chromatography (OH) eluting with 30–70% EtOAc/hexane and then purified by silica gel column chromatography (NH) eluting with 50–70% EtOAc/hexane to give **14** (235 mg, 39%) as a pale yellow powder: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (s, 9H), 4.69 (s, 2H), 5.10 (s, 2H), 7.27–7.32 (m, 2H), 7.50–7.58 (m, 1H), 7.68–7.76 (m, 1H), 7.83–7.89 (m, 2H), 8.08–8.20 (m, 2H), 8.47 (s, 1H); MS (ESI/APCI Dual) m/z 350 $(M+H)^+$.

***tert*-Butyl 4-[(4-Formylisoquinolin-1-yl)methyl]benzoate (15)** To a solution of **14** (235 mg, 0.674 mmol) in $CHCl_3$ (7 mL) was added Dess–Martin periodinane (344 mg,, 0.812 mmol) at 0°C, and the mixture was stirred for 1 h. The reaction mixture was washed with H_2O and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 20–30% EtOAc/hexane to give **15** (200 mg, 85%) as a yellow amorphous: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (9H, s), 4.79 (2H, s), 7.32 (d, 2H, J=8.3 Hz), 7.62–7.68 (m, 1H), 7.82–7.92 (m, 3H), 8.23 (d, J=8.3 Hz, 1H), 8.90 (s, 1H), 9.24–9.28 (m, 1H), 10.36 (s, 1H); MS (ESI/APCI Dual) m/z 380 $(M+Na)^+$.

***tert*-Butyl 4-[[4-[(*E*)-3-Ethoxy-3-oxoprop-1-enyl]isoquinolin-1-yl]methyl]benzoate (16)** To a solution of ethyl 2-(diethoxyphosphoryl)acetate (0.161 mL, 0.806 mmol) in THF (4 mL) was added sodium hydride (38 mg, 0.950 mmol) at 0°C, and the mixture was stirred for 30 min. The reaction mixture was added a solution of **15** (200 mg, 0.576 mmol) in THF (2 mL) and stirred for 1 h. The reaction was quenched by adding a saturated ammonium chloride solution, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (NH) eluting with 20–30% EtOAc/hexane to give **16** (198 mg, 82%) as a pale yellow oil: ^{1}H-NMR ($CDCl_3$) δ: 1.36–1.41 (m, 3H), 1.55 (s, 9H), 4.29–4.36 (m, 2H), 4.73 (s, 2H), 6.56–6.61 (m, 2H), 7.31 (d, 2 H, J=8.7 Hz), 7.55–7.61 (m, 1H), 7.72–7.77 (m, 1H), 7.86–7.91 (m, 2H), 8.12–8.18 (m, 1H), 8.34–8.39 (m, 1H), 8.72 (s, 1H); MS (ESI/APCI Dual) m/z 418 $(M+H)^+$.

***tert*-Butyl 4-[[4-(3-Ethoxy-3-oxopropyl)isoquinolin-1-yl]-methyl]benzoate (17)** To a solution of **16** (181 mg, 0.434 mmol) in MeOH (9 mL) was added $CuCl_2$ (44 mg, 0.326 mmol) and sodium borohydride (91 mg, 2.17 mmol) at 0°C, and the mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 20–40% EtOAc/hexane to give **17** (110 mg, 60%) as a pale yellow powder: ^{1}H-NMR ($CDCl_3$) δ: 1.23–1.28 (m, 3H), 1.55 (s, 9H), 2.73–2.77 (m, 2H), 3.34–3.39 (m, 2H), 4.14–4.20 (m, 2H), 4.68 (s, 2H), 7.30 (d, J=8.3 Hz, 2H), 7.50–7.55 (m, 1H), 7.67–7.72 (m, 1H), 7.85–7.89 (m, 2H), 7.98–8.02 (m, 1H), 8.10 (d, J=8.3 Hz, 1H), 8.37 (s, 1H); MS (ESI/APCI Dual) m/z 420 $(M+H)^+$.

4-[[4-(3-Ethoxy-3-oxopropyl)isoquinolin-1-yl]methyl]benzoic Acid (18) To a solution of **17** (110 mg, 0.262 mmol) in $CHCl_3$ (2 mL) was added TFA (1 mL) at 0°C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was basified with 1N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give **18** (95 mg) as a yellow powder. This compound was used for the next reaction without further purification: ^{1}H-NMR ($CDCl_3$) δ: 2.70–2.74 (m, 3H), 3.25–3.30 (m, 2H), 4.02–4.07 (m, 2H), 4.68 (s, 2H), 7.38 (d, J=8.3 Hz, 2H), 7.62–7.67 (m, 1H), 7.77–7.84 (m, 3H), 8.10 (d, J=8.3 Hz, 1H), 8.30–8.33 (m, 2H); MS (ESI/APCI Dual) m/z 364 $(M+H)^+$.

Ethyl 3-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]methyl]isoquinolin-4-yl]propanoate (19) A mixture of **18** (95 mg), 2-(4-chlorophenyl)amine (0.055 mL, 0.393 mmol), WSC (75 mg, 0.393 mmol), and HOBT (75 mg, 0.393 mmol) in THF (3 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH) eluting with 10–50% $CHCl_3$/AcOEt to give **19** (107 mg, 82%) as a pale yellow powder: ^{1}H-NMR ($CDCl_3$) δ: 1.25 (t, J=7.1 Hz, 3H), 2.73–2.78 (m, 2H), 2.87 (t, J=6.9 Hz, 2H), 3.34–3.39 (m, 2H), 3.62–3.68 (m, 2H), 4.14–4.19 (m, 2H), 4.67 (s, 2H), 6.02 (brs, 1H), 7.11–7.32 (m, 6H), 7.52–7.59 (m, 3H), 7.68–7.73 (m, 1H), 8.01–8.03 (m, 1H), 8.09–8.12 (m, 1H), 8.37 (s, 1H).

3-[1-[[4-[2-(4-Chlorophenyl)ethylcarbamoyl]phenyl]-methyl]isoquinolin-4-yl]propanoic Acid (9y) To a solution of **19** (106 mg, 0.214 mmol) in THF (4 mL) was added 1 N NaOH (2 mL), and the mixture was stirred for 6 h at room temperature. The mixture was acidified with 1 N HCl aq. and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give **9y** (84 mg, 83%) as a colorless powder: mp 208.0–210.0°C; ^{1}H-NMR (DMSO-d_6) δ: 2.65 (t, J=7.7 Hz, 2H), 2.80 (t, J=7.1 Hz, 2H), 3.24 (t, J=7.7 Hz, 2H), 3.40–3.46 (m, 2H), 4.65 (s, 2H), 7.20–8.43 (m, 14H), 12.23 (brs, 1H); MS (ESI/APCI Dual) m/z 473 $(M+H)^+$; *Anal.* Calcd for $C_{28}H_{25}ClN_2O_3 \cdot 0.2H_2O$: C, 70.57; H, 5.37; N, 5.88. Found: C, 70.35; H, 5.22; N, 5.81.

***In Vitro* Pharmacological Studies. Binding Assay** CRTH2 transfected cells were washed and suspended in HBSS containing 10 mM HEPES, pH 7.3 (2×10^5 cells/100 μL). The cells were incubated with 5 nM ^{3}H-PGD$_2$ (GE Healthcare, Little Chalfont, United Kingdom) for 60 min on ice. The cells in suspension were rapidly filtered under vacuum through a glass fiber filter plate GF/C (Whatman Inc., Clifton, NJ, U.S.A.) using a cell harvester Filtermate (Packard, Meriden, CT, U.S.A.). The filters were then washed four times with ice-cold phosphate buffered saline containing 0.1% bovine serum albumin. Bound radio activity was measured with a liquid scintillation counter Top Count NXT (PerkinElmer, Inc., Boston, MA, U.S.A.) using a liquid scintillation cocktail Micro-scinti-O (Packard, Meriden, CT, U.S.A.).

Intracellular Ca^{2+} Influx Assay Human CRTH2 transfectant KB8 cells (BML Co. Kawagoe, Japan) were incubated with 1 μM Fluo-4 AM for 30 min at 37°C in the dark. After incubation, the cells were washed and suspended

in HBSS containing 10 mM HEPES, pH 7.3, 1 mM $CaCl_2$ (2×10^5 cells/100 μL). The compound and 100 nM of PGD_2 were added, and the increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) was measured using the functional drug screening system FDSS6000 (Hamamatsu Photonics, Shizuoka, Japan).

Th2 Cells Migration Assay Human Th2 cells were prepared as follows: the CD4+T lymphocytes separated from PBMCs using anti-CD4 mAb were stimulated with anti-CD3 mAb and anti-CD28 mAb in the presence of IL-4 and neutralizing anti-IFNγ mAb for 3 d and, then, expanded by IL-2 and IL-4 for 7 d. Th2 cells highly expressing CRTH2 were separated with anti-CRTH2 mAb, and 2 d after the separation, these cells were used in the cell migration assay. Compound-treated Th2 cells and 100 nM solution of PGD_2 were applied to top and bottom wells of the 5 μm-pore filter Chemo Tx-96 chamber (Neuroprobe, Gaithersburg, MD, U.S.A.), respectively. After incubation at 37°C for 1 h, the number of the cells in the bottom wells was counted by Burker–Turk hemocytometer.

Pharmacokinetics. Animals Male NC/Nga mice (SLC, Japan) 11–13 weeks old were used in the experiments. All animals were used after acclimation for at least 4 d. The mice were given free access to water and atandard laboratory diet (MF, Oriental Yeast Co., Japan) during the acclimation period. Environmental conditions were controlled at a relative humidity of 50±20% and temperature of 23±3°C.

Male guinea pigs (SLC, Japan) 9–11 weeks old were used in the experiments after acclimation that described above.

All the studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee.

Plasma Concentrations of 1 in Mice Compound **1** was orally administered to male NC/Nga mice. The animals were anesthetized with isoflurane, and blood samples were collected from the postcava and stored at −80°C until the bioanalysis. The plasma concentration of **1** was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI). A 50 μL aliquot of plasma was added with 200 μL of organic solvent (acetonitrile–methanol (9 : 1, v/v)) containing an internal standard and vortex-mixed. After centrifugation, the supernatant (5 μL) obtained was directly injected into a LC-MS/MS system composed of a CTC HTS-PAL autosampler (CTC Analytics AG, Zwingen, Switzerland), HP1100 binary pump system (Agilent Technologies, CA, U.S.A.), and an API 3000 tandem mass spectrometer equipped with a Turbo Ionspray interface (Applied Biosystems, CA, U.S.A.). Chromatographic separation with linear gradient elution was performed on a Shim-pack XR-ODS (2.2 μM, 3.0×30 mm, Shimadzu) using a 0.1% aqueous solution of formic acid and acetonitrile. The column temperature and mobile phase flow rate were set at 50°C and 1.2 mL/min, respectively. Approximately 2/7 of the column effluent was directed to the ion source, and multiple reaction monitoring (MRM) with negative ion detection was performed. The lower limit of quantification (LLOQ) for mouse plasma was 1 ng/mL.

Plasma Concentrations of 9l in Mice and Guinea Pigs Plasma concentrations of **9l** in mice and guinea pigs were measured by almost the same method as above.

***In Vivo* Pharmacological Studies. Antigen-Induced Bronchoconstriction in Guinea Pigs** Guinea pigs were sensitized by aerosolized 1% OVA for 10 min once daily for 8 consecutive days. One week after the final sensitization, the animals were challenged for 5 min with aerosolized 2% OVA. TASP0412098 was administered 30 min before and 2 h after the OVA challenge, and dexamethasone was administered 3 h before the OVA challenge. One week after the first OVA challenge, animals were challenged OVA again with the same protocol and asthmatic responses were measured using a two-chambered, double-flow plethysmograph system. Antigen-induced bronchoconstriction was evaluated as the change in sRaw at 1 min after the antigen challenge for the immediate airway response (IAR), and the area under the change in the sRaw curve (*AUC*) between 4 and 7 h (AUC_{4-7h}) after the antigen challenge for the late airway response (LAR).

Cell Counts in Bronchoalveolar Lavage Fluid of Guinea Pigs Twenty-four hours after the last antigen challenge, the guinea pigs were sacrificed by anesthesia and recovered bronchoalveolar lavage fluid (BALF). The BALF was hemolyzed and cell count per 1 mL was taken as the total cell count. The slides were prepared and stained using May–Grunwald stain and Giemza stain. Approximately 300 cells were counted and the ratios of neutrophils, eosinophils, macrophages and lymphocytes relative to the total were calculated.

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