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Isoquinoline derivatives as potent CRTH2 receptor antagonists: Synthesis and SAR

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ABSTRACT

Synthesis and structure–activity relationship of a novel series of isoquinoline CRTH2 receptor antagonists are described. One of the most potent compounds, **TASP0376377** (**6m**), showed not only potent binding affinity (IC_{50} = 19 nM) but also excellent functional antagonist activity (IC_{50} = 13 nM). **TASP0376377** was tested for its ability of a chemotaxis assay to show the effectiveness (IC_{50} = 23 nM), which was in good agreement with the CRTH2 antagonist potency. Furthermore, **TASP0376377** showed sufficient selectivity for binding to CRTH2 over the DP1 prostanoid receptor (IC_{50} >1 μM) and COX-1 and COX-2 enzymes (IC_{50} >10 μM).

Chemoattractant receptor–homologous molecule expressed on Th2 lymphocytes (CRTH2) has been identified as a G protein-coupled receptor, predominantly expressed on Th2 cells, and plays a key role in allergic diseases, driving the IgE response, eosinophilia, and release of proinflammatory cytokines.[1–3] Activation of CRTH2 promotes the release of histamine from basophils and degranulation of eosinophiles.[4–6] Thus, CRTH2 is involved in complex inflammatory processes, and a CRTH2 antagonist might have beneficial effects in a variety of inflammatory diseases.[7–9]

Ramatroban (**1**), a thromboxane A2 receptor (TP) antagonist currently used for the treatment of allergic rhinitis, was shown to be a potent CRTH2 antagonist. Indomethacin (**2**), a well-known analgesic and cyclooxygenase 1 and 2 (COX-1 and COX-2) enzyme inhibitor, was reported to show CRTH2 agonist potency. Since then, a large number of CRTH2 antagonists including several series of indole acids have been reported. These CRTH2 antagonists have a variety of fused 6–5-membered ring chemotypes as a core structure such as indole (**3**),[10–13] 5-azaindole (**4**),[14] 7-azaindole (**5**),[15] benzimidazole,[16,17] indolizine,[18] spiro–indoline;[19] in addition, they have an acetic acid moiety (Fig. 1).

In the course of our program, which was aimed at developing CRTH2 antagonists for the treatment of allergic diseases, we have been pursuing a new class of potent and selective CRTH2 antagonist lead based on the structures of CRTH2 antagonists known to date (**3**, **4**, **5**). Using a fused 6–6-membered ring system as a core structure, we newly designed a 1,4-disubstituted isoquinoline lead bearing a benzoyl group at the 1-position and an acetic acid moiety essential to CRTH2 potency at the 4-position (Fig. 2). As a result, we have identified a novel compound **6a** showing moderate binding affinity for the CRTH2 receptor in a radioligand binding assay (^{3}H-PGD_2) using CHO cells stably transfected with the human CRTH2 receptor (IC_{50} = 330 nM). In this paper, we describe the synthesis and structure–activity relationship (SAR) of the new class of isoquinoline derivatives.

Scheme 1 shows the synthesis of compounds **6a–m**, **14**, and **15** from commercially available 2-(4-aminophenyl)acetonitrile (**7a**). Protection of the starting material **7a** with di-*t*-butyl dicarbonate afforded **8**. The reaction of **8** with methyl 1-chloroisoquinoline-4-carboxylate in the presence of sodium hexamethyldisilazane, followed by oxidative decyanation under oxygen atmosphere produced **9**[20] in 83% yields. The carboxylic acid **10**, prepared by the basic hydrolysis of **9**, was converted into the corresponding acid chloride, and 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 **11a**[21] in 69% yield from **9**. Deprotection of the *t*-butoxycarbonyl group in **11a** under an acidic condition (TFA), and the subsequent acylation of the resulting aniline intermediate **12** with various kinds of acid chlorides gave **13**. Finally, hydrolysis of the ester moiety afforded the target isoquinoline derivatives **6b–m**, **14**, and **15** in 22–96% yields. On the other hand, compound **6a**

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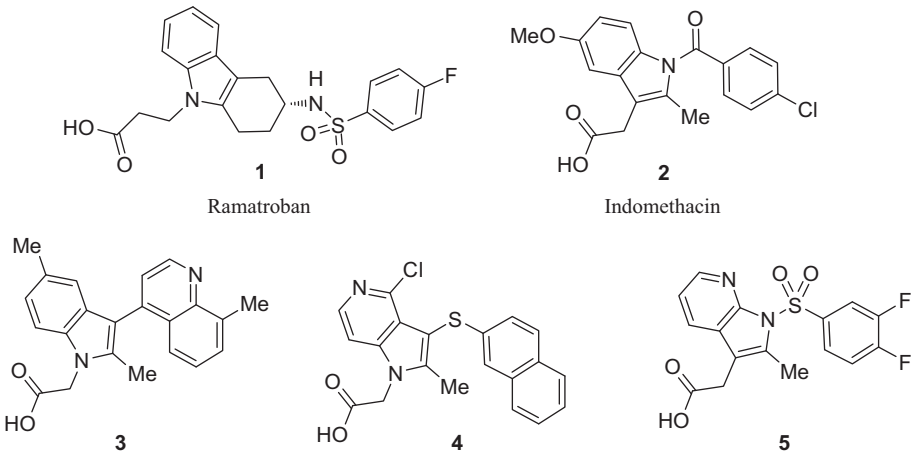


Figure 1. Ligands for the CRTH2 receptor.

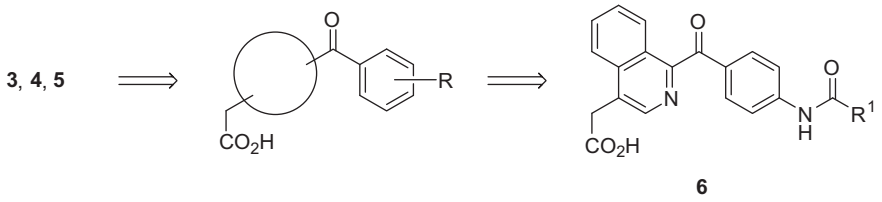
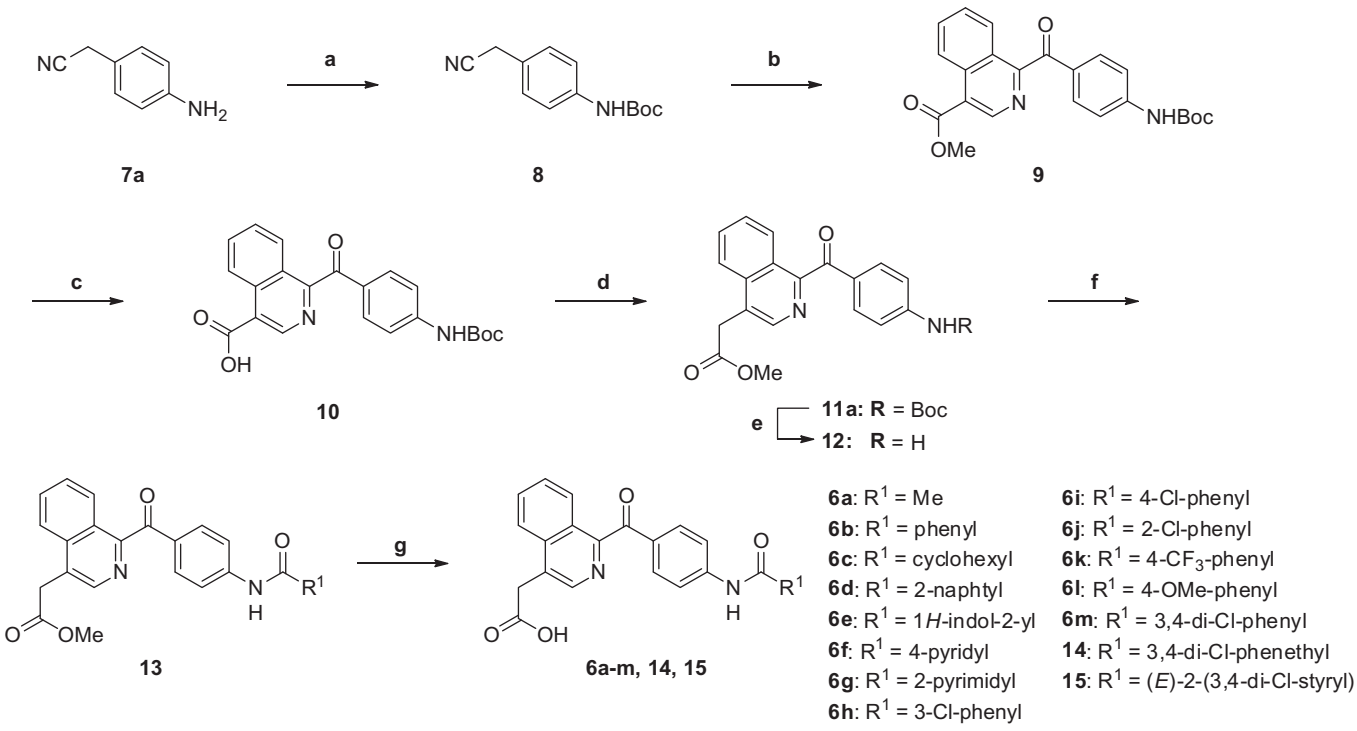


Figure 2. Design of the novel isoquinoline chemotype for the CRTH2 receptor.



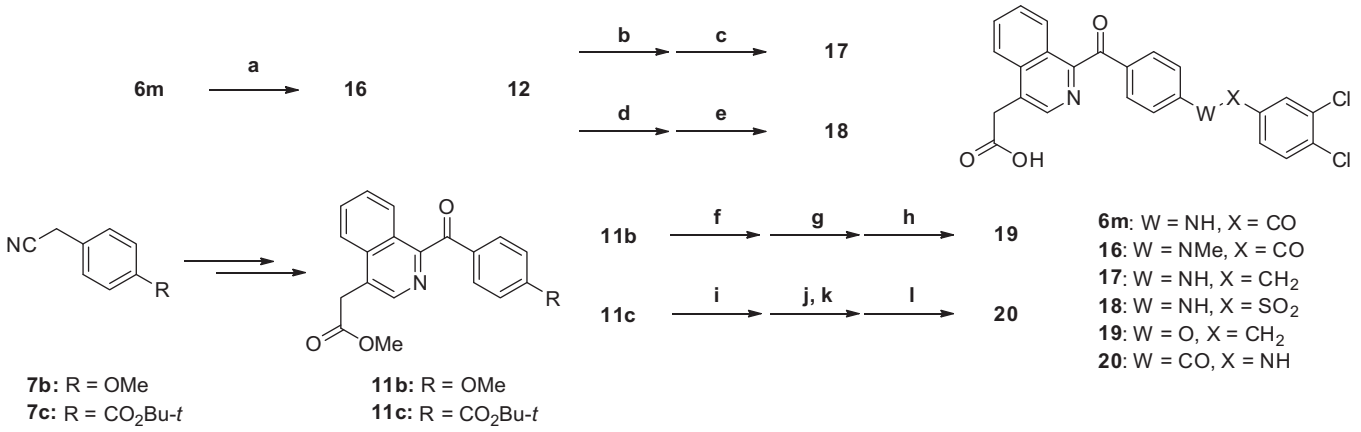
Scheme 1. Reagents and conditions: (a) Boc_2O, EtOH, rt, 57%; (b) methyl 1-chloroisoquinolinc-4-carboxylate, NaHMDS, THS. 0 °C, then, O_2, rt, 83%; (c) 1 N NaOH aq, MeOH, 0 °C to rt, 94%; (d) $(COCl)_2$ $CHCl_3$, rt, $TMSCMN_2$, THF/MeCN, rt, silver acetate, H_2O/dioxane, 60 °C then $TMSCHN_2$, MeOH, rt 69% (4 steps); (e) TFA, $CHCl_3$, 0 °C to rt; (f) for **6b**–**m**, **14**, **15**, RCOCl, pyridine, $CHCl_3$, 0 °C to rt, 39–99%; for **6a**, Av_2O, pyridine, 70 °C, 97%; (g) 1 N NaOH aq, THF, 0 °C to rt, 22–96%.

was obtained by the treatment of **12** with acetic anhydride and the subsequent basic hydrolysis.

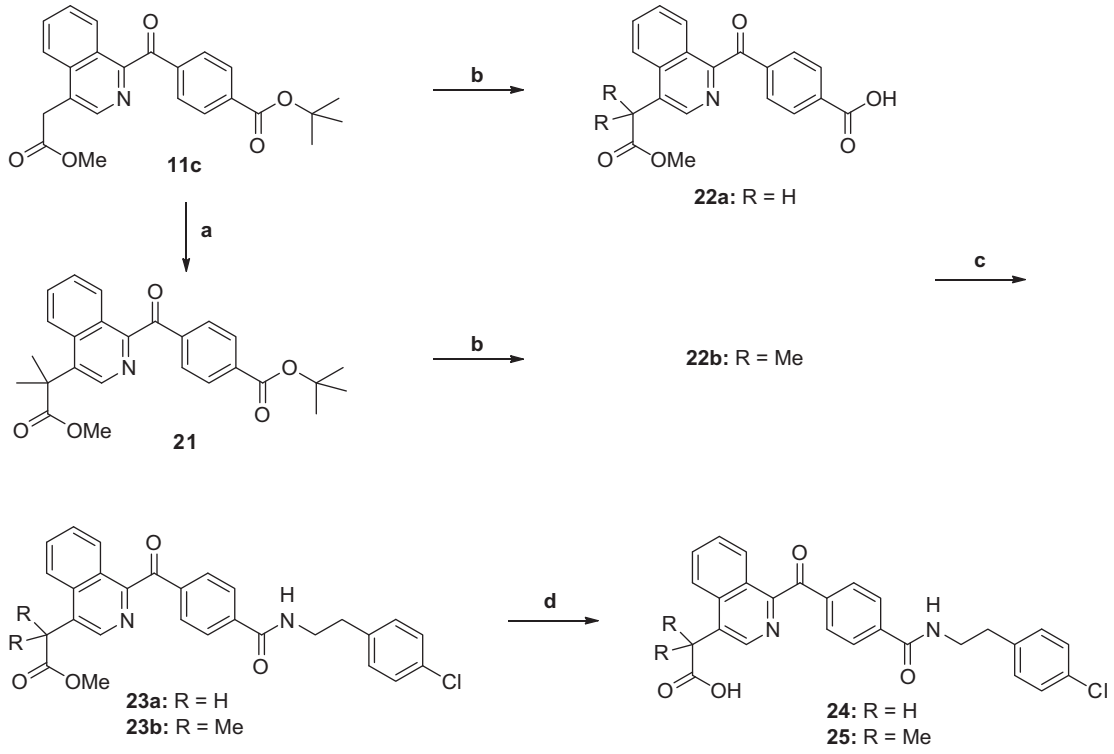
Scheme 2 summarizes the synthesis of compounds **16**–**20**. Compound **16** was prepared by selective N-methylation of the amide of **6m**. With regard to the synthesis of compound **17**, the common intermediate **12** was treated with 3,4-dichlorobenzylbromide in the presence of potassium carbonate, and the ester moiety was hydrolyzed by sodium hydroxide. Compound **18** was synthesized

by the reaction of the aniline intermediate **12** with 3,4-dichlorobenzene-1-sulfonyl chloride in the presence of pyridine followed by basic hydrolysis of the ester moiety. Synthesis of compound **19** was achieved by demethylation of **11b** by boron tribromide, which was derived from methyl 1-chloroisoquinoline-4-carboxylate and 2-(4-methoxyphenyl)acetonitrile in a procedure similar to the synthesis of **11a** (Scheme 1), and the subsequent 3,4-dichlorobenzylation of the resulting phenol. Intermediate **11c** was prepared from methyl 1-chloroisoquinoline-4-carboxylate and *t*-butyl 4-(cyanomethyl)benzoate, in a manner similar to the synthesis of **11a** as described in Scheme 1. By using **11c**, compound **20** was prepared as follows: (1) acidic removal of the ester moiety (TFA), (2) conversion into acyl chloride (oxalyl chloride), (3) condensation of the acyl chloride with 3,4-dichloroaniline.

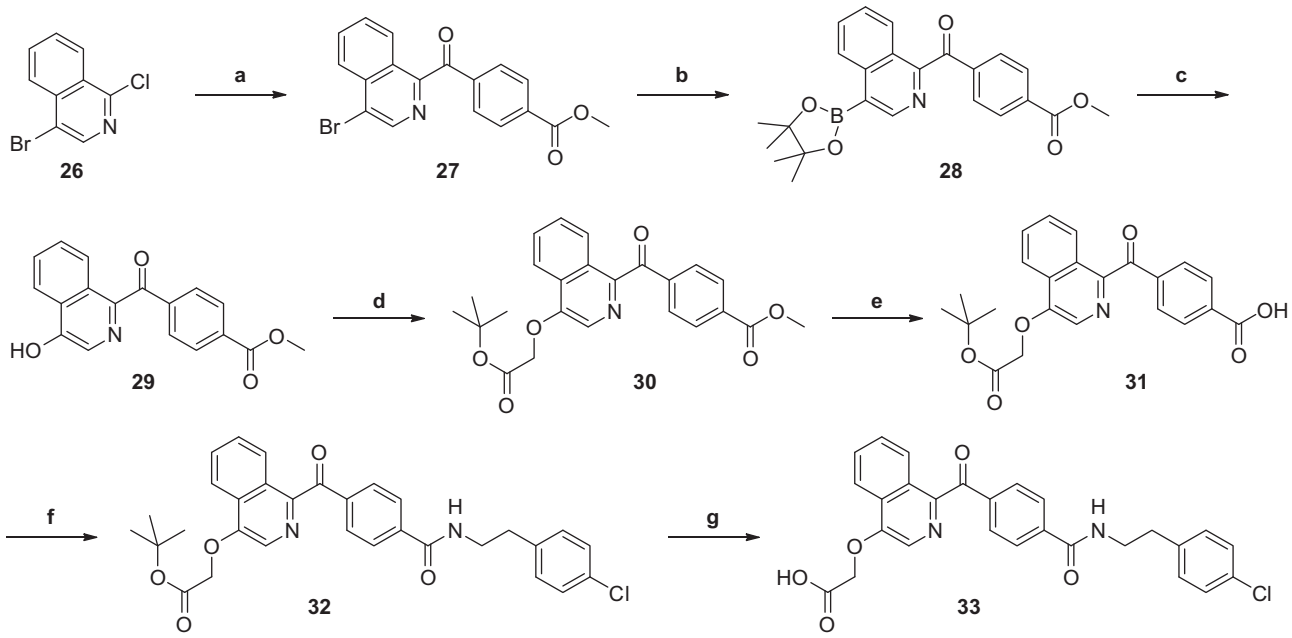
Compounds **24, 25** and **33**, in which the acetic acid part at the 4-position of the isoquinoline core was modified, were synthesized as shown in Schemes 3 and 4. Geminal dimethylation of **11c** by sodium hydride and iodomethane produced **21** in a 58% yield. Deprotection of the *t*-butyl ester moiety using TFA, followed by condensation reaction of **22b** with 4-chlorophenethylamine (WSC-HCl, HOBT-H_2O), gave **23b** in a 44% yield. Finally, hydrolysis of the ester moiety afforded the target derivative **25**. In a similar manner, compound **24** was prepared from **22a**. Compound **33** was synthesized from commercially available 4-bromo-1-chloro-



Scheme 2. Reagents and conditions: (a) NaH, MeI, THF, rt, 31%; (b) 3,4-dichlorobenzylbromide, K_2CO_3, DMF, 90 °C, 35%; (c) 1 N NaOH aq, THF, rt, 58%; (d) 3,4-dichlorobenzene-1-sulfonyl chloride, pyridine, 0 °C to rt, 39%; (e) 1 N NaOH aq, THF, rt, 12%; (f) BBr_3, $CHCl_3$, 0 °C to rt, 47%; (g) 3,4-dichlorobenzylbromide, K_2CO_3, DMF, 80 °C, 23%; (h) 1 N NaOH aq, THF, MeOH, rt, 48%; (i) TFA, $CHC1_3$, 0–50 °C, quant; (j) oxalyl chloride, DMF, $CHCl_3$, rt; (k) 3,4-dichloroaniline, pyridine, $CHCl_3$, 0 °C to rt, 35%; (l) 1 N NaOH aq, MeOH, 0 °C, 87%.



Scheme 3. Reagents and conditions: (a) NaH, MeI, $CHCl_3$, rt, 58%; (b) TFA, $CHCl_3$, rt; (c) for **23a**, oxalyl chloride, DMF, $CHCl_3$, rt then 4-chlorophenethylamine, pyridine, $CHCl_3$, rt, 2 steps 45%; for **23b**, 4-chlorophenethylamine, WSC-HCl, HOBT-H_2O, $CHCl_3$, rt, 2 steps 44%; (d) for **24**, 1 N NaOH aq THF, rt, 78%; for **25**, 1 N LiOH aq, dioxane, 100 °C, 70%.



Scheme 4. Reagents and conditions: (a) Methyl 4-(cyanomethyl)benzoate, NaHMDS, THF, 0 °C to rt, then O_2, rt, quant; (b) bis(pinacolato)diboron, $PdCl_2$(dppf), AcOK, 80 °C, 55%; (c) oxone, $NaHCO_3$, acetone/H_2O, 0 °C to rt, 9%; (d) *t*-butyl 2-bromoacetate, K_2CO_3, DMF, rt, 94%; (e) 0.2 N NaOH aq, THF, rt, 21%; (f) 4-chlorophenethylamine, WSC-HCl, HOBT-H_2O, $CHCl_3$, rt, 59%; (g) 1 N NaOH aq, THF, rt, 39%.

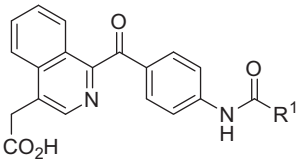
isoquinoline (**26**), which was reacted with methyl 4-(cyanomethyl)benzoate in the presence of sodium hexamethyldisilazane. Subsequent oxidative decyanation afforded **27**. Compound **27** was converted to **29** through the boronic acid ester **28** by the following two-step reaction method: (1) treatment with bis(pinacolato)diboron in the presence of $PdCl_2$(dppf) and potassium acetate, and (2) oxidation of **28** using oxone. Transformation of **29** into the target compound **33** was accomplished by the following conventional method: (1) alkylation of the hydroxyl moiety in **29** with *t*-butyl 2-bromoacetate in the presence of potassium carbonate, (2) basic hydrolysis of the methyl ester (NaOH), (3) condensation of the acid with 4-chlorophenethylamine (WSC-HCl and HOBT-H_2O), and (4) hydrolysis of the *t*-butyl ester moiety.

As described above, we designed and synthesized a new 1,4-disubstituted isoquinoline lead compound, and **6a** was identified as a ligand with moderate potency for the CRTH2 receptor. We initially we examined the effects of the steric factor of R^1 group on the CRTH2 binding potency (Table 1). When the methyl group was replaced with a bulky substituent such as a phenyl (**6b**), cyclohexyl (**6c**), 2-naphthyl (**6d**), or 1*H*-indol-2-yl group (**6e**), the binding affinity of the resulting compounds was dramatically enhanced (**6b**: IC_{50} = 7.9 nM, **6c**: IC_{50} = 14 nM, **6d**: IC_{50} = 4.0 nM, **6e**: IC_{50} = 3.5 nM) as compared with the potency of **6a**. By contrast, a 4-pyridyl (**6f**) or 2-pyrimidyl (**6g**) moiety led to little improvement in potency. These results suggested that the steric factor of R^1 group played a key role in increasing CRTH2 potency.

With these in vitro data in hand, further SAR studies of **6b** were conducted to examine the effects of substituents of the terminal phenyl ring on CRTH2 binding affinity. Incorporation of a chlorine atom into the *meta* or *para* position of the phenyl group led to an increase in binding potency (**6h**: IC_{50} = 3.2 nM, **6i**: IC_{50} = 3.4 nM), while the installation of a chlorine atom into the *ortho* position resulted in a 6-fold decrease in potency compared with **6b**. With regard to the *para* substituent, a trifluoromethyl group or a methoxy group was tolerated, and these compounds exhibited single digit nanomolar potency (**6k**: IC_{50} = 4.9 nM, **6l**: IC_{50} = 7.3 nM). In addition, the *meta*- and *para* di-substituted phenyl group was found to be well tolerated (**6m**: IC_{50} = 19 nM).

Next, we examined the rough SAR of the amide linker moiety of **6m** (Table 2). Replacement of the amide linker with an propanamide linker was tolerated in CRTH2 binding affinity (**14**: IC_{50} = 13 nM). When the propenamide linker was incorporated in place of the amide linker, the binding affinity of the resulting compound **15** (IC_{50} = 4.0 nM) increased by a factor of 4. By contrast, an N-methylamide **16**, which was devoid of the hydrogen bond donor NH, showed a 15-fold decrease in binding affinity (IC_{50} = 210 nM). These data suggested that the hydrogen bond donor (NH) played an important role in maintaining the high binding affinity. Furthermore, replacement of the amide moiety of **6m** with an aminomethylene tether (**17**) maintained potent CRTH2 binding affinity (IC_{50} = 6.1 nM), while the sulfonamide linker (**18**) led to a significant reduction in the binding (**18**: IC_{50} = 340 nM). Replacement with

Table 1
In vitro data of isoquinoline derivatives with R^1 substitution modification



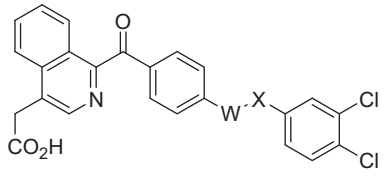
Compund	R^1	hCRTH2 binding IC_{50}[a] (nM)
6a	Methyl	330
6b	Ph	7.9
6c	Cyclohexyl	14
6d	2-Naphthyl	4.0
6e	1*H*-Indol-2-yl	3.2
6f	4-Pyridyl	83
6g	2-Pyrimidyl	290
6h	3-Cl-Ph	3.2
6i	4-Cl-Ph	3.4
6j	2-Cl-Ph	45
6k	4-CF_3-Ph	4.9
6l	4-OMe-Ph	7.3
6m	3,4-Di-Cl-Ph	19

[a] Mean values from at least two independent experiments. IC_{50} values were determined from full 10-point, half-log concentration–response curves.

an oxymethylene linker resulted in a slight loss of the potency (**19**: IC_{50} = 38 nM). Interestingly, the inverse amide linker (**20**) retained potent binding affinity (IC_{50} = 10 nM). These data supported the importance of the hydrogen bond donor (NH) in the linker moiety of the isoquinoline chemotype.

Subsequently, we examined the effects of substituents around the carboxylic acid moiety on CRTH2 binding affinity (Table 3). The carboxylic acid moiety is shared with the representative CRTH2 antagonists and is essential for CRTH2 activity. Germinal dimethylation of the methylene moiety next to the carboxylic acid resulted in a 25-fold drop in potency (**25**: IC_{50} = 200 nM) compared with the original compound **24**. Insertion of an oxygen atom between the carboxymethyl moiety and heteroaryl group resulted in a slight loss in potency (**33**: IC_{50} = 25 nM). These data suggest that the binding space of the CRTH2 receptor, where the acid moiety of the antagonists interacted, is limited.

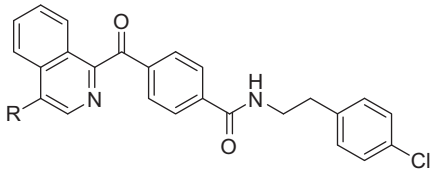
Table 2
In vitro data of isoquinoline derivatives with linker modification

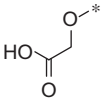


Compound	W	X	hCRTH2 binding IC_{50}[a] (nM)
6m	NH	CO	19
14	NH		13
15	NH		4.0
16	NMe	CO	210
17	NH	CH_2	6.1
18	NH	SO_2	340
19	O	CH_2	38
20	CO	NH	10

[a] Mean values from at least two indepentent experiments. IC_{50} values were determined from full 10-point, half-log concentration–response curves.

Table 3
In vitro data of isoquinoline derivatives with R substitution modifications



Compound	R	hCRTH2 binding IC_{50}[a] (nM)
24		7.8
25		200
33		25

[a] Mean values from at least two indepentent experiments. IC_{50} values were determined from full 10-point, half-log concentration–response curves.

In addition, these isoquinoline derivatives were functionally active and behaved as antagonists of PGD_2 driven Ca^{2+} flux in KB8 cells expressing human CRTH2.[3,22] One of the most potent antagonists, **6m** (IC_{50} = 19 nM), was tested in a chemotaxis assay[23] to determine its effectiveness (IC_{50} = 23 nM), which was in good agreement with the CRTH2 antagonist potency. Furthermore, sufficient level of selectivity was found for binding to CRTH2 over the DP1 prostanoid receptor[24] (IC_{50} >1 μM) and COX-1 and COX-2 enzymes[25] (IC_{50} > 10 μM).

In conclusion, we have identified the novel isoquinoline acetic acid chemotype **6** as a potent CRTH2 antagonist. SAR of the scaffold was explored, resulting in the identification of the compound **6m** (**TASP0376377**), which is a selective functional antagonist of CRTH2. Studies are ongoing to explore the utility of these compounds in inflammatory disease models and will be reported in due course.

References and notes

1. Nagata, N.; Tanaka, K.; Ogawa, K.; Kemmotsu, K.; Imai, T.; Yoshie, O.; Abe, H.; Tada, K.; Nakamura, M.; Sugamura, K.; Takano, K. *J. Immunol.* **1999**, *162*, 1278.
2. Nagata, K.; Hirai, H.; Tanaka, K.; Ogawa, K.; Aso, T.; Sugamura, K.; Nakamura, M.; Takanoa, S. *FEBS Lett.* **1999**, *459*, 195.
3. Pettipher, R.; Hansel, T. T.; Armer, R. *Nat. Rev. Drug Disc.* **2007**, *6*, 313.
4. Hirai, H.; Tanaka, K.; Yoshie, O.; Ogawa, K.; Kenmotsu, K.; Takamori, Y.; Ichimasa, M.; Sugamura, K.; Nakamura, M.; Takano, S.; Nagata, K. *J. Exp. Med.* **2001**, *193*, 255.
5. Monneret, G.; Gravel, S.; Diamond, M.; Rokach, J.; Powell, W. S. *Blood* **1942**, *2001*, 98.
6. Xue, L.; Gyles, S. L.; Wettey, F. R.; Gazi, L.; Townsend, E.; Hunter, M. G.; Pettipher, R. *J. Immunol.* **2005**, *175*, 6531.
7. Evi Kostenis, E.; Ulven, T. *Trend Mol. Med.* **2006**, *12*, 148.
8. Satoh, T.; Moroi, R.; Aritake, K.; Urade, Y.; Kanai, Y.; Sumi, S.; Yokozeki, H.; Hirai, H.; Nagata, K.; Hara, T.; Utsuyama, M.; Hirokawa, K.; Sugamura, K.; Nishioka, K.; Nakamura, M. *J. Immunol.* **2006**, *177*, 2621.
9. Stearns, B. A.; Baccei, C.; Bain, G.; Broadhead, A.; Clark, R. C.; Coate, H.; Evans, J. F.; Fagan, P.; Hutchinson, J. H.; King, C.; Lee, C.; Lorrain, D. S.; Prasit, P.; Prodanovich, P.; Santini, A.; Scott, J. M.; Stock, N. S.; Truong, Y. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4647.
10. Birkinshaw, T. N.; Teague, S. J.; Beech, C.; Bonnert, R. V.; Hill, S.; Patel, A.; Reakes, S.; Sanganee, H.; Dougall, I. G.; Phillips, T. T.; Salter, S.; Schmidt, J.; Arrowsmith, E. C.; Carrillo, J. J.; Bell, F. M.; Paine, S. W.; Weaver, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4287.
11. Tumey, L. N.; Robarge, M. J.; Gleason, E.; Song, J.; Murphy, S. M.; Ekema, G.; Doucette, C.; Hanniford, D.; Palmer, M.; Pawlowski, G.; Danzig, J.; Loftus, M.; Hunady, K.; Sherf, B.; Mays, R. W.; Stricker-Krongrad, A.; Brunden, K. R.; Bennani, Y. L.; Harrington, J. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3287.
12. Richard, E.; Armer, R. E.; Ashton, M. R.; Boyd, E. A.; Brennan, C. J.; Brookfield, F. A.; Gazi, L.; Gyles, S. L.; Hay, P. A.; Hunter, M. G.; Middlemiss, D.; Whittaker, M.; Xue, L.; Pettipher, R. *J. Med. Chem.* **2005**, *48*, 6174.
13. Ulven, T.; Gallen, M. J.; Nielsen, M. C.; Merten, N.; Schmidt, C.; Mohr, K.; Tränklec, C.; Kostenis, E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5924.
14. Armer, R. E.; WO 2005/121141; *Chem. Abstr.* **2005**, *144*, 51458.
15. Sandham, D. A.; Adcock, C.; Bala, K.; Barker, L.; Brown, Z.; Dubois, G.; Budd, D.; Cox, B.; Fairhurst, R. A.; Furegati, M.; Leblanc, C.; Manini, J.; Profit, R.; Reilly, J.; Stringer, R.; Schmidt, A.; Turner, K. L.; Watson, S. J.; Willis, J.; Williams, G.; Wilson, C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4794.
16. Fretz, H. WO 2006/021418; *Chem. Abstr.* **2006**, *144*, 274271.
17. Bennani, Y. L. WO 2006/034418; *Chem. Abstr.* **2006**, *144*, 350544.
18. Hynd, G. WO 2006/136859; *Chem. Abstr.* **2006**, *146*, 100556.
19. Crosignani, S.; Page, P.; Missotten, M.; Colovray, V.; Cleva, C.; Arrighi, J.-F.; Atherall, J.; Macritchie, J.; Martin, T.; Humbert, Y.; Gaudet, M.; Pupowicz, D.; Maio, M.; Pittet, P.-A.; Golzio, L.; Giachetti, C.; Rocha, C.; Bernardinelli, G.; Filinchuk, Y.; Scheer, A.; Schwarz, M. K.; Chollet, A. *J. Med. Chem.* **2008**, *51*, 2227.
20. Kulp, S. S.; McGee, M. J. *J. Org. Chem.* **1983**, *48*, 4097.
21. Kirmse, W. *Eur. J. Org. Chem.* **2002**, *14*, 2193.
22. 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 $CaCl_2$ (2×10^5cells/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). The IC_{50}s of the representative compounds (**6b**, **6g**, **6k**, **15**, **19**) were 42 nM, 550 nM, 15 nM, 17 nM, and 180 nM, respectively.
23. 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 days and, then, expanded by IL-2 and IL-4 for 7 days. Th2 cells highly expressing CRTH2 were separated with anti-CRTH2 mAb, and 2 days 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, USA), respectively. After incubation at 37 °C for 1 h, number of the cells in the bottom wells were counted by Burker–Turk hemocytometer.

24. The binding assay for DP1 was run by Recerca Biosciences (Bothell, USA) using the profiler service according to the manufacturer's procedures.
25. The enzyme inhibition assays for COX-1 and COX-2 were run by Cerep (Paris, France) using the profiler service according to the manufacturer's procedures.