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New indole amide derivatives as potent CRTH2 receptor antagonists

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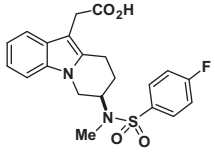
ABSTRACT

A new series of indole amide acting as hCRTH2 receptor ligands had been explored and are described herein. Several amide derivatives displaying low nanomolar activity in hCRTH2 binding and whole blood assays were identified. They were found to behave as a full antagonists, exhibiting good selectivity over related prostaglandin receptors. Also, prototypical compounds in this novel series which displayed acceptable CYP profiles and were orally bioavailable in rats were identified.

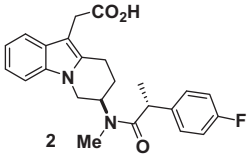
Prostaglandin D2 (PGD2) is produced from arachidonic acid by the combined action of cyclooxygenase and synthase.[1] It is released from mast and Th2 cells in response to an immunological challenge, and has been implicated in different physiological events such as allergic inflammation.[2] PGD2 can activate two distinct receptors, namely the DP receptor,[3] and the chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells (i.e., hCRTH2 also referred as the DP2 receptor).[4] hCRTH2 is a G-protein-coupled receptor[5] and its activation by PGD2 stimulates the recruitment of leukocytes, the release of Th2 cytokines,[6] and the degranulation of basophils and eosinophils.[7] The combined pharmacological action of these biological events plays a key role in late phase allergic inflammation. Such observations represent a sound biological rational for the development of selective hCRTH2 antagonists for the treatment of asthma.[8]

We previously reported the identification of a new class of potent and selective hCRTH2 antagonists. SAR studies in this class of tetrahydropyrido[1,2-*a*] indoles led to the discovery of the clinical candidate **MK-7246** (Fig. 1).[9] Patients who have been exposed to sulfonamide containing antibiotics can develop an immune response leading to adverse effects such as fever, skin rashes, GI disturbance and liver toxicity.[10] As a result, they become hypersensitive to any sulfonamide containing drugs (i.e., sulfa drugs).[10,11] Although such adverse events are considered as idiosyncratic drug reaction, we rationalized that the search for an acceptable replacement group for the sulfonamide moiety present in **MK-7246** was a valid starting point to initiate SAR studies aimed at identifying a back-up series. In this Letter, we would like to report the discovery of a new class of tet-



1: MK-7246

Figure 1. The structure of **MK-7246**.



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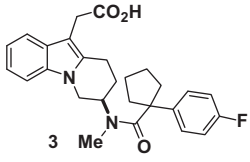

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Figure 2. New series of reverse indole amide (compound **2** (hCRTH2 K_i = 4.0 nM) and compound **3** (hCRTH2 K_i = 1.7 nM)).

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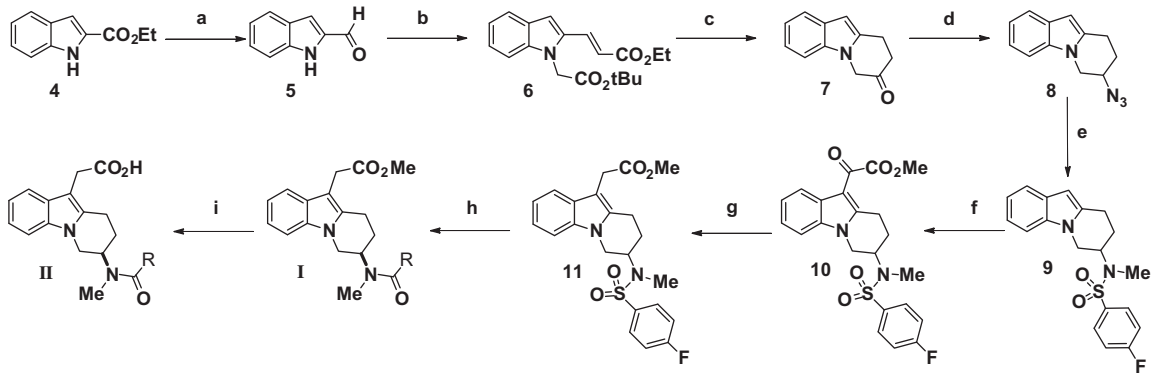


Scheme 1. Reagents and conditions: (a) (i) $LiAlH_4$, THF, −78 °C, 2 h; (ii) MnO_2, CH_2Cl_2, rt, 3 h, 100% (over two steps); (b) (i) carbethoxymethylene triphenylphosphorane, CH_2Cl_2, rt, 12 h, 84%; (ii) *tert*-butyl bromoacetate, Cs_2CO_3, DMF, 60 °C, 18 h, 60%; (c) (i) H_2, Pd/C, EtOH, 1 atm, 12 h, 90%; (ii) KO*t*Bu, THF, rt, 20 h, 97%; (iii) Silica gel, toluene, reflux, 2 h, 82%; (d) (i) $NaBH_4$, THF, 0 °C, 1 h, 100%; (ii) methanesulfonyl chloride, Et_3N,CH_2Cl_2, −40 °C, 30 min, 98%; (iii) sodium azide, DMF, 60 °C, 12 h, 75%; (e) (i) H_2, Pd/C, MeOH, rt, 12 h, 96%; (ii) 4-fluorobenzensulfonyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 4 h, 70%; (iii) MeI, NaH, DMF, 0 °C to rt, 3 h, 96%; (f) (i) oxalyl chloride, CH_2Cl_2, 0 °C, 1 h; (ii) MeOH, CH_2Cl_2, rt, 1 h, 90% (over two steps); (g) (i) $NaBH_4$, THF, 0 °C, 1 h, 100%; (ii) triethylsilane, MeI, CH_3CN, 30 min, 95%; (h) (i) Mg, MeOH, 50 °C, 4 h, 54%; (ii) 4-fluorophenylacetyl chloride, DMAP, Et_3N, CH_2Cl_2, rt, 2 h, 94%; (i) NaOH (2 N), THF/MeOH (2:1), rt, 2 h, 98%.

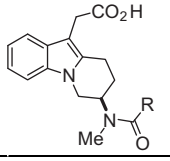
rahydropyrido[1,2-*a*] indoles, bearing an amide moiety, (Fig. 2) acting as potent and selective hCRTH2 antagonists derivatives.[12]

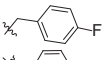
The hCRTH2 antagonists in this novel series were prepared according to the synthetic route described in Scheme 1. Reduction of the ester indole **4** followed by an oxidation with manganese oxide gave the corresponding aldehyde **5**. Wittig reaction followed by *N*-alkylation of the indole **5** afforded the α,β-unsaturated ester **6**, as a mixture (*E*/*Z*) of alkene isomers. This intermediate **6** was then subjected to hydrogenolysis followed by a Dieckman condensation[13] with KO*t*Bu. Decarboxylation using silica gel in refluxing toluene yielded the desired ketone **7**. Reduction of the ketone group to the alcohol followed by activation with methanesulfonyl chloride and S_N2 reaction with sodium azide afforded the desired azide **8**. Compound **9** was generated in three steps from the azide **8**. Hydrogenation of the azide gave the corresponding racemic amine which was *N*-sulfonylated with 4-fluoro benzenesulfonyl chloride under basic conditions. The resulting sulfonamide was finally *N*-methylated using NaH and MeI. C-3-directed nucleophilic addition of indole **9** to oxalyl chloride afforded the glyoxylated derivative **10** following addition of methanol.[14] The resulting keto-ester **10** was reduced with sodium borohydride followed by the dehydration with triethylsilane and iodomethane to yield the racemic ester **11**.[15] Resolution by chiral HPLC allowed the separation of the two enantiomers. The amide functionality of **I** was introduced by radical deprotection of the sulfonamide using magnesium followed by acylation of the resulting amine with an appropriately substituted acyl chloride. Finally, the ester was saponified to afford the desired amide **II**.

SAR studies were initiated to identify an amide group that would be conformationally similar to the sulfonamide moiety present in **MK-7246** (Table 1). Benzamide (**12**), naphthamide (**13**, **14**) and the non-aromatic amide **20**, were all found to be unacceptable alternatives as they all lead to significant loss in potency on the hCRTH2 receptor. On the other hand, the benzyl amides **15** and **16** were satisfactory replacements to **MK-7246**'s sulfonamide moiety. Both compounds exhibited low nanomolar potency against hCRTH2 receptor and behaved as full antagonists as shown by their relative IC_{50}'s in the functional cAMP assay. Additionally, amides **15** and **16** were selective for hCRTH2 versus the DP and TP receptors (K_i's >1 μM). Conformationally restricted benzyl amide analogs such as **17**, **18**, and **19** were also found to be unacceptable replacements.

With our best amide replacement in hand, we pursued SAR studies by exploring the substitution pattern on the amide benzyl group (Table 2). Unfortunately, all attempts to replace the fluorine atoms or to incorporate additional substituents failed to improve potency on the hCRTH2 receptor.

Table 1
Binding affinity and functional antagonism on hCRTH2 for preliminary aryl amides



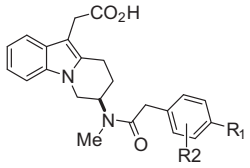
Compd	R	hCRTH2[a] K_i (nM)	cAMP[b] IC_{50} (nM)
MK-7246	--	2.5	3.0
12		340	2500
13		120	540
14		370	2100
15		15	25
16		7.4	12
17		670	810
18		110	140
19		1700	3700
20		240	630

[a] Radioligand competition binding assay using membrane proteins from HEK293 (EBNA) cells stably expressing the receptor CRTH2 in a 10 mM solution of HEPES/KOH (all values are mean of two or more experiments).[16]

[b] Functional assay: the intracellular concentration of cAMP was determined using the ^{125}I-cAMP scintillation proximity assay. The assay was performed in Hank's balanced salt solution 25 mM HEPES containing 5 μM Forskolin (K_i's are an average of at least tow independent titrations).[17]

Consequently, amide **16** was selected for further profiling. We performed pharmacokinetic studies in Sprague–Dawley (SD) rats (Table 4) and found that despite the fact that the clearance, the volume of distribution and half life were comparable to **MK-7246**, the oral bioavailability (14%) needed to be improved. The in vitro metabolism of **16** in SD rat and human hepatocytes[18] was investigated to see if it could shed some light on the origin of the low oral bioavailability. The extent of metabolism observed

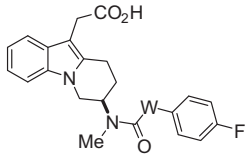
Table 2
SAR on the benzyl ring substitution



Compd	R^1	R^2	hCRTH2[a] K_i (nM)
MK-7246	––	––	2.5
16	F	H	7.4
21	F	2-Cl	184
22	F	2-CF_3	141
23	F	2-F	70
24	F	3Cl	22
25	F	3-CF_3	126
26	F	3-F	19
27	Cl	H	31
28	OMe	H	14
29	CF_3	H	408
30	NMe_2	H	1220

[a] K_i's are an average of at least tow independent titrations.

Table 3
SAR on the amide linker



Compd	W	hCRTH2[a] K_i (nM)	EOS[b,d] IC_{50} (nM)	cAMP[c] IC_{50}(nM)
MK-7246	––	2.5	2.2	3.0
16	CH_2	7.4	4.8	12
2		4.0	0.96	3.0
31		39		30
32		3.0	6.0	6.2
33		2.0	4.4	2.0
34		1.8	1.0	4.0
3		1.7	1.4	4.0
35		4.3	2.4	8.0

[a,b,c] K_i's and IC_{50}'s are an average of at least tow independent titrations.
[d] Whole blood eosinophil shape change assay.[16]

was low in rat hepatocytes with 91% of the parent remaining after 1 h of incubation while 60% of the parent was glucuronidated in human hepatocytes. Neither hydroxylated metabolites, nor amide hydrolysis was observed. Since metabolism does not seem to be implicated in the low oral bioavailability of amide **16** in rats, lack of absorption or active transport in the liver leading to rapid elimination in bile are hypothesized to be involved.

The remaining unexplored segment of the amide moiety was the substitution at the benzylic position. Several compounds were prepared and the results are displayed in Table 3. Introduction of a methyl substituent led to the pair of diastereoisomers **2** and **31**.[19] As a result, we discovered that the chirality of the newly formed asymmetric center had a significant impact on potency. Indeed, the *R*-isomer **2** was found to be 10-fold more potent then the corresponding *S*-isomer **31** as exemplified by their respective hCRTH2 K_i's. More importantly, the activity of **2** in the whole blood assay, measuring eosinophil shape change, was similar to **MK-7246**.[20] The absolute configuration of **MK-7246** was determined by X-ray crystallography.[21] Analogs bearing two benzylic substituents (acyclic and cyclic; i.e., **32**–**35**) were also found to be potent hCRTH2 antagonists with K_i's ranging from 1.7 to 4.3 nM and EOS IC_{50}'s from 2 to 8 nM. To appropriately distinguish these substituted analogs, we performed rat pharmacokinetic studies on all of them. Compounds **2**, **3**, and **34** demonstrated superior pharmacokinetic characteristics, in particular oral bioavailability, and were further profiled (Table 4).

Firstly, the binding affinities of **2**, **3**, and **34** were measured against other prostaglandin receptors (IP, EP1, EP2, EP3, EP4 and FP) and in all cases they were found to be selective for the hCRTH2 receptor by at least 500-fold. They showed little or no inhibitory activities against the CYP3A4 and 2C9, representing a significantly improvement over **MK-7246**. Finally, compound **2**, **3**, and **34** demonstrated improved oral bioavailability compared to the unsubstituted analog **16**.

In summary, we identified a novel series of tetrahydropyrido[1,2-*a*] indole amides acting as potent and selective hCRTH2 antagonists. In particular, analogs **2**, **3**, and **34** were found to be equipotent to our clinical candidate **MK-7246** in the whole blood eosinophil shape change assay but more importantly were devoid of inhibitory activity against CYP2C9. Finally, we successfully discovered a replacement for the sulfonamide moiety present in **MK-7246** by appropriately substituted amide groups.

References and notes

1. Haeggström, J. Z.; Rinaldo-Matthis, A.; Wheelock, C. E.; Wetterholm, A. *Biochem. Biophys. Res. Commun.* **2010**, *21*, 135.
2. (a) Sood, A.; Arora, R. *J. Clin. Hypertens.* **2009**, *11*, 685; (b) Kostenis, E.; Ulven, T. *Trends Mol. Med.* **2006**, *12*, 148.
3. Walch, L.; Labat, C.; Gascard, J. P.; de Montpreville, V.; Brink, C.; Norel, X. *Br. J. Pharmacol.* **1999**, *126*, 859.
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. (a) Honda, T.; Tokura, Y.; Miyachi, Y.; Kabashima, K. *Curr. Drug Targets* **2010**, *11*, 1605; (b) Jones, R. L.; Giembycz, M. A.; Woodward, D. F. *Br. J. Pharmacol.* **2009**, *158*, 104.
6. Xue, L.; Barrow, A.; Pettipher, R. *Clin. Exp. Immunol.* **2009**, *156*, 126.
7. Boyce, J. A.; Broide, D.; Matsumoto, K.; Bochner, B. S. *J. Allergy Clin. Immunol.* **2009**, *123*, 569.

Table 4
Pharmacokinetic data in SD rat[a] as well as the CYP inhibition for potent hCRTH2 antagonist compounds

Compd	*F* (%)	Cl (mL/min/kg)	V_d (L/kg)	$t_{½}$ (h)	CYP 3A4 IC_{50} (μM)	CYP 2C9 IC_{50} (μM)
MK-7246	138	3.9	1.1	4.1	34	9
2	73	1.1	0.36	4.7	>50	>50
3	74	2.7	0.47	2.2	37	>50
16	14	0.38	0.13	4.6	>50	>50
34	93	12	2.6	2.6	>50	>50

[a] po dosing solution: 5 mg/kg (5 mL/kg) in 0.5% methocel ($n = 2$), IV dosing solution: 1 mg/kg (1 mL/kg) in 60% PEG 200 ($n = 2$).

8. (a) Schuligoi, R.; Sturm, E.; Luschnig, P.; Konya, V.; Philipose, S.; Sedej, M.; Waldhoer, M.; Peskar, B. A.; Heinemann, A. *Pharmacology* **2010**, *85*, 372–382; (b) Pettipher, R.; Hansel, T. T. *Drug News Perspect.* **2008**, *21*, 317.
9. (a) Indole derivatives as CRTH2 receptor antagonists, Wang, Z. WO2007019675 and WO2010031182.; (b) Gervais, F. G.; Sawyer, N.; Stocco, R.; Hamel, M.; Krawczyk, C.; Sillaots, S.; Denis, D.; Wong, E.; Wang, Z.; Gallant, M.; Abraham, W. M.; Slipetz, D.; Crackower, M. A.; O'Neill, G. P. *Mol. Phamacol.* **2011**, *79*, 1.
10. Slatore, C. G.; Tilles, S. A. *Immunol. Allergy. Clin. N. Am.* **2004**, *24*, 477.
11. Castrejon, J. L.; Berry, N.; El-Ghaiesh, S.; Gerber, B.; Pichler, W. J.; Park, B. K.; Naisbitt, D. J. *J. Allergy Clin. Immunol.* **2010**, *125*, 411.
12. Indole derivatives as CRTH2 receptor antagonists, Colucci, J.; Boyd, M.; Zaghdane, M. H. WO2010031183.
13. Christie, B. D.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 1239.
14. Liu, Y.; Wu, J.; Weissig, H.; Betancort, J. M.; Gai, W. Z.; Leventhal, P. S.; Patricelli, M. P.; Samii, B.; Szardenings, A. K.; Shreder, K. R.; Kozarich, J. W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5955.
15. Collot, V.; Schmitt, M.; Marwah, P.; Bourguignon, J.-J. *Heterocycles* **1999**, *51*, 2823.
16. Abramovitz, M.; Adam, M.; Boie, Y.; Carrière, M.-C.; Denis, D.; Godbout, C.; Lamontagne, S.; Rochette, C.; Sawyer, N.; Tremblay, N. M.; Belley, M.; Gallant, M.; Dufresne, C.; Gareau, Y.; Ruel, R.; Juteau, H.; Labelle, M.; Ouimet, N.; Metters, K. M. *Biochim. Biophys. Acta* **2000**, *1483*, 285.
17. Sawyer, N.; Cauchon, E.; Chateauneuf, A.; Cruz, R. P.; Nicholson, D. W.; Metters, K. M.; O'Neill, G. P.; Gervais, F. G. *Br. J. Pharmacol.* **2002**, *137*, 1163.
18. The compounds were incubated at 10 μM in the presence of fresh SD rat or fresh human hepatocytes (1e6 cells/mL, Krebs–Henseleit buffer + 3 g/L Hepes, pH = 7.4) for 2 h at 37 °C. Incubations were stopped by adding an equal volume of acetonitrile + 1% formic acid, centrifuged and supernatants were analyzed by UPLC-PDA-Qtof.
19. The absolute configuration of **2** and **31** was determined using Evans' oxazolidinone chiral auxiliaries (data not included).; Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127; Bull, S. D.; Davies, S. G.; Garner, A. C.; Kruchinin, D.; Key, M.-S.; Roberts, P. M.; Savory, E. D.; Smith, A. D.; Thomson, J. E. *Org. Biomol. Chem.* **2006**, *4*, 2945.
20. (a) Gervais, F. G.; Cruz, R. P.; Chateauneuf, A.; Gale, S.; Sawyer, N.; Nantel, F.; Metters, K. M.; O'Neill, G. P. *J. Allergy Clin. Immunol.* **2001**, *108*, 982; (b) Monneret, G.; Gravel, S.; Diamond, M.; Rokach, J.; Powell, W. S. *Blood* **2001**, *98*, 1942.
21. The absolute configuration of **MK-7246** was determined by X-ray crystallography (Unpublished data).