Bioorganic & Medicinal Chemistry Letters 21 (2011) 841–845

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Azaindoles as potent CRTH2 receptor antagonists

Daniel Simard *, Yves Leblanc, Carl Berthelette, M. Helmi Zaghdane, Carmela Molinaro, Zhaoyin Wang, Michel Gallant, Stephen Lau, Trinh Thao, Martine Hamel, Rino Stocco, Nicole Sawyer, Susan Sillaots, Francois Gervais, Robert Houle, Jean-François Lévesque

Merck Frosst Centre for Therapeutic Research, 16711 Trans Canada Highway, Kirkland, Québec, Canada H9H 3L1

ARTICLE INFO

Article history:
Received 28 October 2010
Revised 14 November 2010
Accepted 17 November 2010
Available online 21 November 2010

Keywords:
CRTH2
Chemoattractant receptor-homologous
PGD2
Prostanoid
Azaindole
Tetrahydropyridoindolizines

ABSTRACT

A new class of 7-azaindole analogs of MK-7246 as potent and selective CRTH2 antagonists is reported. The SAR leading to the identification of the optimal azaindole regioisomer as well as the pharmacokinetics and off-target activities of the most potent antagonists are disclosed.

Prostaglandin D_2 (PGD_2) is known to bind with high affinity to two G protein-coupled seven-transmembrane receptors DP1 and DP2. The latter receptor, also known as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), is responsible for the chemoattractant effects of PGD_2 on eosinophils, basophils and Th2-cells.[1,2] The receptor also induces eosinophils shape change and degranulation, the production of proinflammatory cytokines in Th2 cells, and enhances the release of histamine from basophils. The combined pharmacological action of such biological events plays a key role in late phase allergic inflammation. CRTH2 is thus pursued as a target for respiratory diseases such as asthma and COPD.[3]

We recently reported the identification of a potent and selective CRTH2 antagonist[4] (Fig. 1). As Phase I clinical trials were in progress, our main focus was to develop a potential backup for MK-7246 which would have an improved off-target activity profile. Our lead compound had a propensity to undergo moderate covalent binding on hepatic cells and showed micromolar activity on CYP2C9. Consequently, efforts in a new series were undertaken.

One of the preferred approaches was to synthesize close analogs of the lead compound where the indole core would be replaced by an azaindole.[5] This Letter reports the synthesis of all four possible regioisomers (Fig. 2) as well as their structure–activity relationship. In view of increasing structural diversity of the newly born series and potentially avoiding allergic-reaction-type adverse effects related to the sulfonamide moiety,[6] an amido-azaindole series was also explored and is reported.

The original lengthy synthesis of MK-7246 proved to be incompatible with azaindoles as starting material. The key Dieckmann condensation could not be applied to that system and as a result a new synthetic route was devised.[4b]

Efforts in other series had led to the development of a new route taking advantage of the multi-nucleophilic nature of the indole (Scheme 1).[7] The use of an electrophilic aziridine bearing all of the carbons necessary for the construction of the carbocycle as well as the stereogenic center required in the final product, dramatically reduced the synthetic efforts needed to pursue SAR in that series. The number of linear steps fell from 18 to only eight steps.

The synthesis of the aziridine **8** (Scheme 2) started with D-aspartic acid as chiral building block. Methylation of the carboxylic acids followed by the introduction of the required sulfonamide yields the diester **9**. The diester was then reduced to reveal diol **10** which

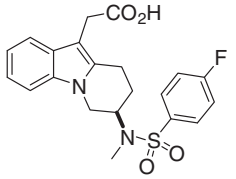


Figure 1. Lead compound MK-7246.

* Corresponding author. Tel.: +1 514 428 8508; fax: +1 514 428 4900.
E-mail address: daniel.simard@merck.com (D. Simard).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.084

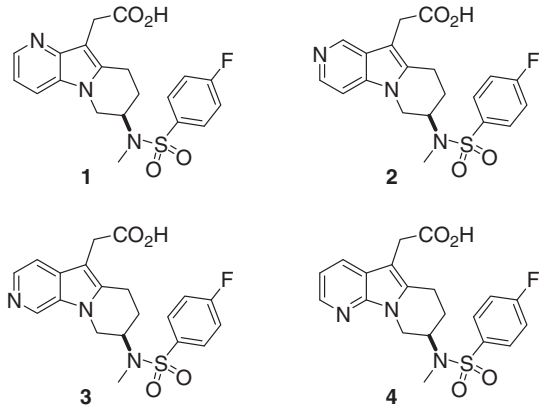
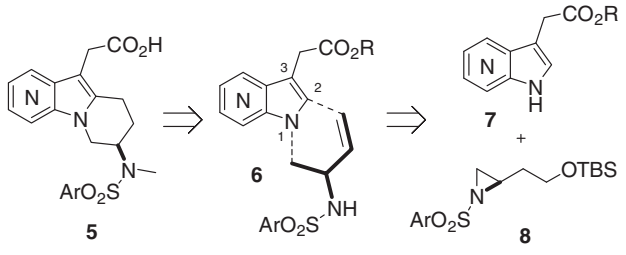


Figure 2. Azaindole analogs.

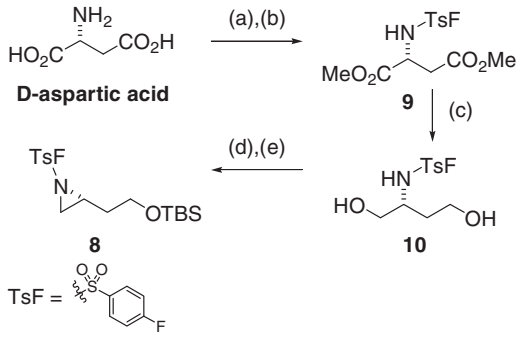
underwent selective intramolecular Mitsunobu reaction with the vicinal sulfonamide. The resulting alcohol bearing aziridine could then be protected with a TBS group and used for rapid construction of our inhibitors.

The synthesis began with the addition of the aziridine **8** onto the azaindole core **11** with sodium hydride in DMF. Simple synthetic transformations then allowed the formation of an electrophilic aldehyde **14** (Scheme 3). Although, this aldehyde was known to cyclize in high yield to generate the corresponding tricyclic compound with the indole core, the same reactivity was not observed with all azaindole regioisomers.

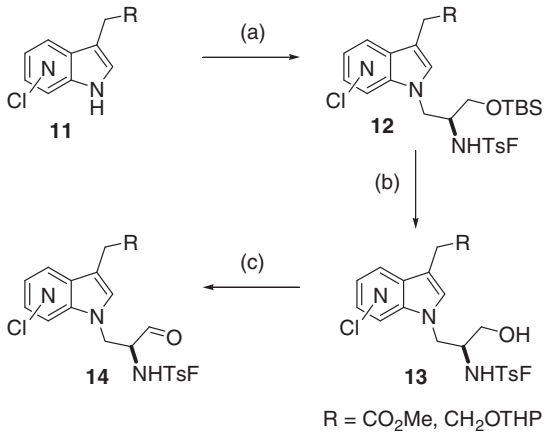
Indeed, most regioisomers cyclized with low yields except 7-azaindoles **18** and **19** (Scheme 4). The 4-azaindole **15** failed to condense under normal reaction condition (PPTS, toluene, reflux). Only when the solvent was switched to NMP, a low yield cyclization was observed. 4-Azaindole and 6-azaindole cyclizations failed in all



Scheme 1. Retrosynthetic analysis of key step.



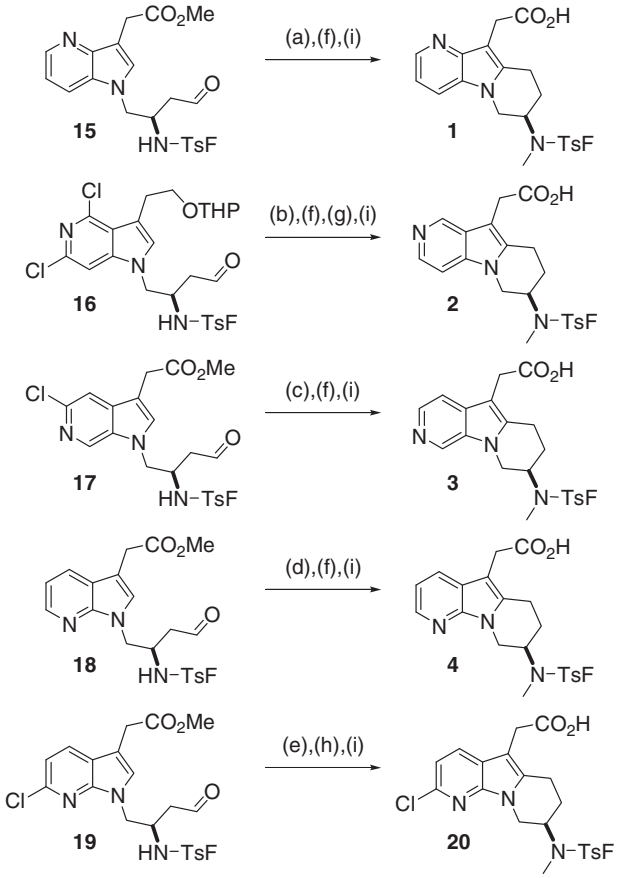
Scheme 2. Synthesis of chiral aziridine **8**. Reagents and conditions: (a) $SOCl_2$, MeOH, 95%; (b) 4-fluorobenzene sulfonyl chloride, TEA, THF, 95%; (c) $NaBH_4$, EtOH, 70%; (d) PBu_3, 1,1′-(azodicarbonyl)dipiperidine (ADDP), THF, 85%; (e) TBSCl, imidazole, THF, 80%.



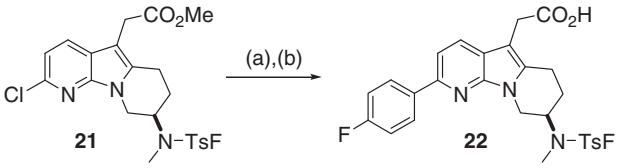
Scheme 3. General synthesis of condensation precursor from aziridine **8**. Reagents and conditions: (a) NaH, **8**, DMF, 38–85%; (b) TBAF, THF, 86–100%; (c) DMP, DCM, 45–95%.

conditions attempted. Cyclization was observed in low yield only when a chlorine substituent was incorporated onto the azaindole core (i.e., **16** and **17**).

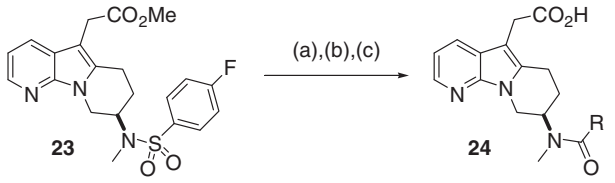
Next, the reduction of the condensation double bond and the carbon–chlorine bonds was investigated (for condensation products of compounds **16** and **17**). Condensation product of compound **19** was singularly hydrogenated using Monsanto catalyst[11] to avoid dehalogenation of chlorine.



Scheme 4. Condensation to tricylic azaindoles. Reagents and conditions: (a) PPTS, NMP, 180 °C, 31%; (b) PPTS, toluene, 110 °C, 27%; (c) PPTS, 4:1 toluene/NMP, 150 °C, 6%; (d) PPTS, toluene, 110 °C, 86%; (e) PPTS, toluene, 110 °C, 80%; (f) Pd/C, H_2, MeOH, 50–95%; (g) DMP, DCM, 99% then $NaClO_2$, H_3PO_4, 2-methyl-2-butene, 72%; (h) $[Rh(COD)(R,R)\text{-DIPAMP}]^+BF_4^-$, 40 psi H_2, MeOH, 95%. (i) NaH, MeI, THF, 70–95% then aq NaOH, MeOH, THF, 90–99%.



Scheme 5. Diversification at position-6 of the 7-azaindole core. Reagents and conditions: (a) 4-fluorophenyl boronic acid, $NaHCO_3$, $Pd(dppf)_2(CH_2Cl_2)_2$, 40%; (b) aq NaOH, MeOH, THF, 85%.



Scheme 6. Synthesis of azaindole amides. Reagents and conditions: (a) Mg, MeOH, 80%; (b) RCO_2H, HATU, DIPEA, DMF, 65–96%; (c) aq. NaOH, THF, MeOH, 85–99%.

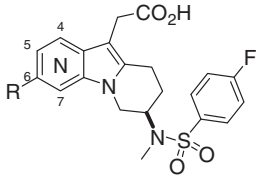
Compound **16** was generated using a literature procedure[12] and required two-step Dess–Martin-periodinane/Pinnick oxidation prior to the methylation of the sulfonamide. Compound **22** (Scheme 5) was obtained from the Suzuki coupling of the methyl ester intermediate **21** (en route to compound **20**). Hydrolysis of all methyl ester intermediates with sodium hydroxide yielded compounds **1**–**4**, **20** and **22** which were assayed for binding on prostanoid receptors (Table 1).

From the results shown in Table 1, only the 7-azaindoles **4**, **20** and **22** demonstrated low nanomolar activity in the hCRTH2 binding assay. Similar results in the cAMP functional assay clearly indicated that this class of compounds behaves as a full antagonist. Additionally, compounds **4**, **20** and **22** showed superior selectivity for hCRTH2 against DP and TP prostanoid receptors in comparison to compound MK-7246. Conversely, the 4- and 5-azaindole regioisomers **1** and **2** showed very low affinity on all prostanoid receptors tested, and although the 6-azaindole **3** displayed some affinity for *h*CRTH2 (139 nM), we focused our efforts on the more promising 7-azaindole series. Finally, compound **4** was found not to be a CYP2C9 inhibitor (IC_{50} >50 μM) as compared to the lead MK-7246 (IC_{50} = 9.4 μM) but failed to address the in vivo covalent binding issue associated with MK-7246 (vide infra).

Functionalization at the position-6 of the 7-azaindole with chlorine is well tolerated. In the EOS whole blood assay, the azaindole **20** exhibited an activity similar compared to MK-7246. Since the presence of a 6-chloro substituent creates an electrophilic site on the azaindole core, we considered introducing a metabolically more robust substituent that would maintain the gain in potency while reducing the possibility of a nucleophilic addition mediated metabolism. With the introduction of an aromatic ring at position-6, as in compound **22** (Scheme 5), the binding affinity for *h*CRTH2 was conserved, but an eight fold shift in the whole blood EOS assay was observed.

Although the overall profile of **4** was encouraging, we pursued its optimization based on SAR studies imported from a novel class of compounds in which the sulfonamide moiety of MK-7246 was replaced by an amide group.[13] Potential liabilities related to the

Table 1
Activities of azaindole sulfonamides



Compd	N[a]	R	*h*CRTH2[b] K_i (nM)	cAMP[c] IC_{50} (nM)	EOS[d] IC_{50} (nM)	DP[b] K_i (nM)	TP[b] K_i (nM)
MK-7246	—	H	2.5	3.0	2.2	373	3804
1	4	H	7189	—	—	>1300	>7000
2	5	H	6725	—	—	>3400	>6800
3	6	H	139	351	—	>12000	>22000
4	7	H	3.3	3.4	7.0	>47000	>22000
20	7	Cl	1.8	3.2	3.3	>18000	>27000
22	7	4F-Ph	1.9	3.6	15.8	>3800	>1000

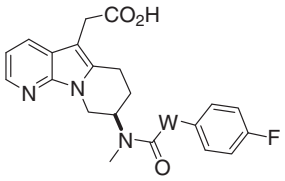
[a] Azaindole nitrogen position.
[b] Radioligand competition binding assay using membrane proteins from HEK293 (EBNA) cells stably expressing the receptor *h*CRTH2 in a 10 mM solution of HEPES/KOH (all values are mean of two or more experiments).[8]
[c] Functional assay: the intracellular concentration 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 (all values are mean of two or more experiments).[9]
[d] Human whole blood eosinophil shape change assay (all values are mean of two or more experiments).[10]

Table 2
Covalent binding and CYP profiles of selected antagonists[a]

Compd	In vivo CB (pmol equiv/mg)		CYP2C9 IC_{50} (μM)	CYP3A4 IC_{50} (μM)	3A4 TDI K_{obs} (min^{-1}) at 50 mM
	6 h	24 h			
MK-7246	61	30	9.4	34	0.047
4	52	50	>50	>50	—
24a	8	6	>50	>50	<0.004

[a] IC_{50} are averages of at least two experiments.

Table 3
Activities of azaindole amides



Compd	W	*h*CRTH2[a] K_i (nM)	cAMP[b] IC_{50} (nM)	EOS[c] IC_{50} (nM)	DP[a] K_i (nM)	TP[a] K_i (nM)
24a		3.4	5.7	1.2	>38000	>71000
24b		21.1	73.8	—	>12000	>22000
24c		4.7	3.5	2.3	>10000	>21000
24d		3.6	7.5	3.1	>3700	>6800
24e[d]		5.1	4.4	2.4	>4000	>7200
24f		3.4	4.7	1.2	>4000	>7200
24g		3.9	4.5	3.4	>4000	>7200
24h		11.5	7.0	-	>4000	>7200

[a–c] See Table 1.
[d] Diastereomeric mixture.

presence of a sulfonamides group (i.e., allergic reaction to 'sulfa' drugs) as well as the potential for in vivo covalent binding found for compound MK-7246 and **4** (Table 2) were the main drivers for exploring this new chemical space.

Following the sequence shown in Scheme 6, we converted the previously obtained sulfonamide intermediate **23** into amides via a magnesium radical reduction of the sulfonamide to the secondary amine. The resulting amine was diversified using a series of different carboxylic acids using HATU coupling conditions followed by hydrolysis under standard conditions to obtain antagonists **24a**–**h**.

Most amides obtained through this process showed low nanomolar activity on both the binding and the whole blood assays (Table 3). They also showed excellent selectivity for the *h*CRTH2 receptor over the other prostanoid receptors. Pharmacokinetic studies in rats on this class of compounds revealed that they were orally bioavailable and exhibited a wide range of clearance rates and half-lives (Table 4).

From these studies, two compounds (**4**, **24a**) stood out for their low clearance and relatively high bioavailability, although, these results are based on only two animals. The improved CYP profile (i.e., lower CYP2C9 inhibition) observed with **4** translated equally well in the amide series as shown by the similar results obtained for compound **24a** (Table 2). In addition, **24a** was found to display a lower activation constant (K_{obs}), a hallmark measurement of CYP3A4 time dependant inhibition (TDI)[14] and a significantly lower in vivo covalent binding as compared to MK-7246 (Table 2). A reduced TDI of CYP3A4 for compound **24a** lowers the risks of potential drug–drug interaction caused by gradual accumulation of irreversible or quasi irreversible binders to the cytochrome active site.

In conclusion, the indole core found in MK-7246 was effectively replaced by a 7-azaindole core while retaining affinity for the *h*CRTH2 receptor and activity in the eosinophil shape change whole blood assay. The selectivity over other prostanoid receptors, mainly DP and TP, was improved as well as the CYP profile. The introduction of an amide group in lieu of the sulfonamide moiety reduced the shift in the EOS whole blood assay but more importantly solved the covalent binding issue that was observed with our lead compound. As a result of the SAR studies described in this manuscript, we identified the azaindole **24a** as a potential backup compound that addressed the potential liabilities associated with MK-7246.

Table 4
Sprague–Dawley (SD) rat pharmacokinetic data for selected CRTH2 antagonists[a]

Compd	*F* (%)	Cl (ml/min/kg)	V_d (L/kg)	$T_{½}$ (h)
MK-7246	138[b]	3.9	1.15	4.1
4	50	0.4	0.23	7.5
24a	84	3.6	0.67	4
24c	156[b]	21	1.54	1.9
24d	63	12	2.20	3.1
24f	20	28	4.58	2.1
24g	38	18	1.72	11.6

[a] PO dosing solution: 5 mg/kg (5 mL/kg) in 0.5% methocel; IV dosing solution: 1 mg/kg (1 mL/kg) in 60% PEG 200 ($n = 2$).
[b] Bioavailability superior to 100% might be explained by the estimation of substrate concentration at T_0 for IV curve.

Acknowledgments

The authors would like to thank Nicolas Lachance, Scott Peterson and Michel Gallant, for proofreading this Letter.

References and notes

1. Nagata, K.; Tanaka, K.; Ogawa, O.; Kemmotsu, K.; Imai, T.; Yoshie, O.; Abe, H.; Tada, K.; Nakamura, M.; Sugamura, K.; Takano, S. *J. Immunol.* **1999**, *162*, 1278–1286.
2. Nagata, K.; Hirai, H.; Tanaka, K.; Ogawa, K.; Aso, T.; Sugamura, K.; Nakamura, M.; Takano, S. *FEBS Lett.* **1999**, *459*, 195–199.

3. Kostenis, E.; Ulven, T. *Trends Mol. Med.* **2006**, *12*, 148–158.
4. 4a Gallant, M.; Beaulieu, C.; Berthelette, C.; Colucci, J.; Crackower, M. A.; Dalton, C.; Denis, D.; Ducharme, Y.; Friesen, R. W.; Guay, D.; Gervais, F. G.; Hamel, M.; Krawczyk, C. M.; Kosjek, B.; Lau, S.; Leblanc, Y.; Lee, E.; Mellon, C.; Molinaro, C.; Mullet, W.; O'Neill, G. P.; O'Shea, P.; Sawyer, N.; Sillaots, S.; Simard, D.; Slipetz, D.; Stocco, R.; Sørensen, D.; Truong, V. L.; Wong, E.; Zaghdane, M. H.; Wang, Z. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 288; (b) Wang, Z. WO 2007/019675 Al, 2007.; (c) Wang, Z. WO 2010/031182 A1, 2010.
5. (a) Sandham, D. A.; Adcock, C.; Kamlesh, B.; 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; (b) 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.
6. (a) Slatore, C. G.; Tilles, S. A. *Immunol. Allergy Clin. N. Am.* **2004**, *24*, 477; (b) Hess, D. A.; Sisson, M. E.; Suria, H.; Wijsman, J.; Puvanesasingham, R.; Madrenas, J.; Rieder, M. *FASEB J.* **1999**, *13*, 1688; (c) Castrejon, J. L.; Berry, N.; El-Ghaiesh, S.; Gerber, B.; Pichler, W. J.; Kevin Park, B.; Naisbitt, D. J. *J. Allergy. Clin. Immunol.* **2010**, *125*, 411; (d) Reider, M. J.; Krause, R.; Bird, I. A. *Toxicology* **1995**, *95*, 141.
7. (a) Molinaro, C., in preparation.; (b) Okano, A.; Oishi, S.; Tanaka, T.; Fujii, N.; Ohno, H. *J. Org. Chem.* **2010**, *75*, 3396; (c) Onistschenko, A.; Stamm, H. *Chem. Ber.* **1989**, *122*, 2397.
8. Sturino, C. F.; O'Neill, G.; Lachance, N.; Boyd, M.; Berthelette, C.; Labelle, M.; Li, L.; Roy, B.; Scheigetz, J.; Tsou, N.; Aubin, Y.; Bateman, K. P.; Chauret, N.; Day, S. H.; Lévesque, J.-F.; Seto, C.; Silva, J. H.; Trimble, L. A.; Carriere, M. C.; Denis, D.; Greig, G.; Kargman, S.; Lamontagne, S.; Mathieu, M. C.; Sawyer, N.; Slipetz, D.; Abraham, W. M.; Jones, T.; McAuliffe, M.; Piechuta, H.; Nicoll-Griffith, D. A.; Wang, Z.; Zamboni, R.; Young, R. N.; Metters, K. M. *J. Med. Chem.* **2007**, *50*, 794.
9. 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.
10. Blood was collected in vaccutainers containing EDTA. The antagonist was added to blood and incubated for 10 min at room temperature. DK-PGD_2 was then added to blood for 4 min at 37 °C in a running water bath. Blood cells were then fixed in presence of cold 0.25% (v/v) paraformaldehyde prepared in 75% (v/v) PBS for 1 min on ice. Fixed blood (175 μL) was transferred into 870 μL of cold 155 mM NH_4Cl lysis solution and incubated at 4 °C for at least 40 min. The solution was then centrifuged at 430*g* for 5 min and the supernatant was discarded. Centrifuged cells were analyzed with a FACs Calibur flow cytometer (Becton Dickinson). Flow cytometry raw data were analyzed with FlowJo software by isolating the eosinophils from the neutrophils based on their intrinsic autofluorescence and determining the percent of total eosinophils with increased forward scatter (FSC-H) value. Maximum (100%) and minimum (0%) shape change were determined in the presence of 10 μM DK-PGD_2 and PBS, respectively. MK-7246 was tested in 10-point dose titration curves in the presence of 30 nM DK-PGD_2 ($\sim EC_{80}$) to determine the IC_{50}.
11. (a) Schmidt, U.; Wild, J. *Angew. Chem.* **1984**, *96*, 996; (b) Knowles, W. S.; Vineyard, B. D.; Sabacky, M. J.; Bachman, G. L.; Weinkauff, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 5946.
12. (a) Ujjainwalla, F. *Tetrahedron Lett.* **1998**, *39*, 5355; (b) Park, S. S. *Tetrahedron Lett.* **1998**, *39*, 627.
13. Zaghdane, M. H.; Boyd, M.; Colucci, J.; Simard, D.; Berthelette, C.; Leblanc, Y.; Wang, Z.; Houle, R.; Levesque, J.-F.; Molinaro, C.; Hamel, M.; Stocco, R.; Sawyer, N.; Sillaots, S.; Gervais, F. G.; Gallant, M., in preparation.; (b) Colucci, J.; Boyd, M.; Zaghdane, M. H. WO 2010/031183 A1, 2010.
14. Riley, R. J.; Grime, K.; Weaver, R. *Expert Opin. Drug Metab. Toxicol.* **2007**, *3*, 51.