Discovery of Dual Antagonists of CRTH2 and DP Receptors: Novel Potential Treatment for Asthma

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Amgen Inc.

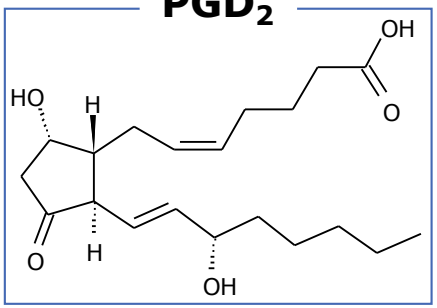
Asthma Overview

- **Asthma affects over 100 million people world-wide**
  - Approximately 5%-10% of adults and 10%-15% of children suffer from asthma globally
  - Prevalence continues to increase
  - Annual expenditure for respiratory drugs in US alone totals $14 billion
- **Asthma is a chronic inflammatory disease of the airways with both:**
  - Acute responses
    - Contraction of airway smooth muscle
    - Mucus hypersecretion
    - Vasodilation
    - Microvascular leakage
    - Airway hyperresponsiveness
  - Chronic
    - Injury/repair of bronchial epithelium
    - Recurrent exacerbations
    - Airway remodeling
    - Permanent airflow obstruction

Prostaglandin D_2 (PGD_2)

- Synthesized by mast cells, Th2 cells and dendritic cells
- Released by mast cells in large amounts during asthmatic responses
  - Major prostanoid released by mast cells following immune challenge[1]
  - Bronchoalveolar lavage fluid levels of PGD2 increase dramatically, reaching as much as ~30 ng/mL (~85 nM; mean ~9 ng/ml or ~26 nM)[2]
- PGD2 mediates pathophysiological effects relevant to asthma:
  - Bronchoconstriction
  - Microvascular leakage
  - Eosinophil, basophil and Th2 chemotaxis
  - Mucus secretion
- Transgenic mice overexpressing PGD synthase display elevated pulmonary infiltration of inflammatory cells and Th2 cytokine production after antigen challenge[3]

PGD_2



[1] ***J Immunol* 1982; 129:1627.**

[2] ***Am J Respir Crti Care Med* 2000; 162:637.**

[3] ***J Immunol* 2002; 168:443.**

DP (DP1) Overview

DP (D Prostanoid receptor)

- Gαs-type G protein-coupled receptor (cAMP)
- Cloned in 1995[1]

Expression

- Airway epithelium, smooth muscle, platelets and at low levels on basophils, eosinophils, mast cells and dendritic cells, NK T cells

Functions

- Vasodilation and mucus secretion
- DP ko mice display reduced hyperresponsiveness to acetylcholine, reduced pulmonary eosinophilia and reduced Th2 cytokine levels after antigen challenge[2]
- DP antagonist decreases eosinophil infiltration in guinea pig asthma model and decreases nasal resistance in guinea pig rhinitis model[3]

Genetic Validation

- SNPs in DP promoter suggest that increased expression of DP is linked to asthma while decreased expression of DP may be protective

[1] ***J Biol Chem*** **1995; 270:18910.**
[2] ***Science*** **2000; 287:2013.**
[3] ***J Pharmacol Exp Ther*** **2001; 298:411.**

CRTH2 (DP2) Overview

CRTH2 (Chemoattractant receptor-homologous molecule expressed on TH2 cells)

- Gαi-type G protein-coupled receptor (calcium)
- First reported in 1999[1]
- Prostaglandin D2 (PGD2) was identified as the natural ligand in 2001[2]

Expression

- TH2 cells, eosinophils, basophils, mast cells, neutrophils

Functions

- Chemotaxis of eosinophils, basophils, TH2 cells
- Eosinophil activation and degranulation
- Eosinophil mobilization from bone marrow
- Cytokine production from Th2 T cells
- Leukotriene production by mast cells

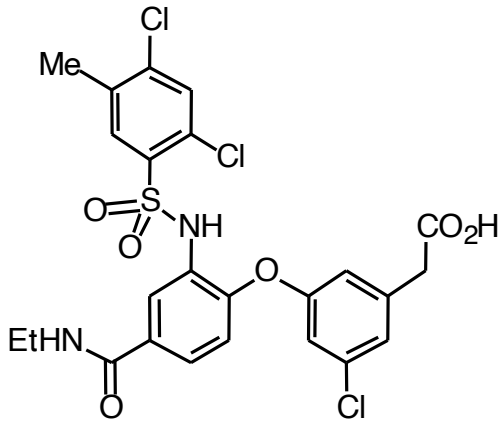
Genetic Validation

- SNPs in 3' UTR of CRTH2 gene result in higher stability mRNA, and are positively linked to asthma in African Americans and Chinese populations

[1] ***J Immunol*** **1999; 162:1278.**
[2] ***J Exp Med*** **2001; 193:255.**

HTS Lead

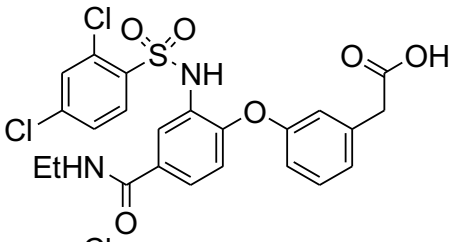
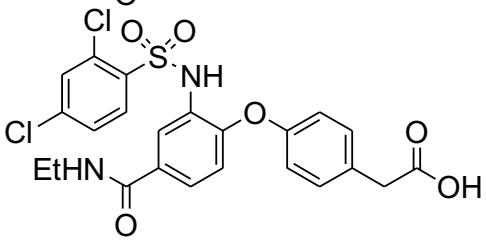
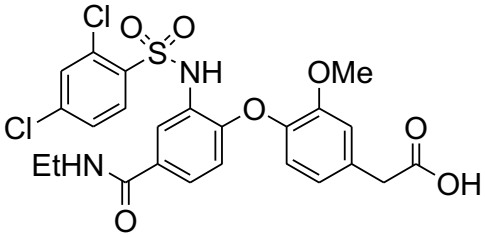
Phenylacetic Acid Derivative

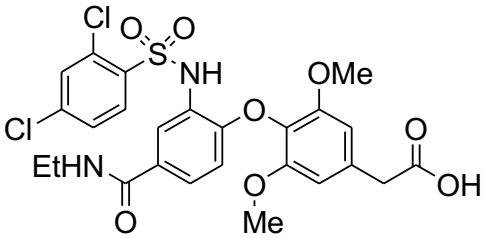
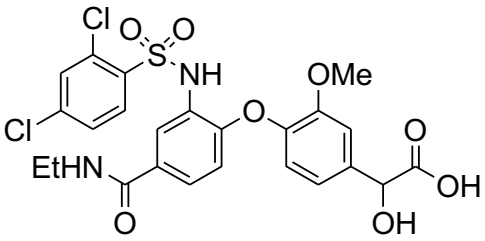


^{3}H-PGD2 binding assays
hCRTH2 IC_{50} = 10 nM (buffer)
hCRTH2 IC_{50} = 2,700 nM (100% h-plasma)
hDP IC_{50} = 8,000 nM (buffer)

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line

CRTH2/DP Cross-Activity

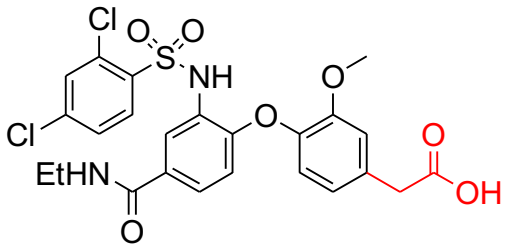
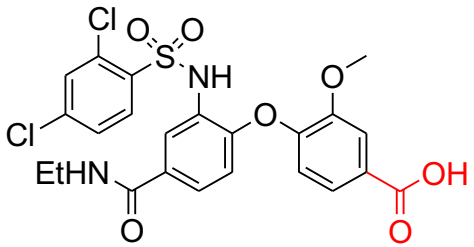
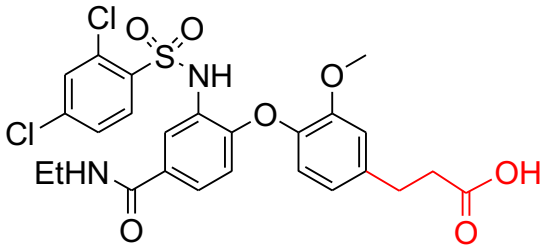
Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.006	>10.0
	0.004	0.120
	0.003	0.055

Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.002	7.04
	1.0	0.050

- *para*-Substituted phenylacetic acids offer good cross-activity between CRTH2 and DP
- α-Substitution of the phenylacetic acid gives a DP-selective compound

^{3}H-PGD_2 binding assay with 293 transfected human cell line

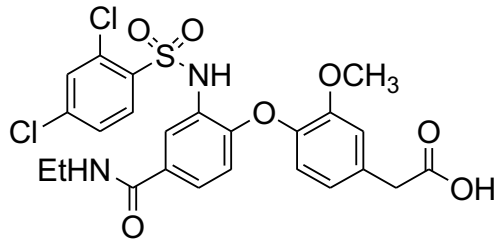
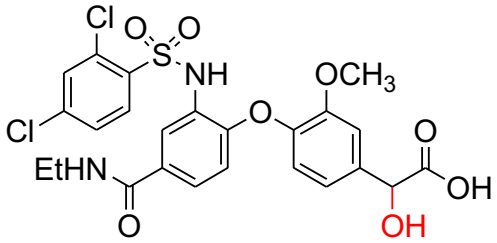
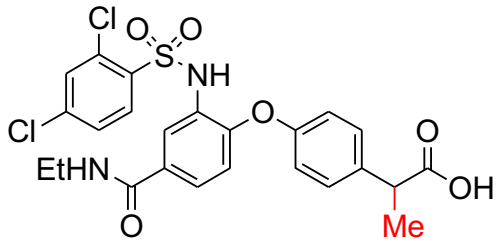
Acid Linker

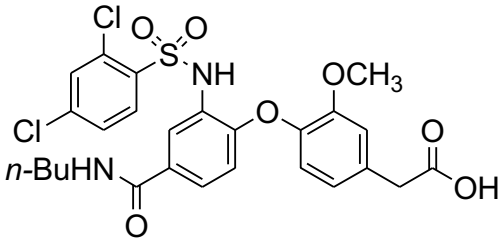
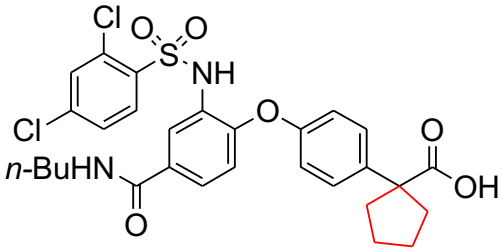
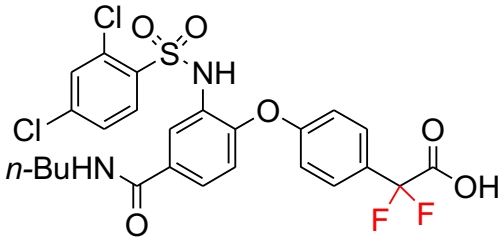
Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	**0.003**	**0.055**
	>50	>12.5
	0.74	2.07

- The optimal distance between the acid and the phenyl ring is one carbon

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line

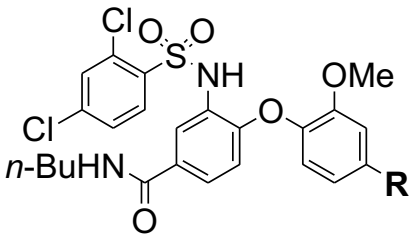
α-Substitution of Phenylacetic Acid

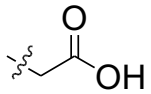
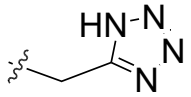
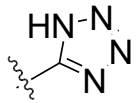
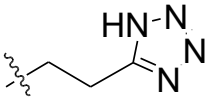
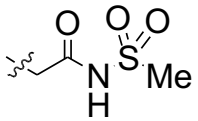
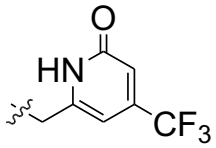
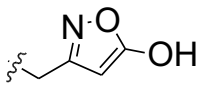
Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.003	0.055
	0.917	0.050
	0.108	0.043

Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.003	0.012
	>12.5	0.013
	0.423	0.003

- α-Substitution tends to maintain DP activity but decrease CRTH2 activity

Carboxylic Acid Replacements

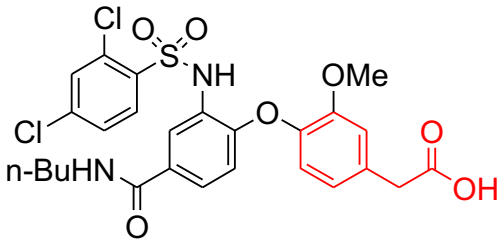
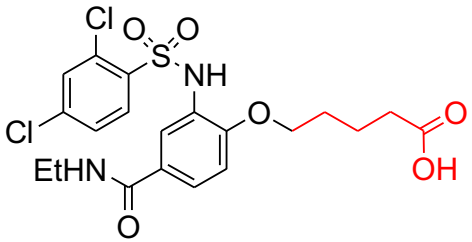
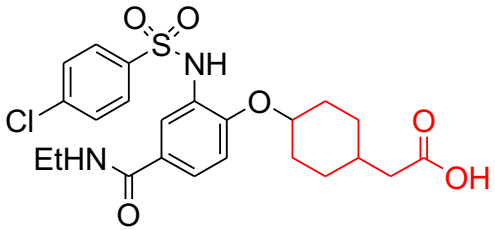


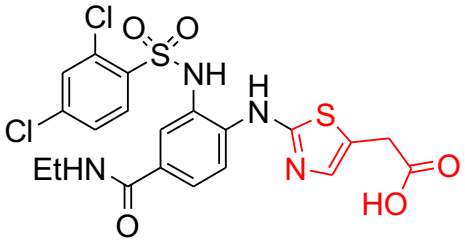
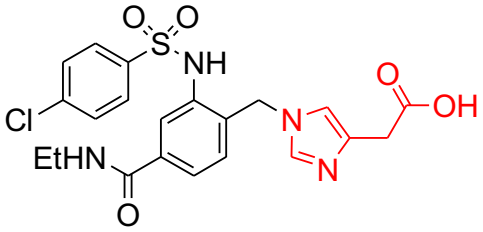
R	CRTH2 binding IC_{50} (μM)*	DP binding IC_{50} (μM)*
	0.003	0.012
	0.351	0.007
	6.41	0.29
	2.09	0.19
	0.33	0.006
	4.58	0.011
	0.43	0.053

- Several carboxylic acid replacements maintain DP activity, decrease CRTH2 activity

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line

Replacements of the Phenyl Acetic Acid Ring

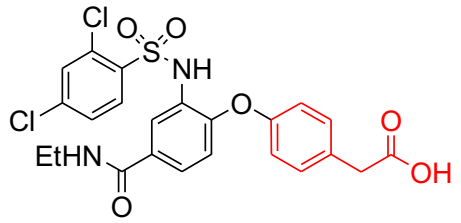
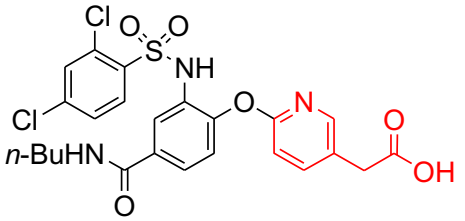
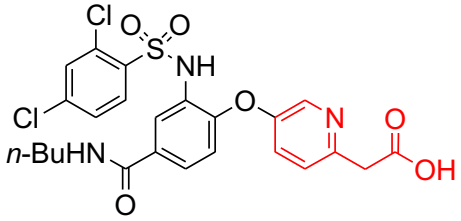
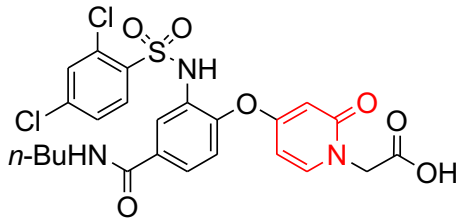
Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.003	0.012
	0.345	1.683
	5	

Compound	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.448	>12.5
	0.251	>12.5

- Saturated alkyl groups impart decreased activity
- Some heterocycles show selective activity for CRTH2 over DP

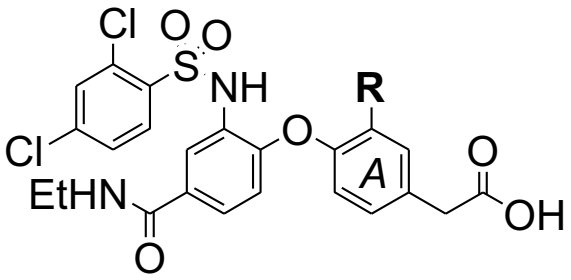
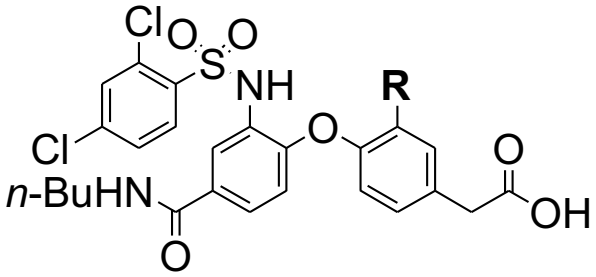
* ^{3}H-PGD_2 binding assay with 293 transfected human cell line

Replacements of the Phenyl Acetic Acid Ring

Structure	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	**0.006**	**0.120**
	0.315	0.479
	**0.010**	**0.231**
	8.25	11.37

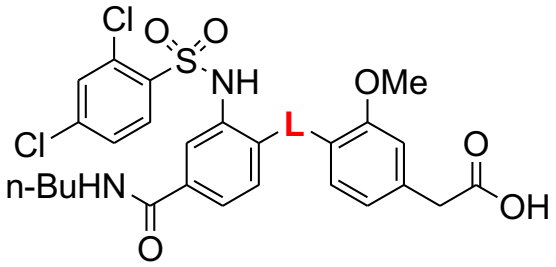
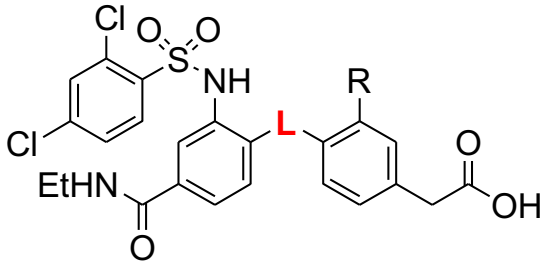
- 2-Pyridine is a good replacement for the *A* ring

3-Substitutions on the *A* Ring

Structure	R	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	H	0.004	0.120
	OMe	0.003	0.055
	OEt	0.002	1.91
	OMe	0.003	0.012
	Cl	0.006	0.008
	F	0.007	0.030
	Me	0.003	0.006

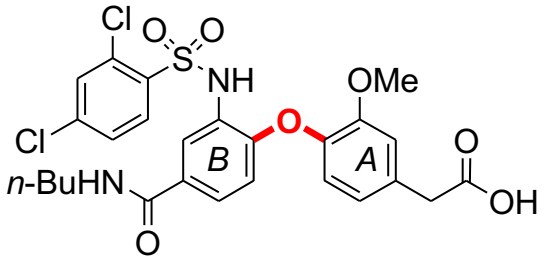
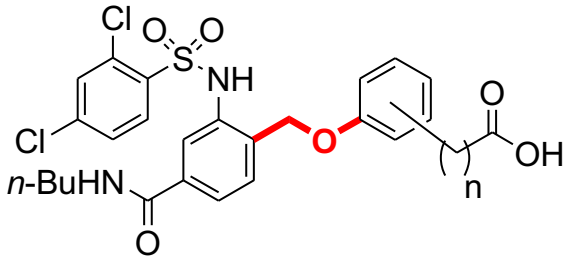
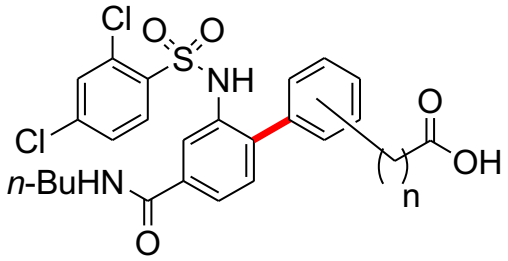
- 3-OMe, Cl, F, or Me enhance or maintain both activities

Modifications of the Oxygen Linker

Structure	L	R	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	O		0.003	0.012
	CH_2		0.002	0.005
	O	H	0.004	0.045
	CH_2	H	0.015	0.569
	SO_2	H	1.73	>12.5
	NH	Br	0.055	0.234

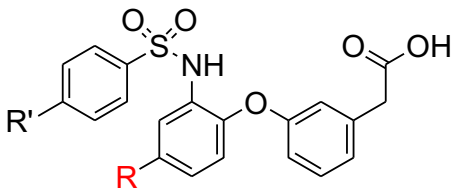
- Methylene linker is a good replacement for the oxygen linker

Modifications of the Oxygen Linker

Structure	Regio-isomer	n	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
			**0.003**	**0.012**
	*p*	1	>12.5	>12.5
	m	1	>12.5	>12.5
	p	2	>12.5	>12.5
	o	2	>12.5	>12.5
	*p*	1	>12.5	**2.81**
	m	1	**0.869**	>12.5
	p	2	>12.5	**1.23**
	m	2	>12.5	**0.120**

- The optimal distance between *A* ring and *B* ring is one atom

Amide Moiety Replacements

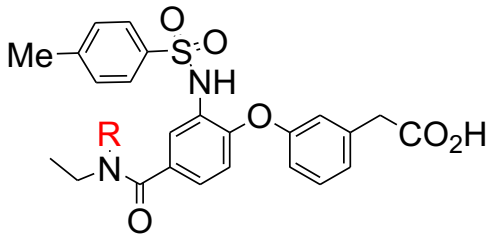
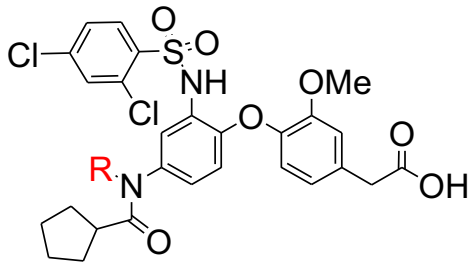
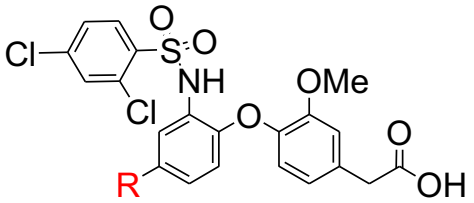
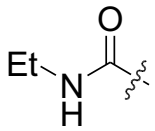
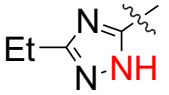
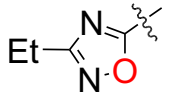
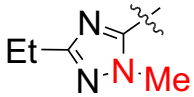


R	R'	CRTH2 binding IC50 (μM) *
Cl	H	0.675
MeO	H	1.40
O Me	H	0.380
OH Me	H	2.00
NHEt	H	>10
O NH_2	H	0.047
O NHEt	H	0.013
O NEt_2	Me	1.10
H N Et O	Cl	0.007
H N NHEt O	Cl	0.016

• The primary or secondary amide at the center phenyl ring can be replaced by reversed amide or urea while maintaining CRTH2 activity

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line.

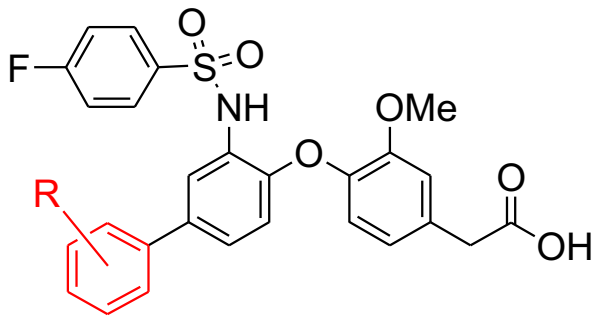
Amide Moiety: The Importance of an H-Donor

Structure	R	CRTH2 binding IC_{50} (μM)*	DP binding IC_{50} (μM)*
	H	0.004	
	Et	1.10	
	H	0.006	0.019
	Me	>12.5	3.47
		0.006	0.120
		0.006	0.015
		0.864	0.016
		0.159	0.010

• H-Bond donor seems to be important to CRTH2 activity but does not necessarily affect DP activity

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line

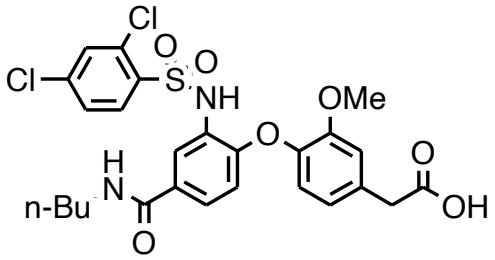
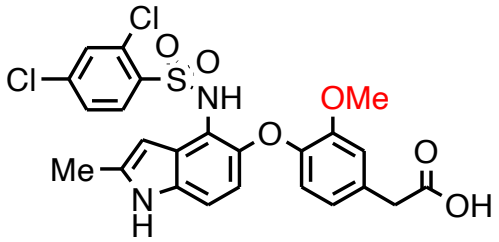
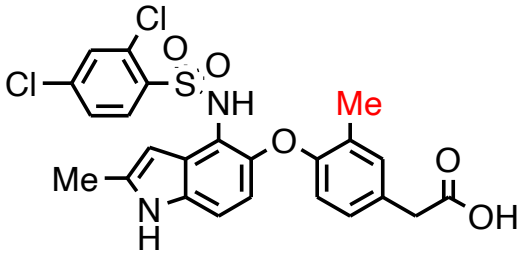
Amide Moiety Replacements: Aromatic Ring Without NH

Structure	R	CRTH2 binding IC_{50} (μM)*	DP binding IC_{50} (μM)*
	4-Et	0.118	0.008
	4-n-Bu	0.200	0.015
	3-i-Pr	0.241	0.043
	4-Cl	0.137	0.088
	3-Cl	0.150	0.041

• Aryl replacements can improve DP activity, but generally decrease CRTH2 activity without NH

* ^{3}H-PGD_2 binding assay with 293 transfected human cell line; N/A: data not available

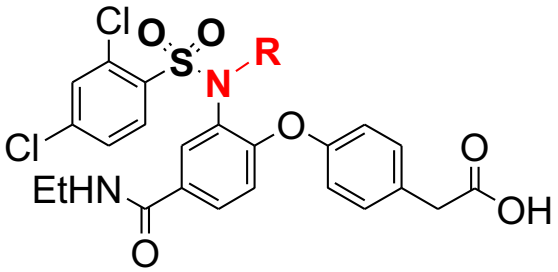
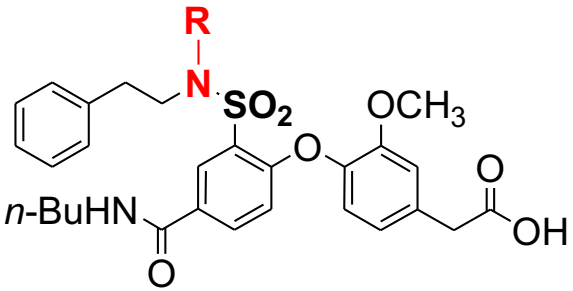
Indole as Amide Replacement

Structure	CRTH2 binding IC_{50} (μ M)*	DP binding IC_{50} (μM)*
	**0.003**	**0.012**
	**0.018**	**0.009**
	**0.021**	**0.018**

- Indole core compounds have comparable potency to that of amide analogs

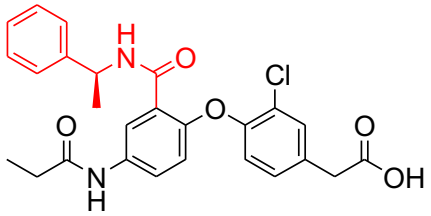
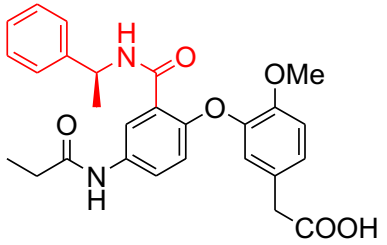
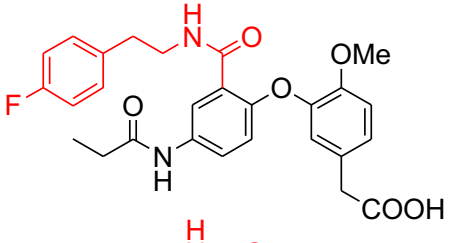
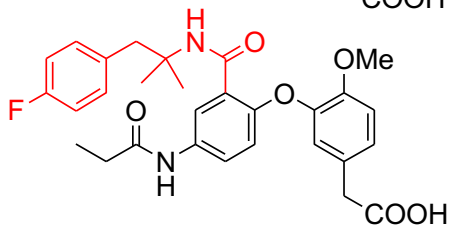
* ^{3}H-PGD_2 binding assay with 293 transfected human cell line.

Sulfonamide Methylation

Compound	R	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	H	0.004	0.045
	Me	0.354	>12.5
	H	0.136	>12.5
	Me	0.029	>12.5

• Sulfonamide NH may not be a requirement for CRTH2 activity, but it is important for DP activity

Reversed Amide Replacement

Structure	CRTH2 binding IC_{50} (μM)	DP binding IC_{50} (μM)
	0.003	>10.0
	0.001	>10.0
	0.002	>12.5
	0.002	>12.5

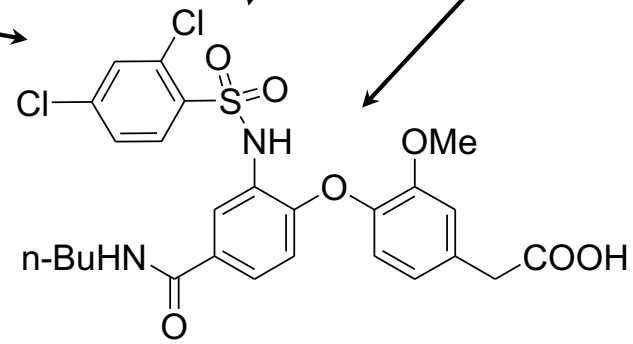
- Reversed amides are excellent replacements with respect to CRTH2 activity but not with respect to DP activity

SAR Summary

Several modifications of the sulfonamide linker are tolerated by CRTH2 receptors but detrimental to DP activity

The ether linker can be replaced by a methylene linker

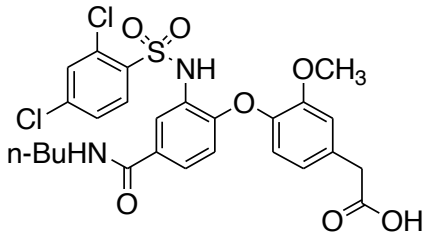
Substitutions can enhance potency



Reversed amides, ureas, carbamates and especially five-membered heterocycles with NH are tolerated

para-Acetic acid is optimal for dual antagonists

CRTH2/DP Dual Antagonist: AMG 009



AMG 009

CRTH2 Activities	IC_{50}(nM)
hCRTH2(293), Buffer Binding:	3
hCRTH2(293), 100% Plasma Binding:	26
hCRTH2(CEM), migration:	5
Sheep CRTH2, Buffer Binding:	2
Guinea Pig CRTH2, Buffer Binding:	3

DP Activities	(nM)
hDP(293), Buffer Binding (IC_{50}):	12
hDP(293), 50% Plasma Binding (IC_{50}):	347
hDP(293), cAMP (Ki, buffer):	0.7
hDP(293), cAMP (Ki, 33% plasma):	143
Sheep DP, cAMP (Ki, platelet rich plasma):	1800

Selectivity / Toxicity

No activity against
- Receptor panel
- CYP450 (1A2, 2D6, 3A4)

HERG (patch clamp): 9% inhibition at 5μM

CYP450 2C9, IC_{50}: 44μM

Ames test	negative
chromosal aber. (*in vivo/in vitro*)	negative

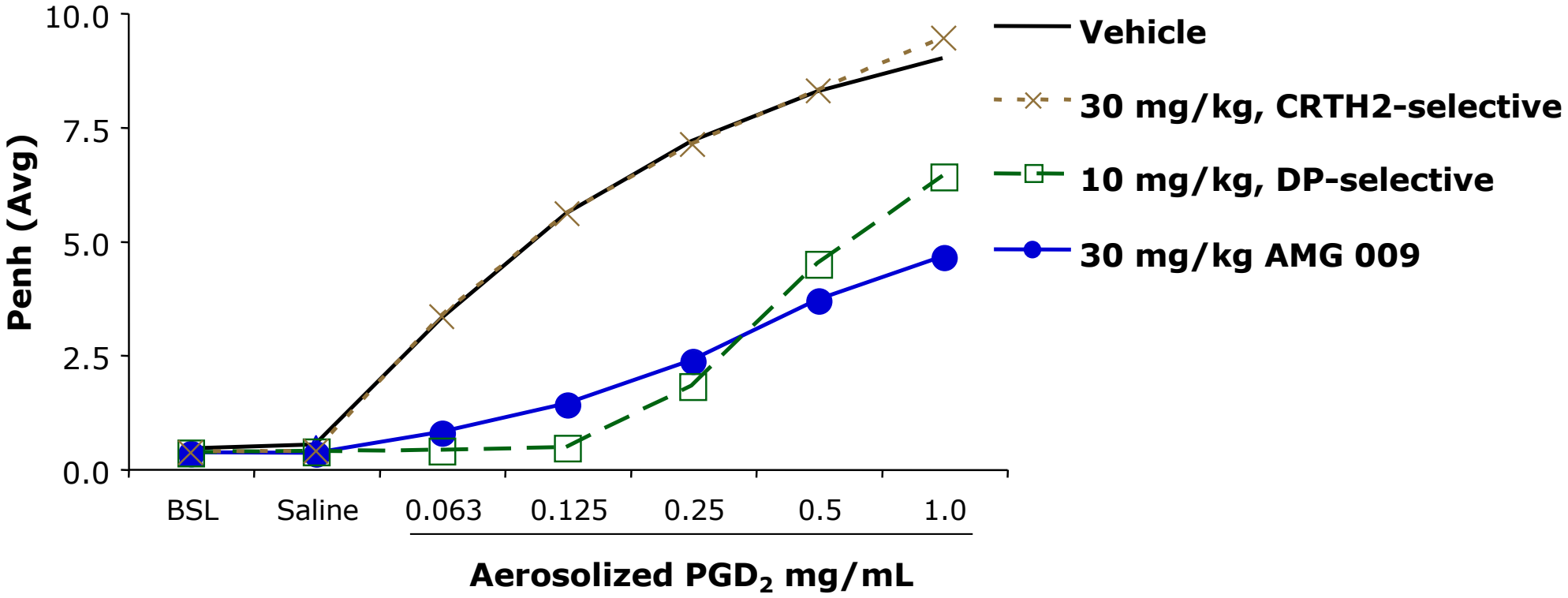
PK	Cls	MRT	Vdss	F_{po}
Rat:	1.2L/h/kg	0.9h	1.0L/kg	28%
Dog:	0.77L/h/kg	1.4h	1.0L/kg	60%
Cyno:	0.27L/h/kg	1.3h	0.37L/kg	39%

Man: low predicted clearance (hepatocytes)

Plasma Protein Binding

Rat:	99.8%	Sheep:	99.4%
Monkey:	99.7%	Human:	99.2%
Dog:	98.9%		

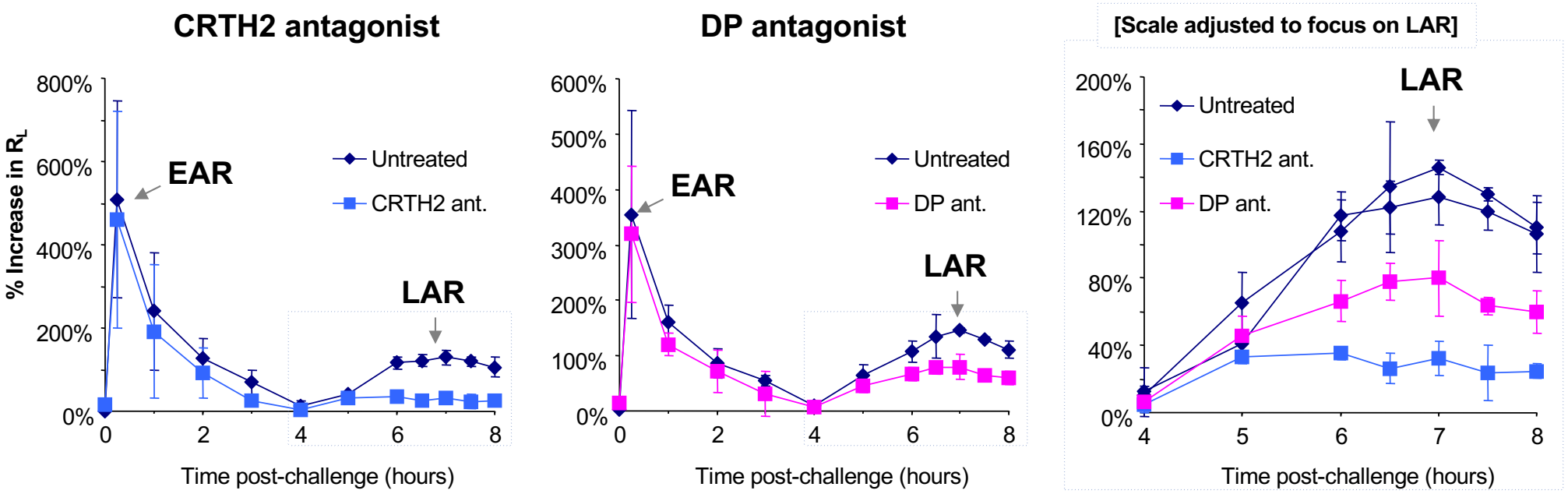
Treatment with AMG 009 Blocks PGD_2-Induced Acute Airway-Constriction in Guinea Pigs



Compounds were dosed SC 15 minutes before challenge with aerosolized PGD_2
n=8 guinea pigs / group

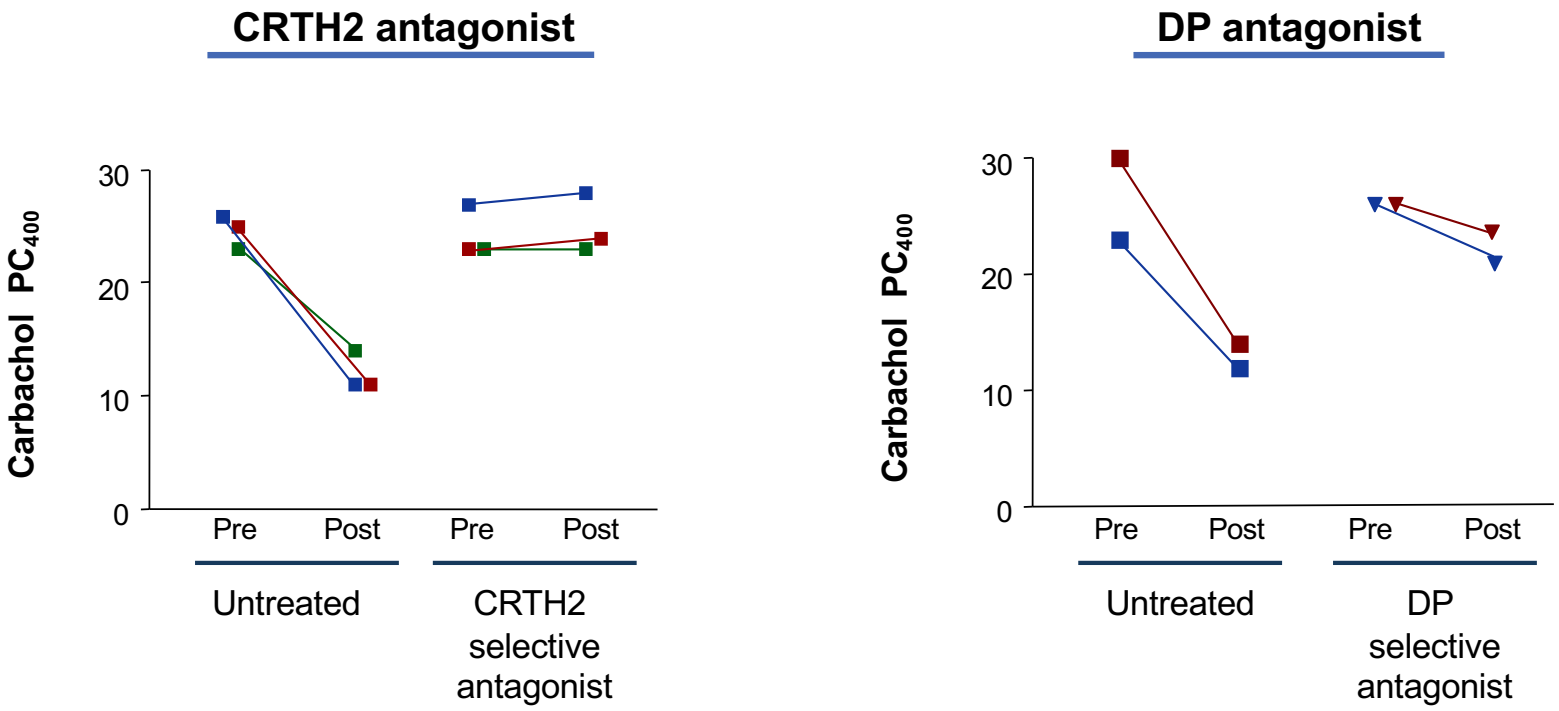
CRTH2 *and* DP are Involved in Late Airway Response to Allergen in Sheep (W.M. Abraham)

Antagonism of either CRTH2 or DP reduces antigen-induced late airway response



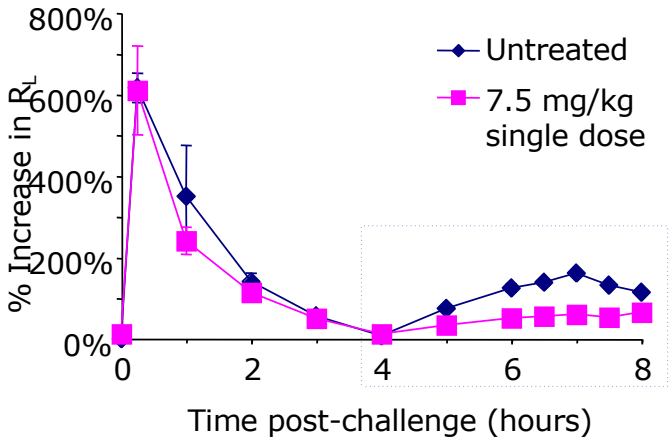
- R_L=Pulmonary resistance (L x cm H_2O/L/s)
- Mean values ± SEM
- Untreated group represents historical data for these individual sheep
- CRTH2 selective antagonist (15 mg/kg) was given SC BID for two days and 30 minutes prior to challenge with aerosolized *Ascaris suum* (n=3)
- DP selective antagonist (7.5 mg/kg) was give SC BID 60 minutes prior to antigen challenge (n=2)

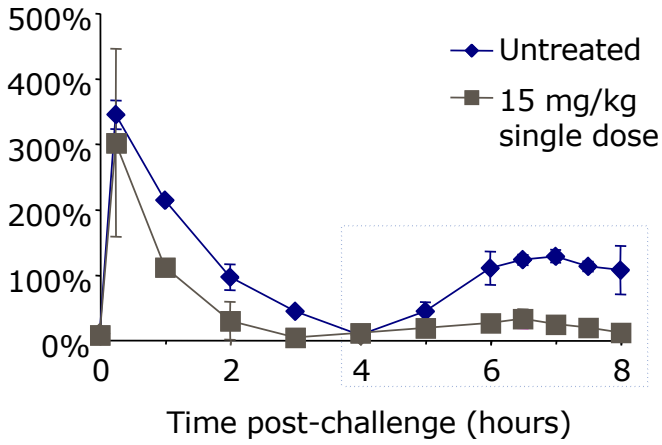
CRTH2 *and* DP are Involved in Mediating Airway Hyper-reactivity to Carbachol (W.M. Abraham)

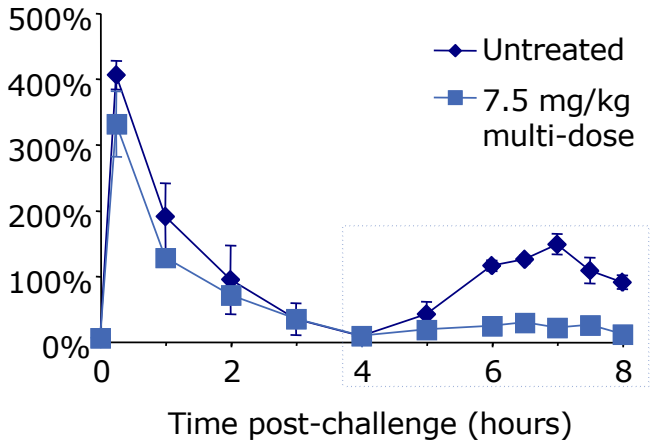


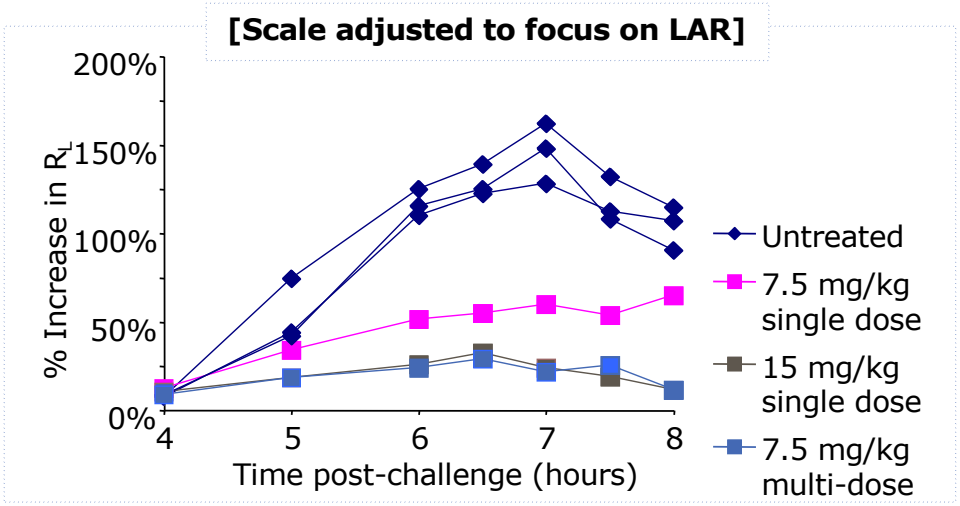
- CRTH2 selective antagonist (15 mg/kg) was given SC BID for two days and 30 minutes prior to challenge with aerosolized *Ascaris suum* (n=3 sheep/group)
- DP selective antagonist was given SC 1 h prior to challenge with aerosolized *Ascaris suum* (n=2 sheep/group)
- Untreated group represents historical data for these individual sheep

Treatment with AMG 009 Inhibits Antigen-induced Late Airway Response in Sheep Allergic Airway Model









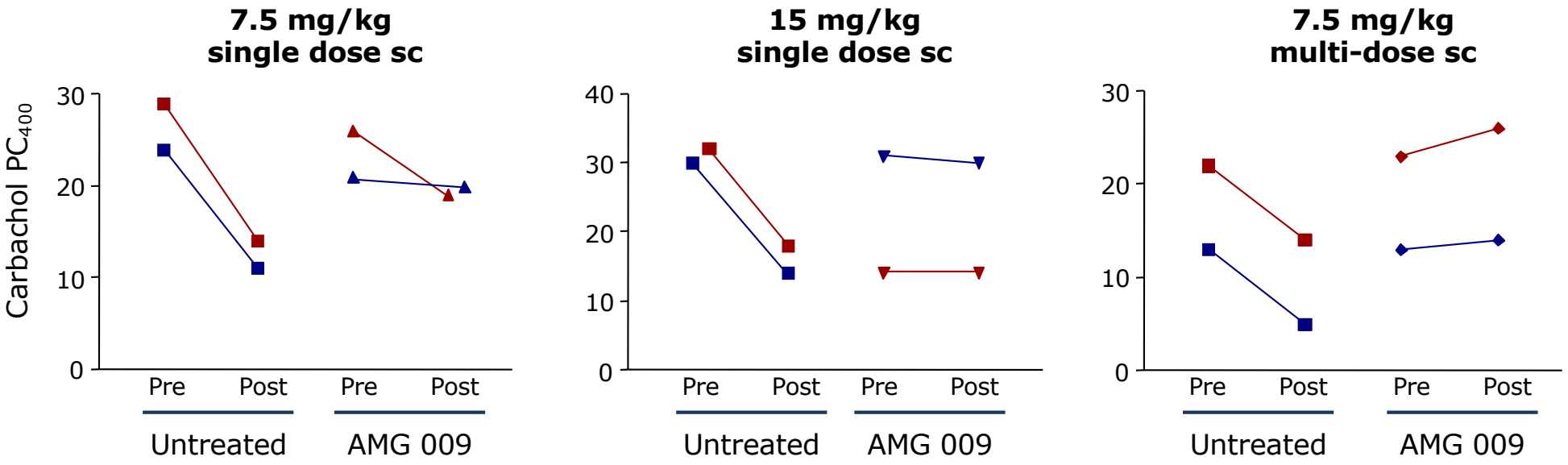
AMG 009 was dosed SC one hour ("single dose") or once-daily for 3 days ("multi-dose") before challenge with aerosolized *Ascaris suum*

n=2 sheep/group

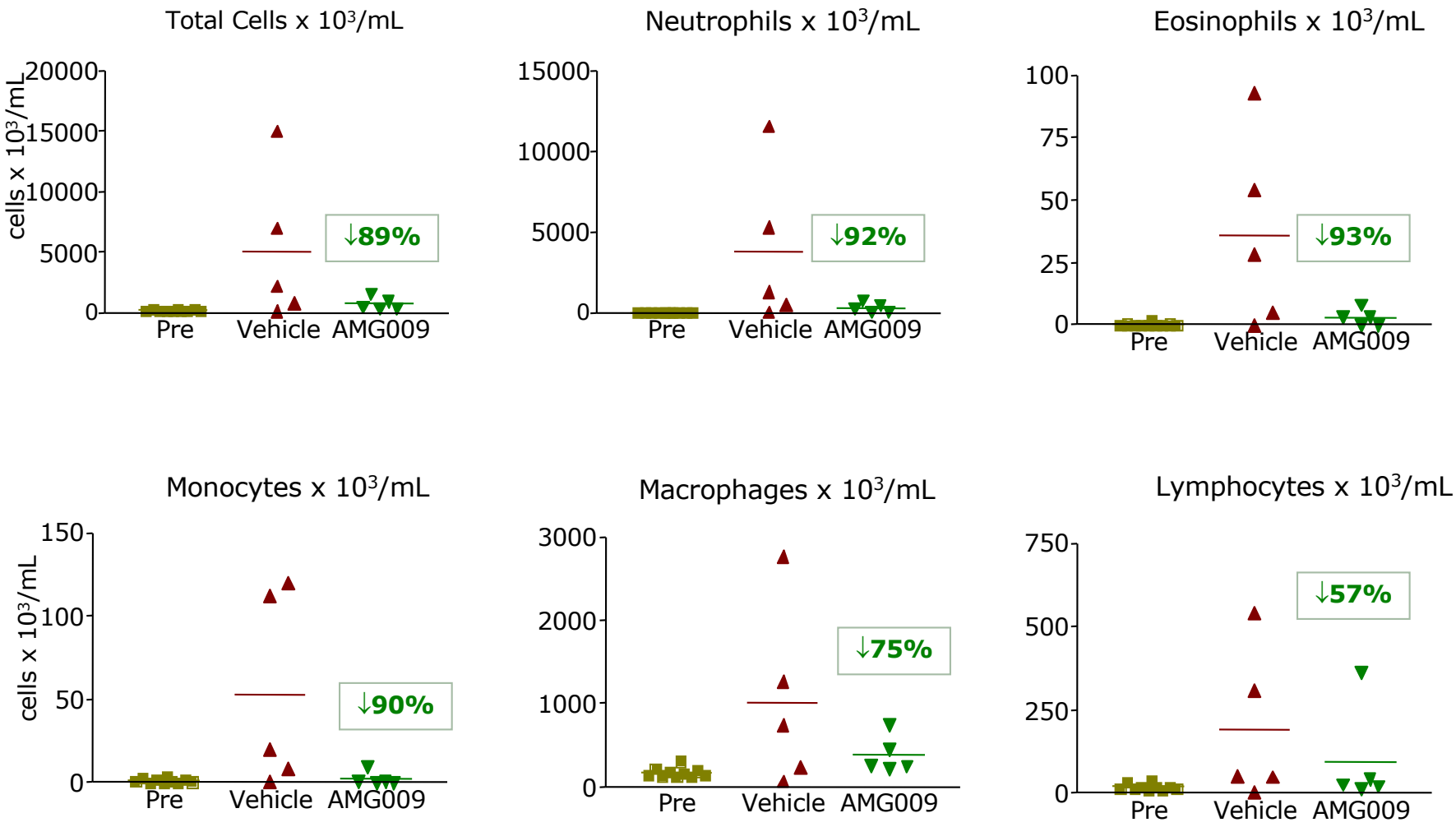
Untreated group represents historical data for these individual sheep

Treatment with AMG 009 Blocks Antigen-Induced Development of Airway Hyperreactivity to Carbachol

(W.M. Abraham)



Treatment with AMG 009 Blocks Allergen-Induced Recruitment of Inflammatory Cells to the Lung in Sheep



- AMG 009 was given SC once-daily for 3 days before segmental challenge with *Ascaris suum*
- n=5 sheep/group in a crossover design

Conclusions

- Identified AMG 009 as a potent dual antagonist of CRTH2 and DP receptors
- Demonstrated efficacy with AMG 009 in a guinea pig model of PGD_2-induced acute airway-constriction
- Demonstrated efficacy with CRTH2- and DP- selective antagonists and AMG 009 in sheep models of asthma
- AMG 009 was well tolerated in preclinical safety models

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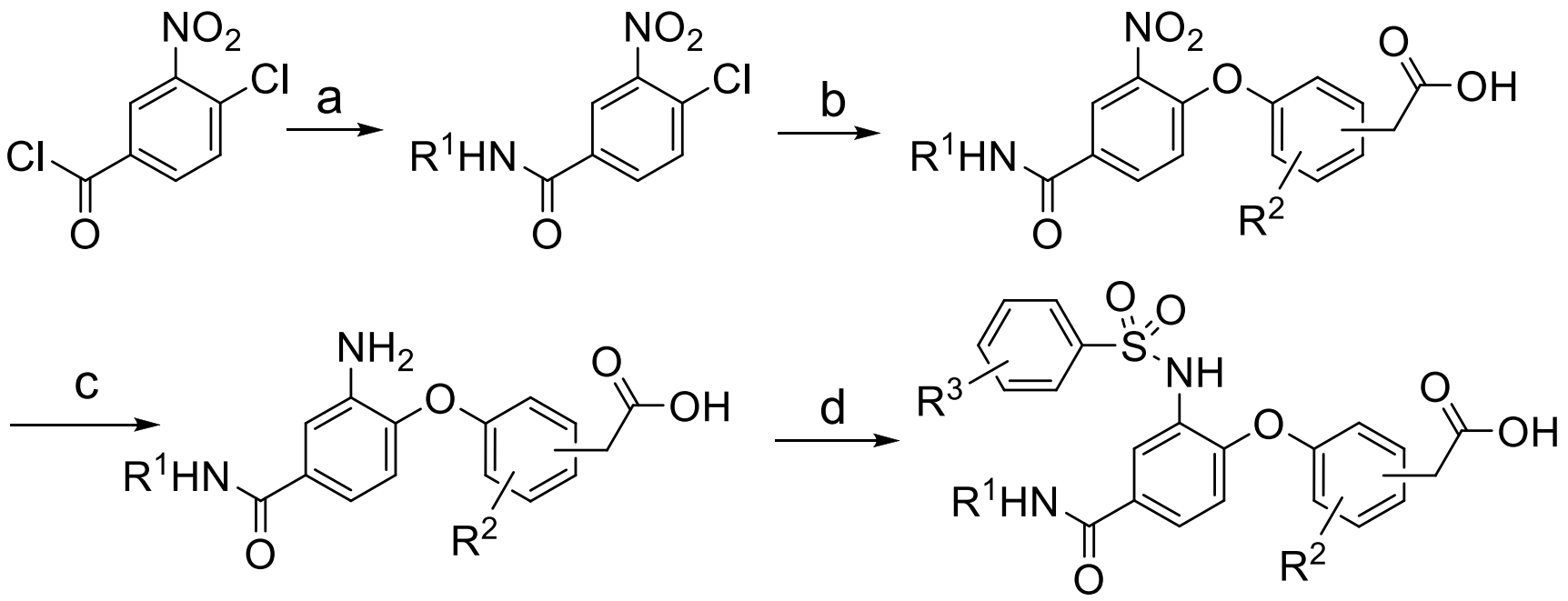
Chemistry
Matt Brown
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George Tonn
Craig Uyeda
Jill Wait
Simon Wong

Mount Sinai Medical Center
William Abraham

Back-ups

General Synthesis



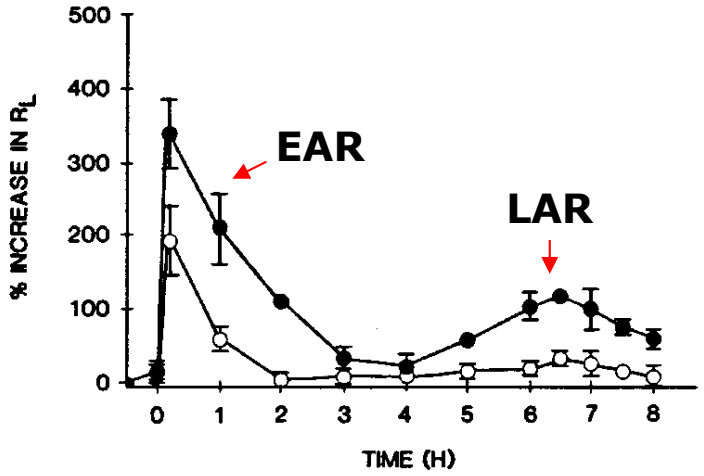
(a) Amines, triethylamine, DCM, rt, 4h, ~90%; (b) phenylacetic acids methyl ester, cesium carbonate, DMSO, 70°C, 6h, ~85%; (c) H_2, Pd/C, EtOH, rt, 1h, 100%; or $SnCl_2$, EtOAc, 60°C, 4h, 80%; (d) sulfonyl chlorides, pyridine, rt, 24h, 70%.

Sheep Airway Response Model

Model overview

- **Animals naturally sensitive to *Ascaris suum***
- **Asthmatic response is induced by challenge with aerosol *Ascaris suum* extract**
  - Early airway response (EAR) occurs immediately
  - Late airway response (LAR) occurs 4-8 hours after challenge
  - Airway hyperreactivity (AHR) to spasmogen (carbachol; measured at 24 hours after challenge)
  - Airway inflammation is measurable 24 hours after challenge

Efficacy of Montelukast



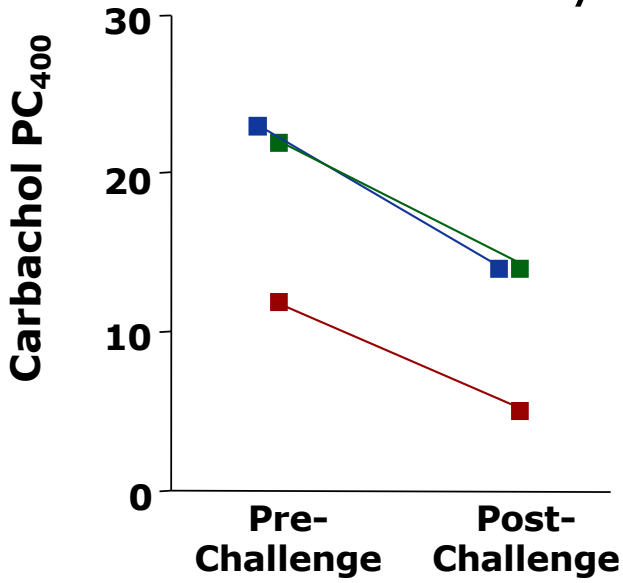
***Can J Physiol Pharmacol* 1995; 73:191.**

Fig. 3. Inhibition of ascaris-induced immediate (early) and late phase bronchoconstriction in allergic sheep by montelukast. Animals were infused with either montelukast (8 mg·kg^{-1}·min^{-1}; ○) or vehicle (●) and changes in lung resistance were recorded for 8 h post antigen challenge. Results are shown as means ± SEM, *n* = 3).

Sheep Airway Response Model

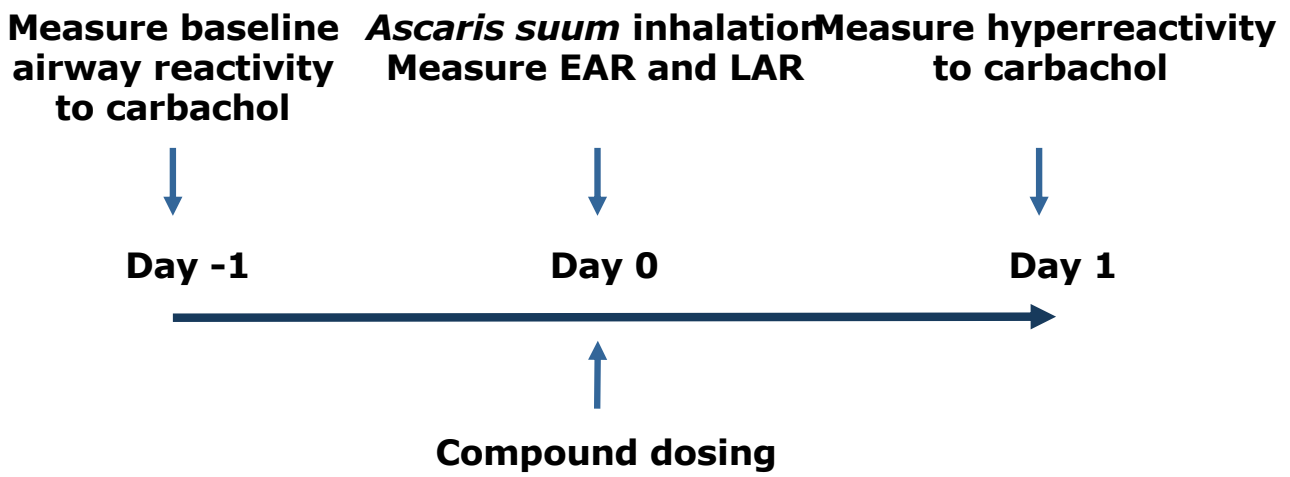
Airway hyperreactivity (AHR)

- Antigen challenge causes enhanced response to non-specific spasmogens (e.g., carbachol, methacholine, acetylcholine)
- AHR manifests experimentally as a reduction in the dose of spasmogen required to trigger full bronchoconstriction
  - 400% change in lung resistance (PC_{400})
- Anti-inflammatory agents block development of AHR



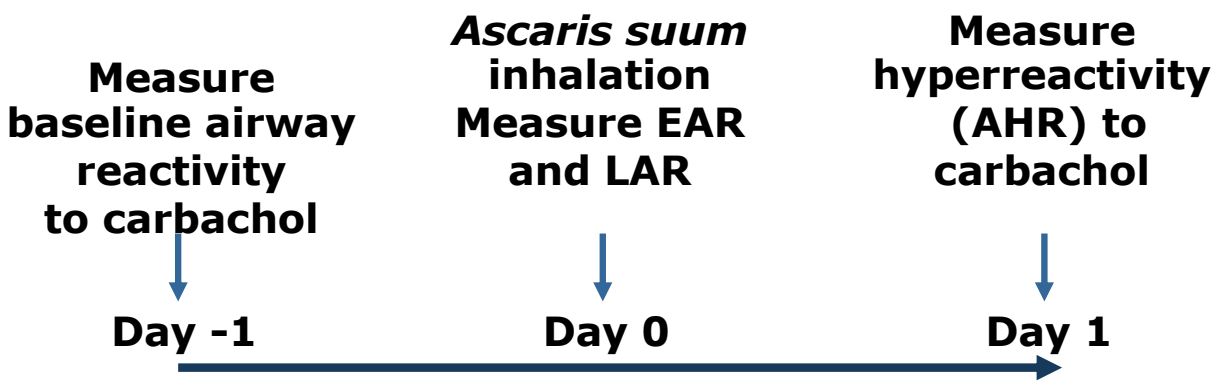
← **Airway hyperresponsiveness in three sheep before and after aerosol challenge with *Ascaris suum.* Allergen exposure typically results in ~ a two-fold decrease in PC_{400} for carbachol**

Evaluation of Antagonists Selective for CRTH2 or DP in the Sheep Model



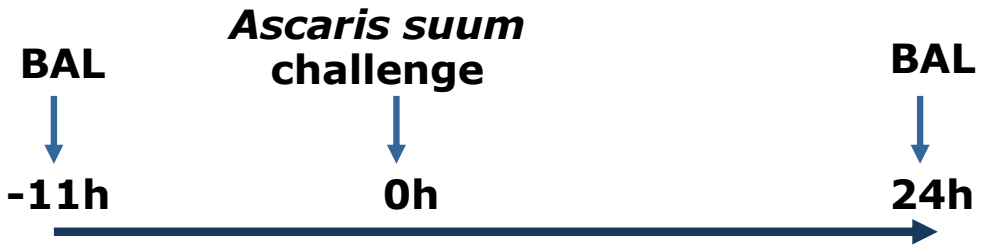
	CRTH2-Selective Antagonist	DP-Selective Antagonist
hCRTH2 (binding assay in buffer) IC_{50}	2 nM	>50,000 nM
hCRTH2 (binding assay in plasma) IC_{50}	59 nM	>50,000 nM
sheep-CRTH2 (binding assay in buffer) IC_{50}	5 nM	15,000 nM
hDP (binding assay in buffer) IC_{50}	1,800 nM	66 nM
hDP (binding assay in plasma) IC_{50}	42,000 nM	520 nM
sheep-DP (platelet rich plasma cAMP) K_i	inactive	59 nM

Sheep Asthma Model: Evaluating EAR, LAR and AHR



- AMG009 was given SC one hour ("single dose") or once-daily for 3 days ("multi-dose") before challenge with aerosolized *Ascaris suum*
- AMG009 plasma drug levels (7.5 mg/kg single dose): C_{max} = 250 ug/L (432 nM); $C_{6\text{-}8h}$ = 130 ug/L (226 nM); C_{24h} = 64 ug/L (110 nM)
- n=2 sheep/group

Sheep Asthma Model: Evaluating Airway Inflammation



- AMG009 administered once-daily for three days prior to allergen challenge at 15mg/kg SC
- Two-legged crossover (vehicle x AMG009)
- 4-weeks period between dosing groups
- n = 5 animals
- BAL: bronchoalveolar lavage

Leg 1	Leg 2
Vehicle	AMG009
Vehicle	AMG009
Vehicle	AMG009
AMG009	Vehicle
AMG009	Vehicle