# The Discovery and Evaluation of PF-3893787: A Novel Histamine H4 Receptor Antagonist

*Nigel Swain*

*nigel.swain@pfizer.com*

Pfizer World Wide Medicinal Chemistry

16th SCI-RSC Medicinal Chemistry Symposium

Churchill College, Cambridge, UK

Tuesday 13th September 2011

# Outline

- Introduction
- Tools for CIR & CIS studies
- New series from HTS
- Hit to lead studies
- Early toxicity studies
- Final optimisation & compound selection
- Enablers for clinical studies
- Initial clinical results
- Summary and learning

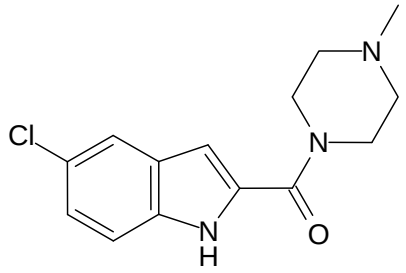
# Histamine H4 Receptor

- H4R is an aminergic GPCR
  - cloning & characterisation reported 2000-1
  - 40% homology with hH3R
- Expressed on immune cells
  - eosinophils, neutrophils, T-cells, mast cells & basophils
- H4R antagonists implicated in treatment of inflammatory diseases
  - Asthma, pruritus, inflammatory skin diseases, pain, AR, IBD, Cancer,...
- High ‘drugability’ – increase CIR/CIS

# Objectives

- Test mechanistic rationale & safety
  - Human and animal cellular experiments
  - In vivo disease models
  - In vitro safety assessments
  - In vivo safety studies
- Require tool compounds suitable for *in vitro* and *in vivo* CIS & CIR studies
  - Mechanism
  - Chemical series
  - Specific compound

# A Literature Tool




MW 277
LE **0.47**
clogP 2.5, LogD 2.6
Caco-2 AB/BA 44/29cms$^{-1}$

**JNJ-7777120**

hH4R Binding Ki 8.0nM (Lit. Ki 4nM[1])

H4R Functional Ki 6.8nM

Highly selective vs. H1/H2/H3 and in WLP

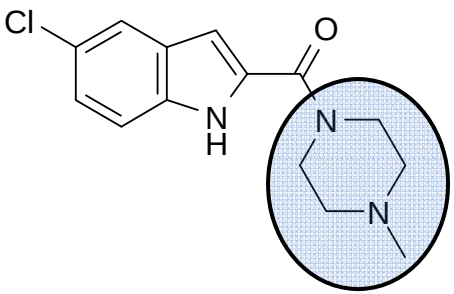
Literature[2]:
HLM $T_{1/2}$ 28min
RLM $T_{1/2}$ 4.5min

Rat (10mpk, PO) $T_{1/2}$ = 2.3h, F = 22%

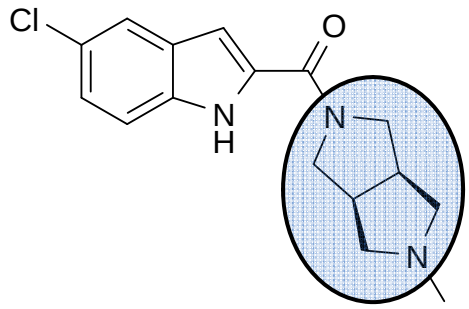
- Very useful early tool but sub-optimal metabolic stability and pharmacokinetics
- Seek novel compounds with improved pk properties
- Avoid indole

[1]Jablonowski et. al., Journal of Medicinal Chemistry, 2003, 46 (19), 3957-3960.
[2] Zhang et. al., Pharmacology & Therapeutics 2007, 113 (3), 594-606.

# Octahydropyrrolopyrrole Discovery

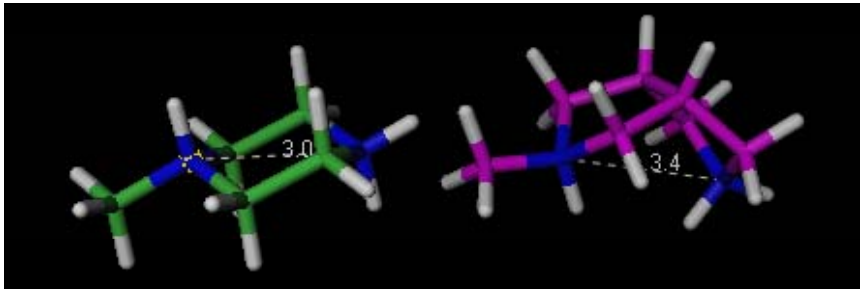



**JNJ-7777120**
clogP 2.5, logD 2.6, pKa 6.9
H4R Binding Ki 8.0 nM (lipE 5.5)*
H4R Functional Ki 6.8nM
HLM 28uL/min/mg
RLM 61uL/min/mg



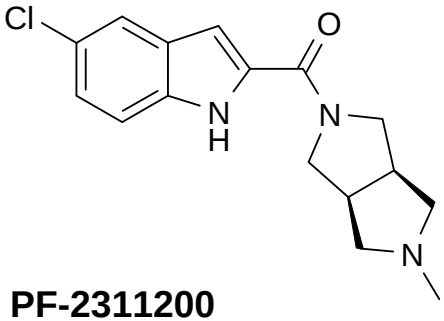

**PF-2311200**
clogP 2.3, logD 1.7, pKa 8.3
H4R Binding Ki 16nM (**lipE 6.1**)
H4R Functional Ki 27nM
**HLM 7uL/min/mg**
RLM 36uL/min/mg
**RM+ve**

Diamine monomer selection
low MW (<200)
Piperazine mimics

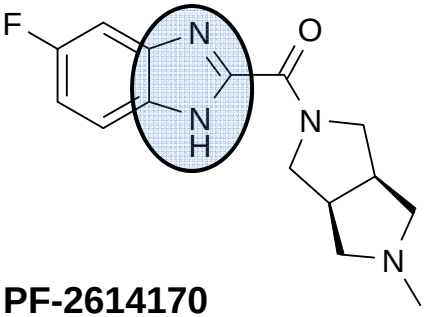



***LipE** = $pIC_{50}$ - LogD

# Benzimidazole Discovery

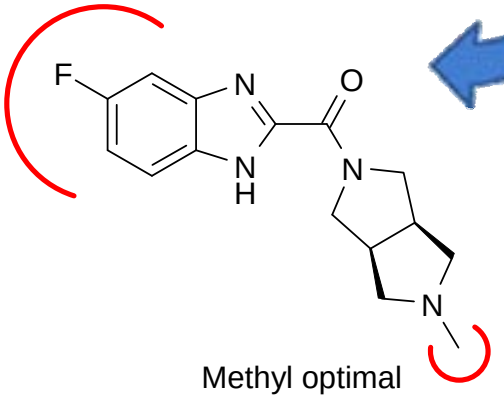



**PF-2311200**
clogP 2.3, logD 1.7
H4R Binding Ki 16nM (lipE 6.1)
H4R Functional Ki 27nM
HLM 7uL/min/mg
RLM 36uL/min/mg
**RM+ve**

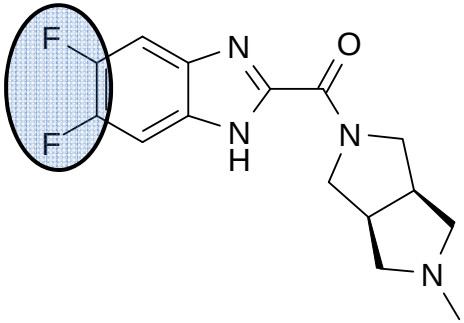



**PF-2614170**
clogP 1.2, logD 0.8
H4R Binding Ki 83nM (lipE 6.3)
H4R Functional Ki 39nM
HLM <7uL/min/mg
RLM 40uL/min/mg
**RM-ve**

F, Cl, Me
mono/di substituted all
well tolerated

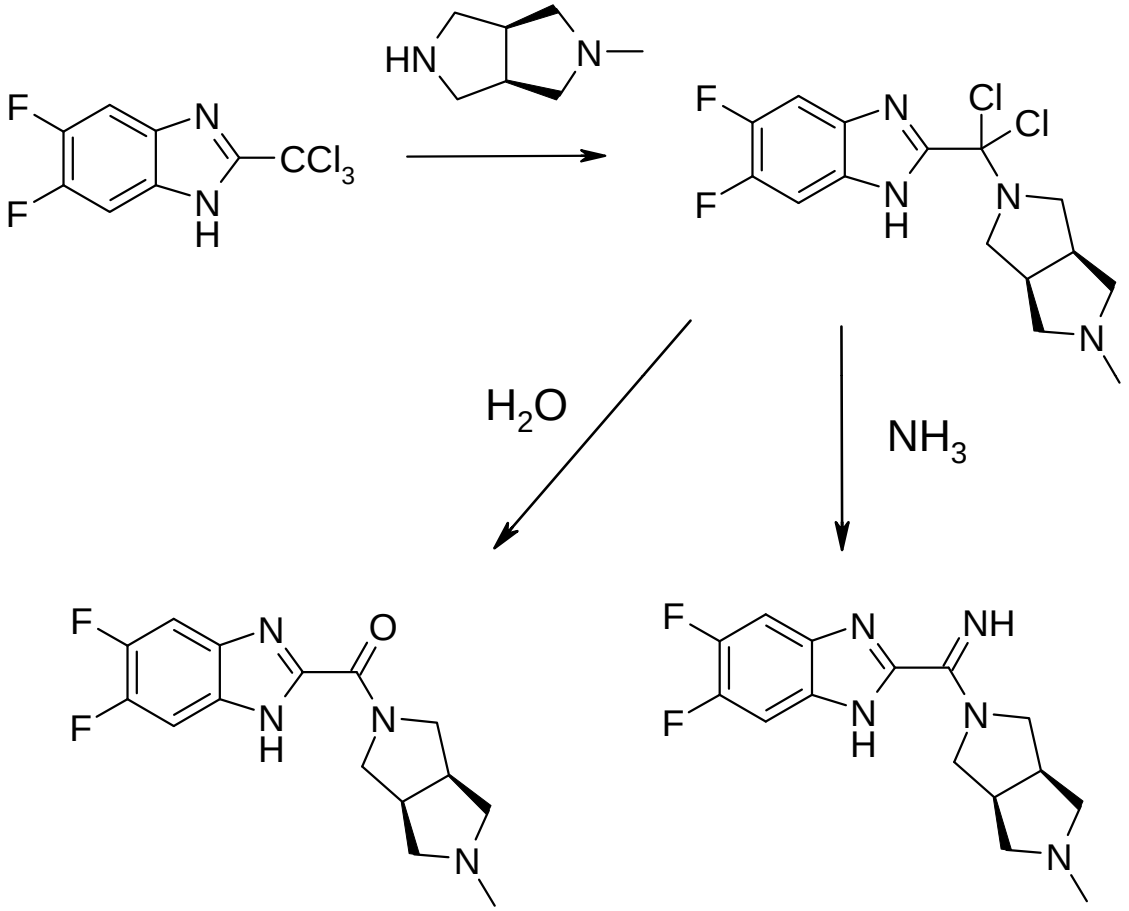



Methyl optimal
>10x loss for H, Et, iPr, cPr



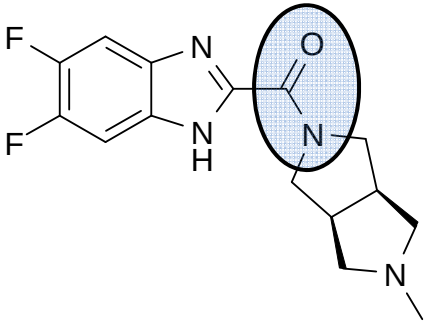

**PF-3306138**
**H4R Binding Ki 10-128nM**
**H4R Functional Ki 10-53nM**
HLM <7uL/min/mg
**RLM 10-40uL/min/mg**

# A Synthetic Bonus



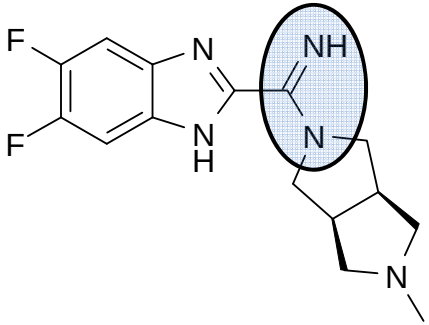

Amidine formation *via* suspected incomplete hydrolysis
Amidine synthesised *via* alternative route and profile verified

# Amide/Amidine Profiles




**PF-3306138**
clogP 1.3, logD 1.2, pKa 8.2
H4R Binding Ki 128nM (lipE 5.7)
H4R Functional Ki 53nM
HLM <7uL/min/mg
RLM 40uL/min/mg

**Rat PK**
Cl 58ml/min/kg
Vd 4.1L/Kg
$T_{1/2}$ 0.8h
F 50%



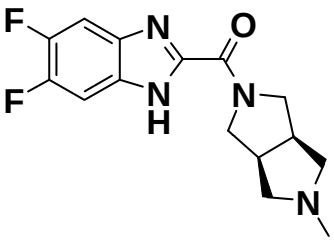

**PF-2988403**
clogP 2.4, logD 1.0, pKa 8.4, 7.1
H4R Binding Ki 10nM **(lipE 7.0)**
H4R Functional Ki 10nM
HLM <7uL/min/mg
**RLM 10uL/min/mg**

**Rat PK**
Cl 31ml/min/kg
Vd 31L/Kg
$T_{1/2}$ 12h
F 20%

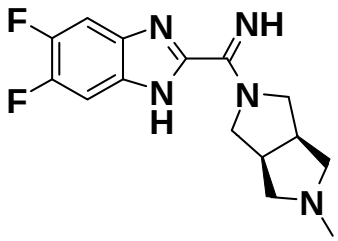
- Novel H4 antagonists, indole removed, suitable for further study

# Benzimidazole ETS Results

- Leads took project to Lead Development milestone
- Clean off-target pharmacology - de-risk novel H4R antagonist mechanism
- Parallel rat PO 4 day Early Toxicology Study (ETS) with amide & amidine
  - Increased confidence in conclusions with n=2 compounds




**PF-03306138**
MW 306, LogD 1.2
H4 bind/func Ki 117/48nM
5HT3 selectivity 8x
HLM/RLM <7/40 uL/min/mg
**Rat** T1/2 0.8h

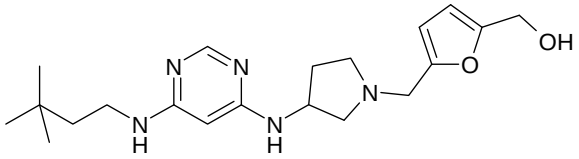



**PF-02988403**
MW 305, LogD 1.0
H4 bind/func Ki 7/10nM
5HT3 selectivity 110x
HLM <7/10 uL/min/mg
**Rat** T1/2 11h

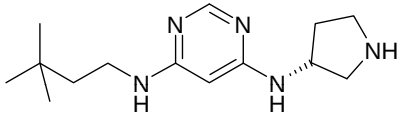
- Serious adverse effects produced by both compounds in rats:
  - Dose-dependent lymphoid depletion from spleen, thymus and gut associated lymphoid tissues
  - Decreased reticulocyte count and decreased erythropoesis at all doses
  - Induce a significant pro-inflammatory response in rat
- TK analysis confirms exposure >H4R Ki drives effects

- Blood cells generated in bone marrow where H4R is expressed
- Is H4 receptor antagonism a toxic mechanism or is it just these compounds?
- Develop a new series from HTS to explore mechanism vs. compounds

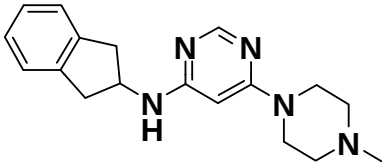
# HTS & H2L



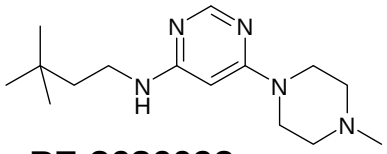
**PF-426713 – HTS hit from a FE library**
HTS H4 func 73% @ 15μM, IC50 >20μM
H4 bind Ki 2110nM
**H3 bind Ki 765nM**
clogP 3.8
LE 0.29, lipE 1.9



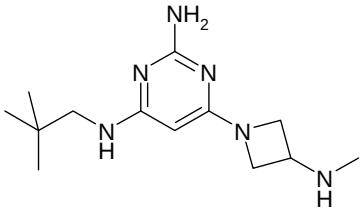
**PF-3604861**
H4 bind Ki 502nM
H4 func Ki 574nM
H3 bind Ki 8220nM
clogP 3.4
**LE 0.46, lipE 2.9**



**PF-2345185**
H4 bind Ki 212nM
clogP 3.2
**LE 0.45, lipE 3.5**

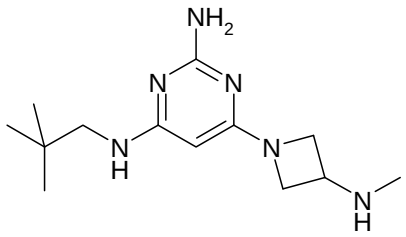


**PF-3686093**
H4 bind Ki 82nM
H4 func Ki 37nM
H3 bind Ki 646nM
clogP 3.6
**LE 0.50, lipE 3.5**



**PF-3826719**
H4 bind Ki 1.3nM
H4 func Ki 0.034nM
H3 bind Ki 213nM (**160x**)
clogP 2.7
**LE 0.65, lipE 6.2**

# PK of PF-03826719




**PF-3826719**

H4 bind Ki 1.3nM
H4 func Ki 0.034nM
H3 bind Ki 213nM
clogP 2.7, logD 0.7
LE 0.65, lipE 8.2
pKa 6.5 & 7.9

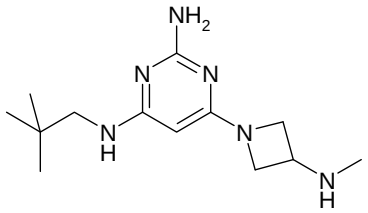
HLM < 7μl/min/mg
RLM <8.5 μl/min/mg

| Data | Rat | Dog |
|---|---|---|
| Blood Cl (Clu) | 77 (196) | 30 (68) |
| Blood Vd (Vdu) | 49 (124) | 48 (109) |
| T1/2 (h) | 6.9 | 19 |
| Bioavailability | 57 | - |
| Renal Clu | 10 | - |

| Prediction | Man (from rat) | Man (from dog) |
|---|---|---|
| Blood Cl | **16** | **16** |
| Blood Vd | 42 | 37 |
| T1/2 | 30 | 27 |
| Bioavailability | 30% | |

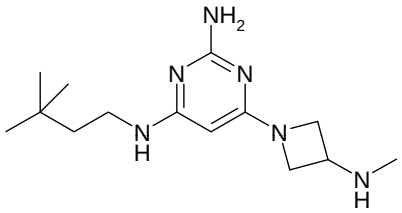
- *Human dose prediction of 15mg od (3 x Ki at trough)*
- But High Confidence of High Clearance and high risk
- But still a good tool to revisit CIS with further rat ETS

# Benzimidazole ETS



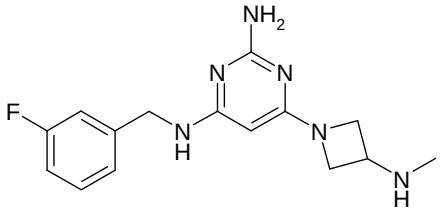
**PF-3826719**
H4 bind Ki 1.3nM
H4 func Ki 0.034nM
RLM 10μl/min/mg

**Active**
23/55/160xhKi
vacuolation observed at highest dose
**No effect on hematopoetic or lymphoid tissues**



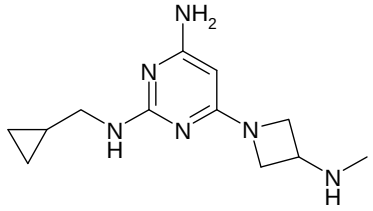
**PF-3861018**
H4 bind Ki 4.6nM
H4 func Ki 1.5nM
RLM 33μl/min/mg

**Active**
14/191xhKi
**No effect on hematopoetic or lymphoid tissues**



**PF-3818195**
H4 bind Ki 20.2nM
H4 func Ki 8.4nM
RLM <8.5μl/min/mg

**Active**
18/160xhKi
vacuolation observed at highest dose
BM cytology changes at high dose

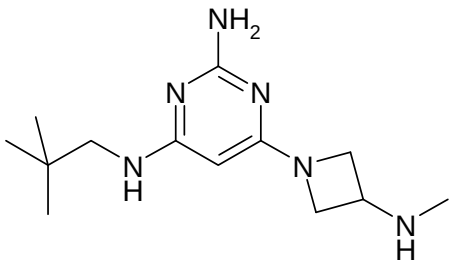


**PF-3818170**
H4 bind Ki 9070nM
H4 func Ki >1820nM
RLM <8.5μl/min/mg

**Structurally related inactive**
Mortality at high dose

- Good news… No effect on hematopoetic or lymphoid tissues
- Still need rH4R to put data fully into context
- Value of multiple compounds increases confidence in conclusions

# Improving PK




**PF-3826719**

H4 bind Ki 1.3nM
H4 func Ki 0.034nM
H3 bind Ki 213nM
clogP 2.7, logD 0.7
LE 0.65, lipE 8.2
pKa 6.5 & 7.9

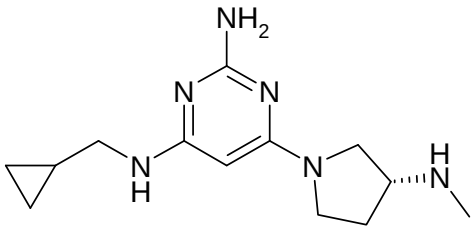
HLM < 7μl/min/mg
RLM <8.5 μl/min/mg

| Data | Rat | Dog |
|---|---|---|
| Blood Cl (Clu) | 77 (196) | 30 (68) |
| Renal Clu | 10 | - |

| Prediction | Man (from rat) | Man (from dog) |
|---|---|---|
| Blood Cl | **16** | **16** |

- PF-3826719 stable in HLM & RLM but *in vivo* Cl~LBF
- Many promising new leads also stable in HLM & RLM
- In vitro ADME screens not sufficient to differentiate
- Profile best candidates in rat PK

# Profile of PF-3893787




**PF-3893787**

H4 bind Ki 1.2nM
H4 func Ki 0.7nM
clogP 1.5, logD -0.1
LE 0.62, lipE 9.0
pKa 6.9 & 8.8

HLM < 7μl/min/mg
RLM <8.5 μl/min/mg

| | **Rat** | **Dog** |
|---|---|---|
| Blood Cl (Clu) | 29 (70) | 7(9) |
| VD (VDu) | 23(56) | 16(21) |
| T1/2 (h) | 9.4 | 27 |
| F% | 57 | 50 |
| Renal CLu | 14 | - |
| | | |
| **Prediction** | **Man** | **Man** |
| Blood Cl | 5 | 5 |
| Vd | 16 | 20 |
| T1/2 | 37 | 40 |
| F% | 85 | - |

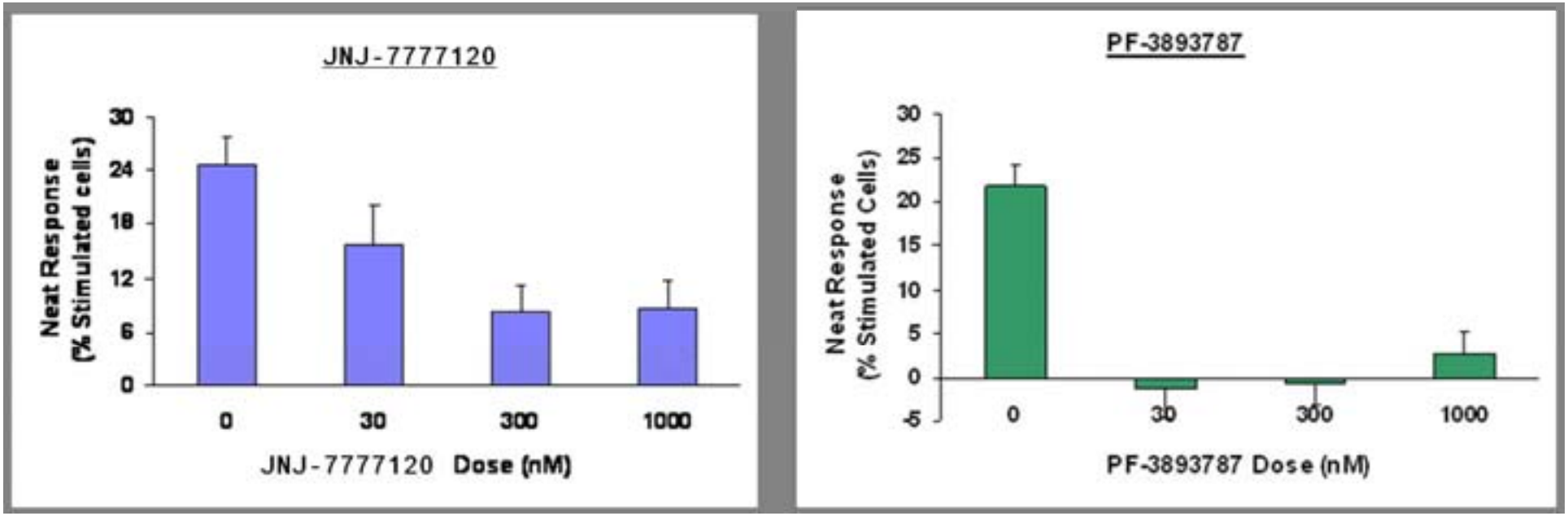
**High confidence of moderate Cl**

**Long T1/2 – low dose prediction**

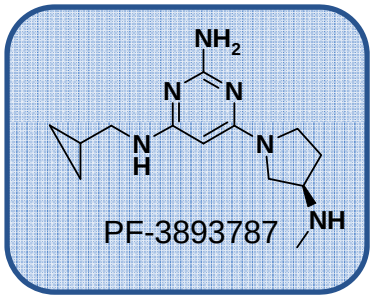
ETS completed Rat & Dog – no adverse events

# Biomarker for H4R Antagonism

- Eosinophil shape change induced by H4R agonists such as Imetit
- Inhibition of Imetit-induced shape change in whole blood *via* Gated Autofluoresence Forward Scatter (GAFS) assay - a validated biomarker of H4R antagonism
- Comparison between JNJ-7777120 and PF-3893787:



# *In vitro* Pharmacology




- Functional antagonist at the human native receptor

- Potency has been established using whole blood or isolated eosinophils on several end points including shape change and actin polymerisation

| | PF-3893787 | | JNJ-7777120 | |
|---|---|---|---|---|
| | Mean $IC_{50}$ | N | Mean $IC_{50}$ | N |
| Histamine-induced *isolated* eosinophil shape change | 0.65nM, 5.3nM | 2 | 199.0nM (86.7 -456.5) | 4 |
| Histamine-induced *isolated* eosinophil actin polymerisation | 1.3nM (0.56-3.0) | 14 | 5.3nM (2.9-9.5) | 9 |
| Imetit-induced *whole blood* eosinophil shape change (GAFS) | <30nM (total) | 3-6 | 30-100nM (total) | 3-6 |

# *In vivo* Pharmacology

- Hard to generate strong CIR in pre-clinical animal models

- lack of disease models

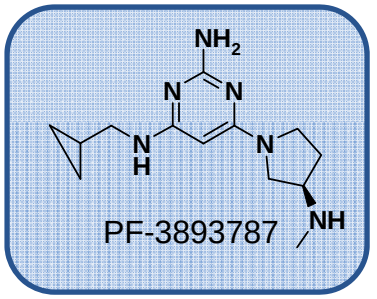
- significant H4R species differences[1]

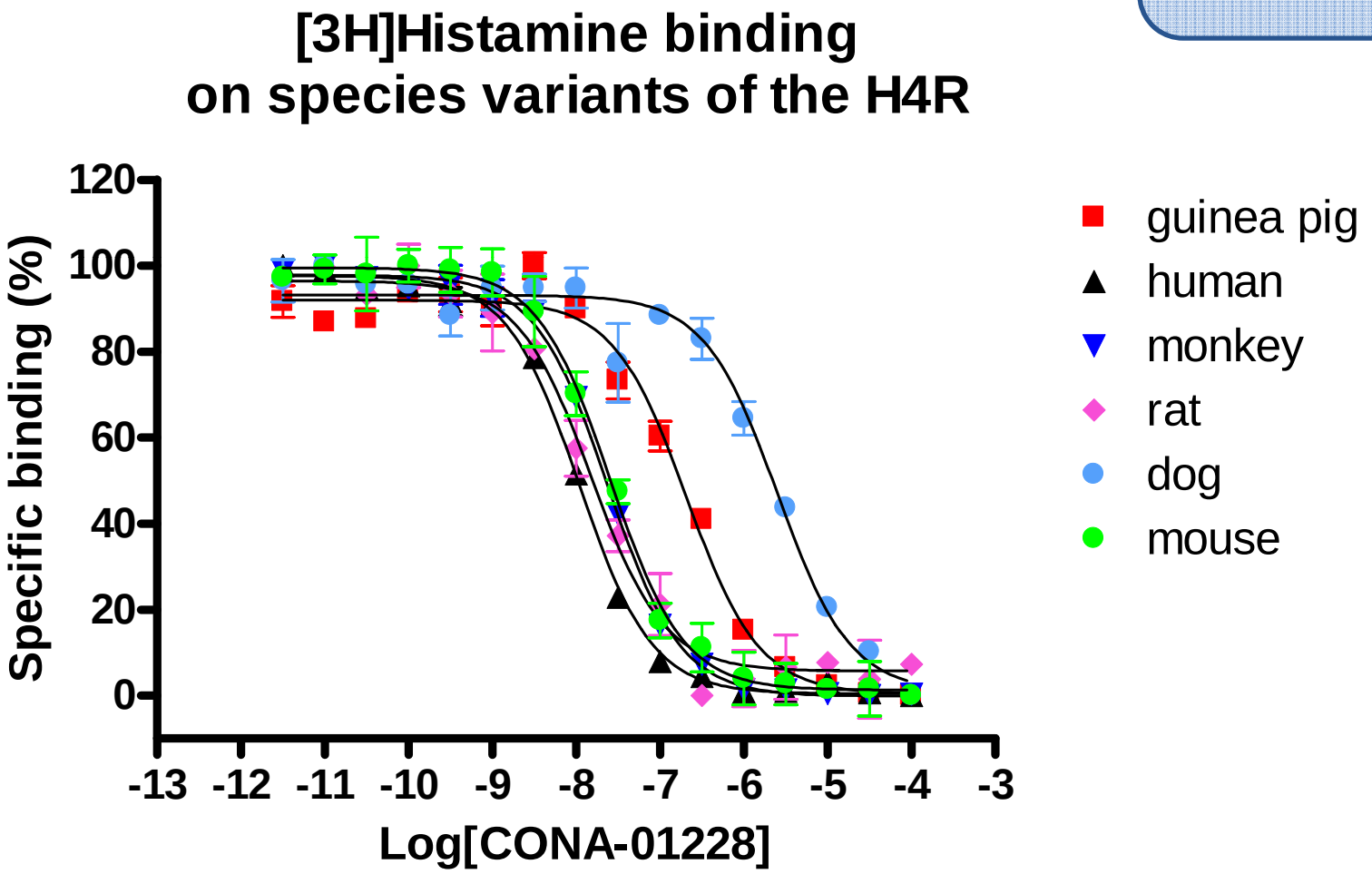
| | | | | | | |
|---|---|---|---|---|---|---|
| Human | 100 | | | | | |
| Macaque | **93** | 100 | | | | |
| Dog | **71** | **71** | 100 | | | |
| G.Pig | **62** | **64** | **61** | 100 | | |
| Rat | **68** | **68** | **65** | **61** | 100 | |
| Mouse | **67** | **66** | **66** | **62** | **85** | 100 |
| | Human | Macaque | Dog | G.Pig | Rat | Mouse |

• Key $H_4$ compounds profiled in binding and functional assays using recombinant $H_4$ from various species

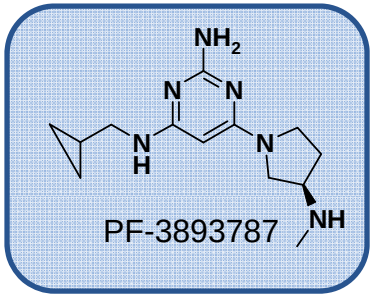
[1]*Liu et. al. JPET, 2001, 299 (1), 121-130*

# PF-3893787 Ki's

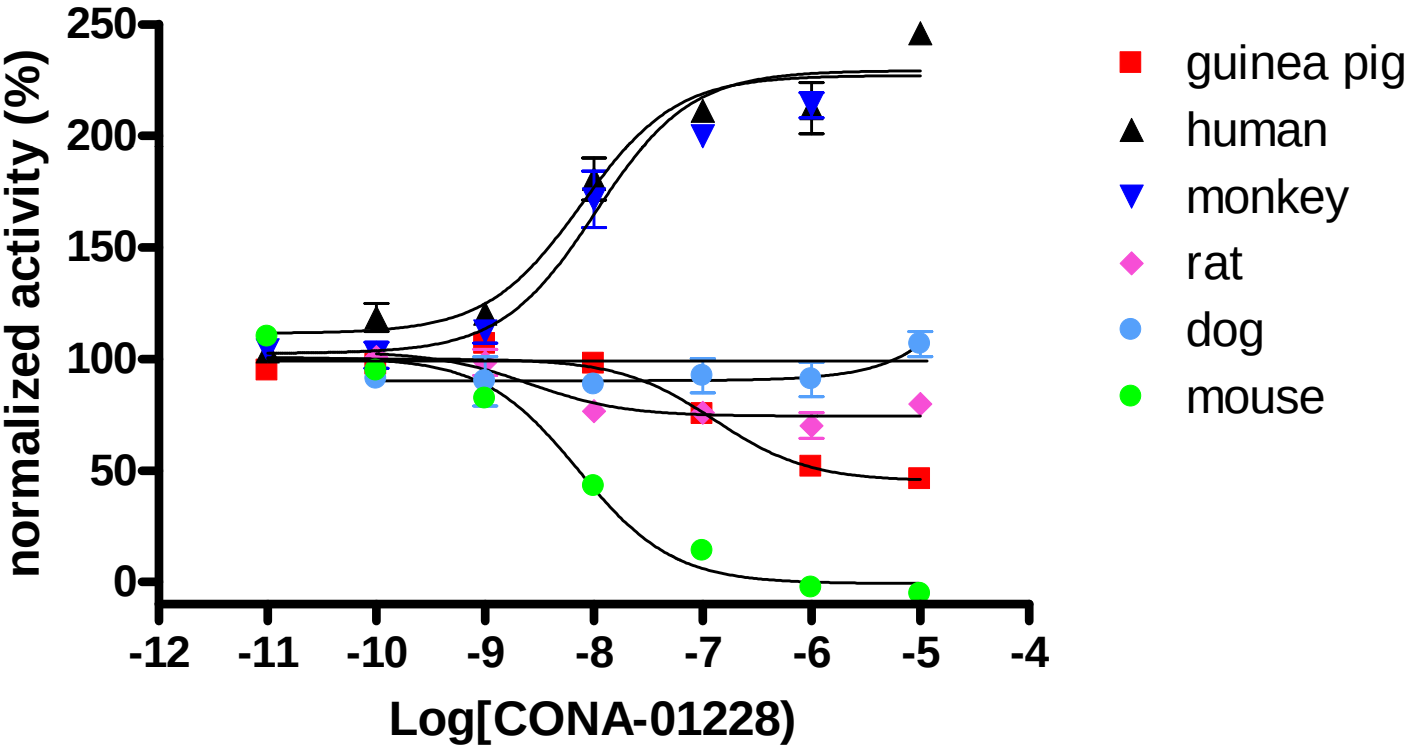


# PF-3893787 Functional Data




Effect on forskolin-induced CRE-reporter gene activity mediated by variants of the H4R




Pharmacology at different species of H4R complex – important implications in interpretation of animal data

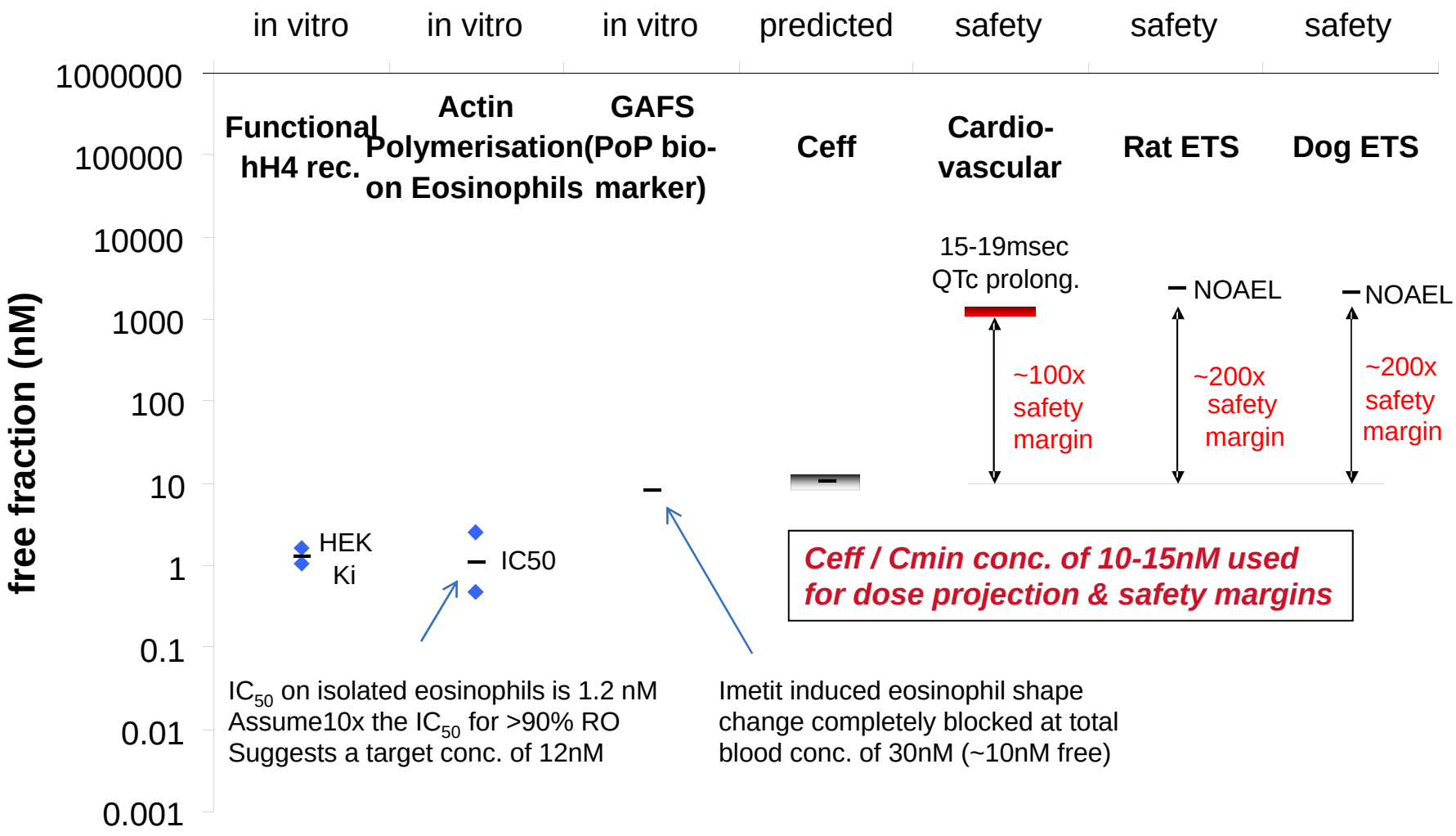
# Orthologues

- Orthologue *in vitro* and sequence data confirmed difficulty in generating CIR for asthma in pre-clinical animal models

- Also now discovered that prototype benzimidazoles (amide & amidine) actually agonists in rat
  - Explains pro-inflammatory findings in rat ETS
  - Also suggests an H4R antagonist should be anti-inflammatory

- Sufficient rationale to take H4R antagonist to clinic

# Ceff / Safety Summary



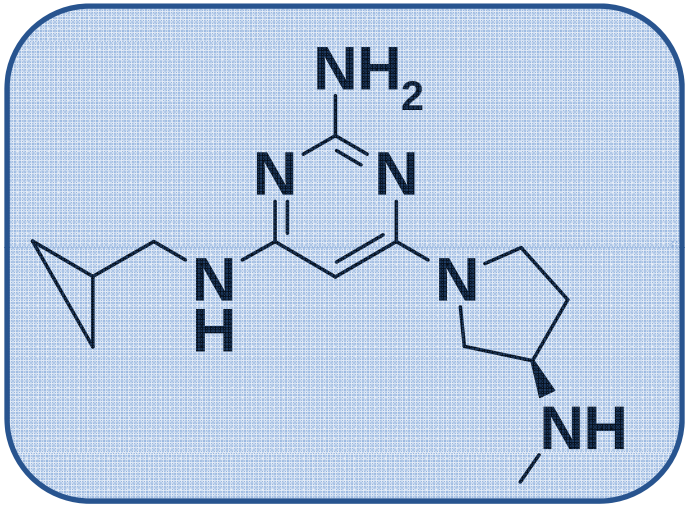
# Enablers for Moving to the Clinic

- Developed robust biomarker assay
- Human dose projection (in absence of PK/PD data)
  - Potency in human native cells
  - Good projected PK profile
- Understanding of affinity and efficacy against orthologues
- Mechanism de-risked in rodent and macaque
  - Regulatory toxicity studies completed in rat and macaque
  - Sufficient margins for progression to human

# PF-3893787-18

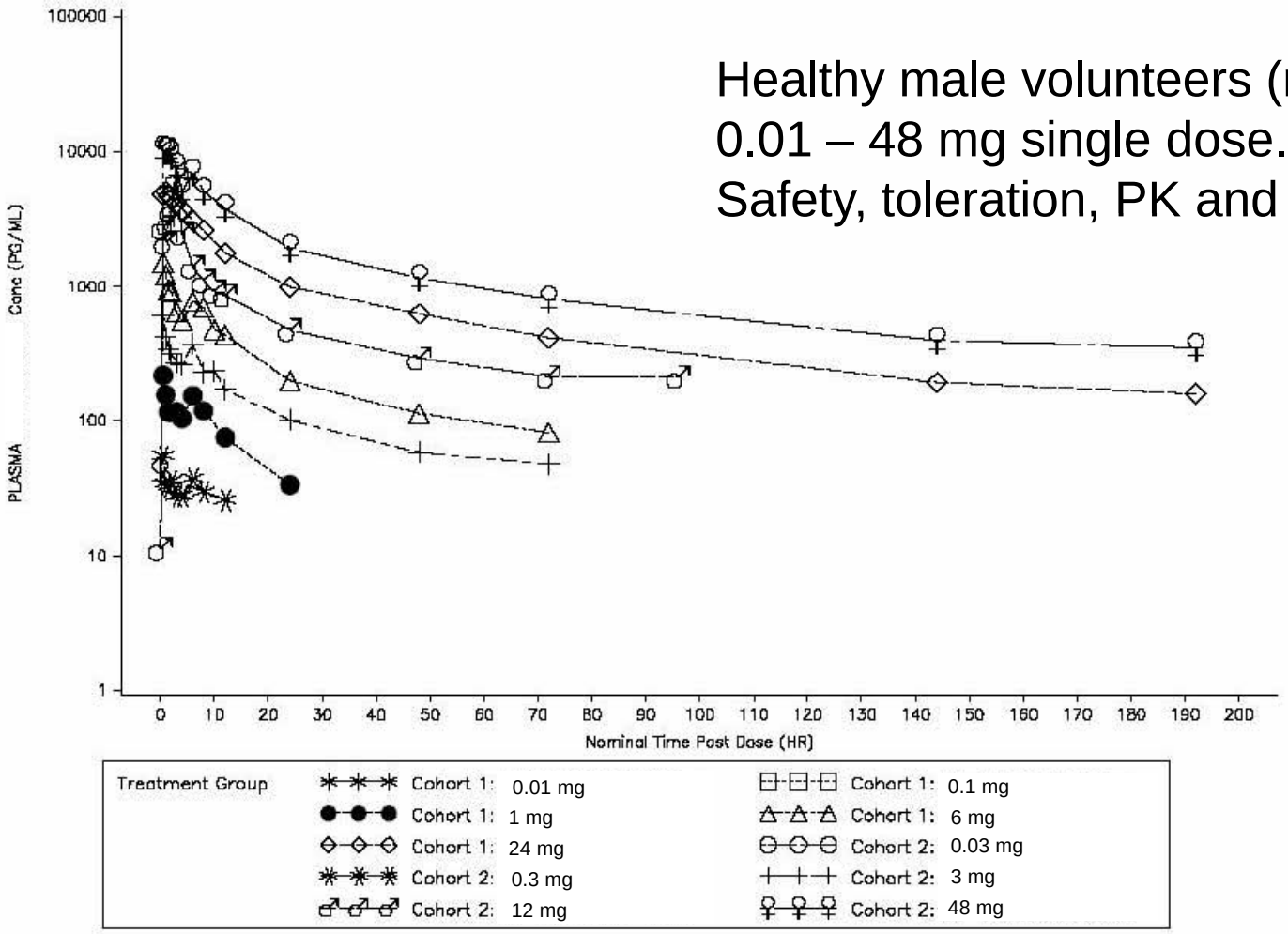
## An oral once-daily histamine $H_4$ antagonist

MW 262
Tartrate salt




- Potent and selective H4 antagonist at native receptor
- Potential QD profile with low projected dose (7-20mg)
- Rat and macaque are suitable Regulatory Tox. species
- Sufficient safety margins for progression to human
- Fit for purpose pharmaceutical properties

# FIH Summary – PK & Safety



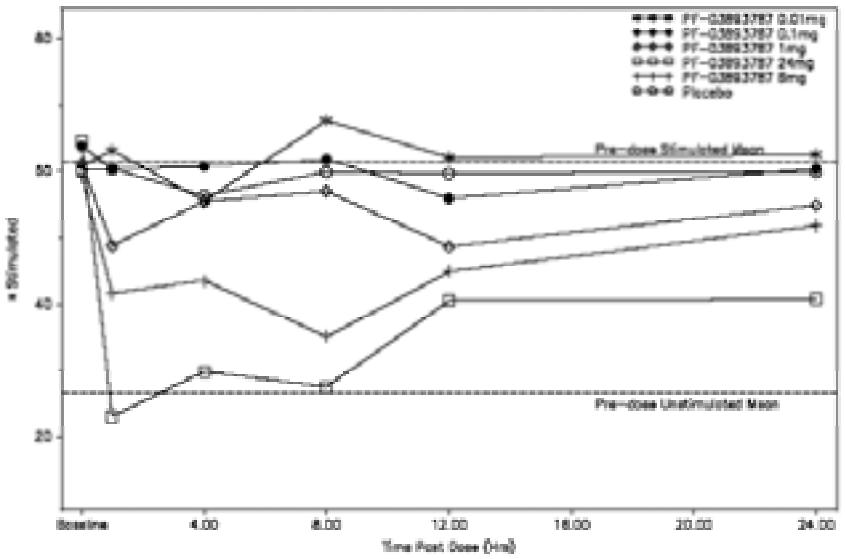

Healthy male volunteers (n = 23).
0.01 – 48 mg single dose.
Safety, toleration, PK and PD

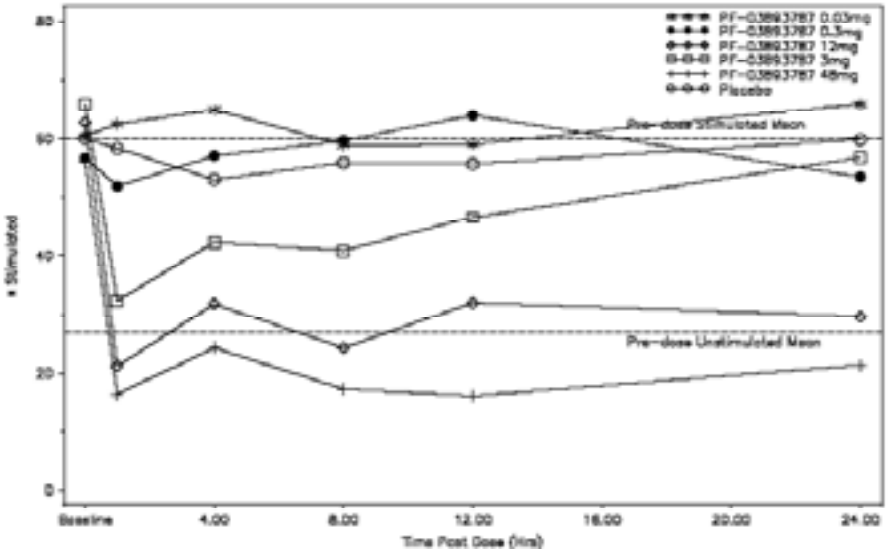
PF-3893787 rapidly absorbed $C_{max}$ 30 to 45 minutes postdose

Well tolerated and safe at all dose levels

# FIH Summary – PoP Biomarker

- Systemic pharmacodynamics of PF-3893787 was assessed *ex vivo* using imetit-stimulated eosinophil shape change measured by the GAFS flow cytometric assay
- PF-3893787 produced dose-and time-dependent inhibition of this assay at doses >1mg, with complete inhibition of the response over the 24 hour period postdose at doses >12mg



# Early Clinical Summary

- PF-3893787 is a validated clinical H4R antagonist
- Doses required to block H4R pharmacology in volunteers are safe and well tolerated
- Studies exploring the utility of PF-3893787 in patients will be reported in due course
- Potential indications include asthma, pruritus, inflammatory skin diseases, pain, AR, IBD, & Cancer

# Learning

- Value of early toxicity studies with parallel compounds
  - N=2 increases confidence in conclusions
- Need to understand orthologue potency & efficacy
  - Enables interpretation of CIR & CIS studies
  - Drives selection of species for safety studies
- An orthogonal second series allowed project continuation
  - Enables test of compound vs. mechanism driven effects
- Value of human pharmacology in native tissue
  - Supports dose prediction
- Power of a biomarker
  - Confidence in pharmacology allows confident test of mechanism in patients

**Biology**
Garry Douglas
Nicole Schacht
Tim Davies
Andy Gray
Kristina Ulrich
John Adcock
Hannah Mace
Isabelle Delescluse
Debbie Meyer
Debbie Heuvelman
Ramla Ali
Adrian Barnard
Chris Brown
Karl Campany
**Nick Clarke**
Matt Deacon
Garry Douglas
Rabia Hidi
Jennifer Hincks
Cheryl Lee
Hannah Mace
David Mcloughlin
Mark O'Reilly
Luis Perez Tosar
Christelle Perros-Huguet
Anne Phelan
Nikki Robas
Gary Salmon
Tim Stroud
Mike Trevethick
Chris Williams
Anne Wilson
**Steve Liu**

**Chemistry**
Andy Bell
Mark Bunnage
Kate Burt
Thomas Dupont
David Dunwoodie
Jonathan Fray
Duncan Hay
Tim Hobson
**Charlotte Lane**
Aibd Masood
Andrew Mansfield
Don Middleton
**Charlie Mowbray**
Sandra Newman
Michael Paradowski
Francesca Perruccio
Rachel Plunkett
David Price
Matt Selby
Nigel Swain
Hannah Vuong
Helen White
David Williams
Kuen Yeap

**Clinical/Development**
Lisa Tan
Jonathan Ward
**Grant Langdon**
Rich Allan

**RTL**
**Mike Bartley**

**Pharm Sci – OPCoE**
Simon Pegg
Michelle Collins
Karin Westin
Neil Feeder
Rita Lodaya
**Pharm Sci – CRD/RAPI**
Chris Ashcroft
Zijhian Zhu

**PDM**
**Rhys Jones**
Daniel Siddle
Ian Gardner
Anthony Harrison
Heather Chassaing
Michelle Gleave
Raj Logan
Ranjit Atwal
Sarah Kempshall
Russell Jones
Hannah Jones
Henry Pertinez
Phil Dalton
Claire Collins
Kuresh Youdin
**DSRD**
Neil Brunton
**Emanuel Schenck**
Mick Sutton
Fiona Spence

**VU University Amsterdam**
Prof. Rob Leurs

# Back Ups

3040.DC1.html

0026-895X/10/7705-734–743$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2010 The American Society for Pharmacology and Experimental Therapeutics
*Mol Pharmacol* 77:734–743, 2010

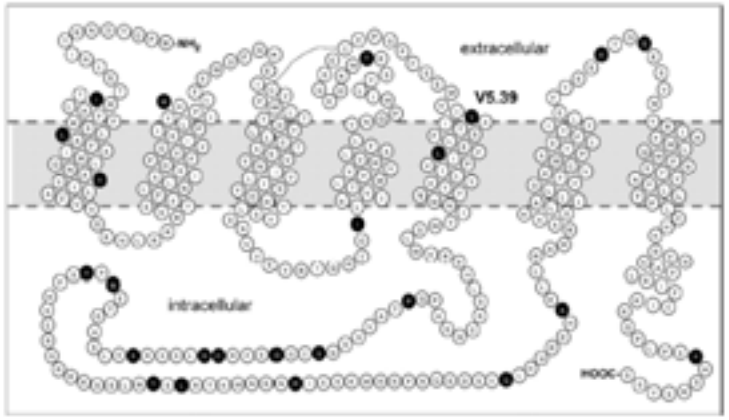
Vol. 77, No. 5
63040/3575066
*Printed in U.S.A.*

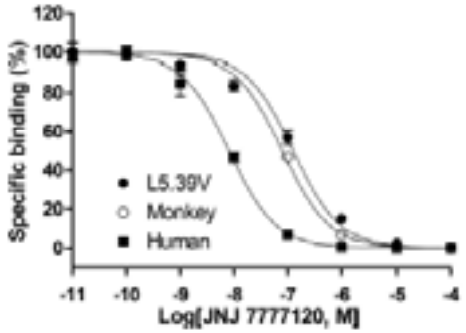
# Molecular Determinants of Ligand Binding to $H_4R$ Species Variants

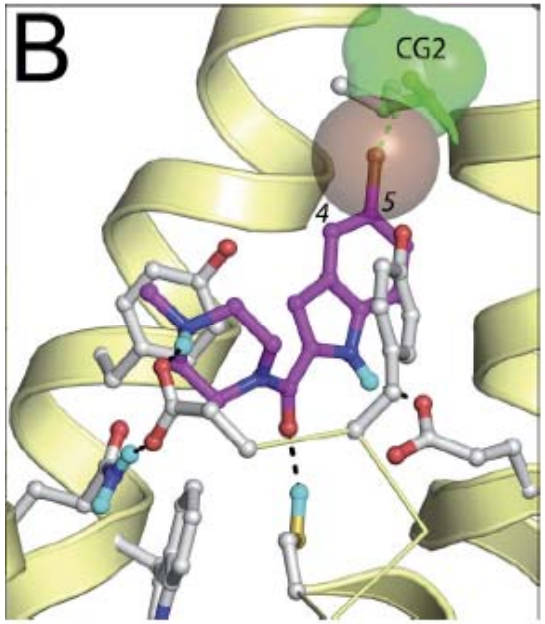
Herman D. Lim, Chris de Graaf, Wen Jiang, Payman Sadek, Patricia M. McGovern, Enade P. Istyastono, Remko A. Bakker,[1] Iwan J. P. de Esch, Robin L. Thurmond, and Rob Leurs

*Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Faculty of Science, VU University Amsterdam, Amsterdam, the Netherlands (H.D.L., C.d.G., E.P.I., P.S., R.A.B., I.J.P.d.E., R.L.) and Johnson & Johnson Pharmaceutical Research and Development, LLC, San Diego, California (W.J., P.M.M., R.L.T.)*

Received December 9, 2009; accepted January 26, 2010



# H4R species binding Kis

**Table 4.** $pK_i$ values for the displacement of [$^3$H]histamine by compound **13** and JNJ-7777120 from H4 receptors from different species.

| Compound/Species | **13** (PF-3893787) | JNJ-7777120 |
|---|---|---|
| Human | 8.21±0.07[a] (n=3) | 8.48±0.01 (n=2) |
| Macaque | 7.81±0.03 (n=3) | 7.17±0.09 (n=3) |
| Dog | 5.79±0.09 (n=3) | 6.89±0.08 (n=3) |
| Guinea pig | 6.91±0.01 (n=3) | 5.97±0.02 (n=3) |
| Rat | 7.91±0.10 (n=3) | 8.37±0.02 (n=4) |
| Mouse | 7.68±0.06 (n=4) | 8.41±0.09 (n=3) |

[a] The displacement binding was performed using [$^3$H]histamine and homogenate of HEK 293 T cells transiently transfected with the cDNA of corresponding H4R variants. The data are presented as mean ± S.E.M (number of experiments).

# Other H4 antagonists

**J&J**

**Phase 1:** SD JNJ-39758979  50, 100, 300, 600mg or Placebo

**12 week asthma study using 300mg QD:** Read-out: August 2010

**Itch study using SD of 600mg J&J-39758979 vs histamine challenge:** Read out May 2010

**Palau**

**Phase 1:** rising SD UR-63325 completed. MD scheduled for 2Q2010

**Cellzome**

Planned Phase I with CZC-13788  was cancelled.