



# The Discovery and Optimisation of a Highly Selective Series of TRPV4 Antagonists

**Sarah Skerratt, Pfizer Neusentis**

Acknowledgements: Gordon McMurray, Wendy Winchester, Paul Brennan, James Mills, Wolfgang Klute, Duncan Armour, Kuen Yeap, Florian Wakenhut, Carolyn Barker, Jayesh Mistry, Karl Gibson, Eileen Blasi, Paul Butler, TCGLS



**WORLDWIDE RESEARCH & DEVELOPMENT**  
Medicinal Chemistry

# Talk outline



- Is TRPV4 a therapeutically relevant target?
- Is TRPV4 a safe target?
- The search for selective TRPV4 antagonists: HTS screening campaign
  - Computational methods to remove “frequent hitters” and prioritise compounds for follow up
- Hit to Lead: Optimising a piperidine series
- PF-05214030: Understanding translation
  - *in vitro/ex vivo/in vivo* characterisation
- Efficacy study data
- CV outcomes
- More emerging human TRPV4 mutation data and a project NO GO decision
- Conclusions

# TRPV4: A therapeutically relevant target?



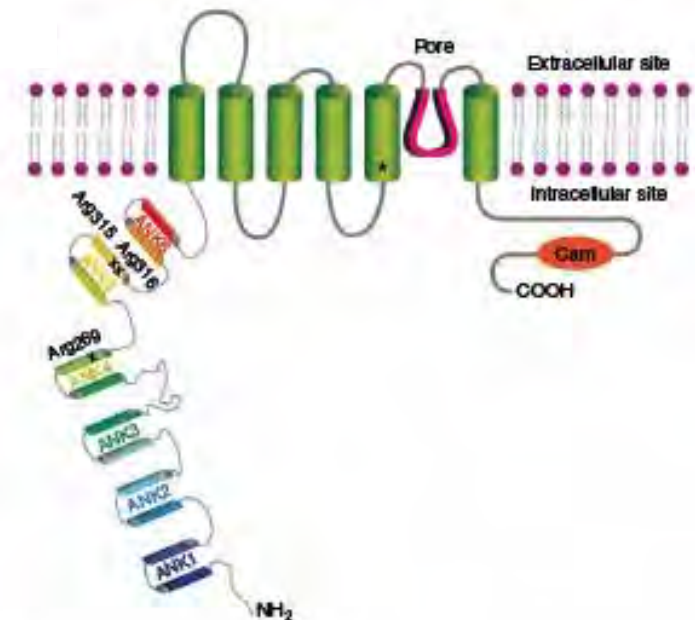
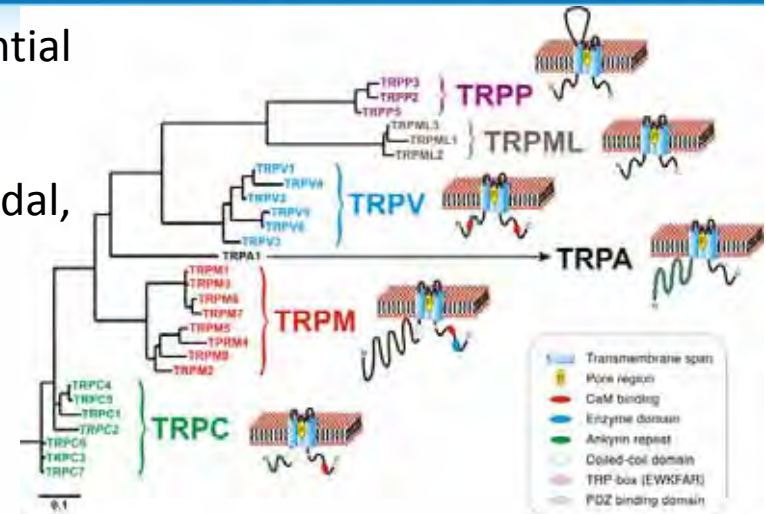
❑ TRPV4 is a member of the **T**ransient **R**eceptor **P**otential family of ligand-gated ion channels

❑ TRPV4 is a non selective cation channel and is polymodal, i.e. activated by a range of stimuli:

- Small molecules
- Temperature (>27°C)
- Hypotonic solutions
- Mechanical stimuli

❑ TRPV4 has a wide expression profile

- Sensory and motor nerves
- Bladder urothelium and kidney (widespread)
- Vascular endothelium and smooth muscle
- Lungs (epithelia, endothelia and smooth muscle)
- Macrophages
- Osteoclasts and osteoblasts
- Ear (hair cells, stria vascularis, auditory ganglion)



# TRPV4: A therapeutically relevant target?



## □ TRPV4 KO mouse data (literature):

- KO mice are generally healthy and reproductive

- Altered urinary function:

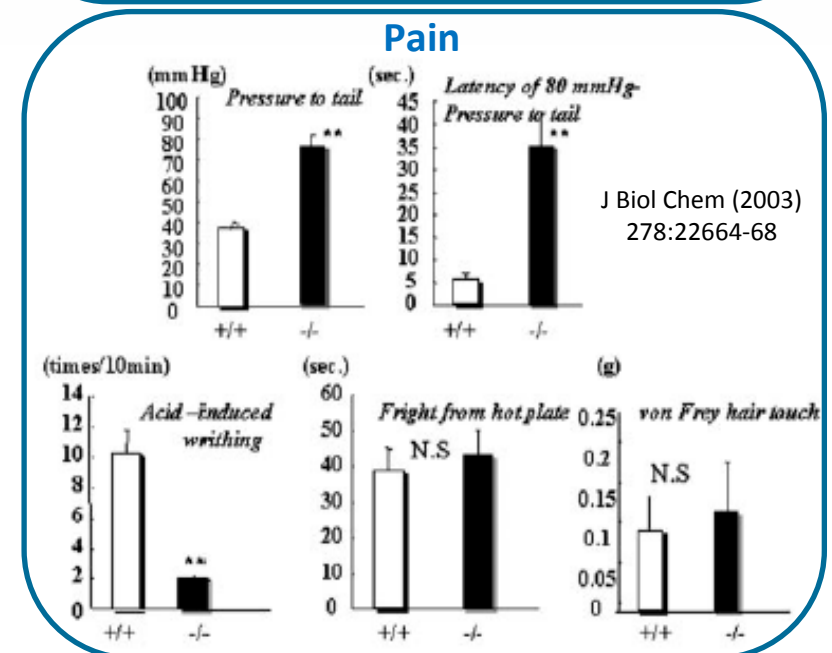
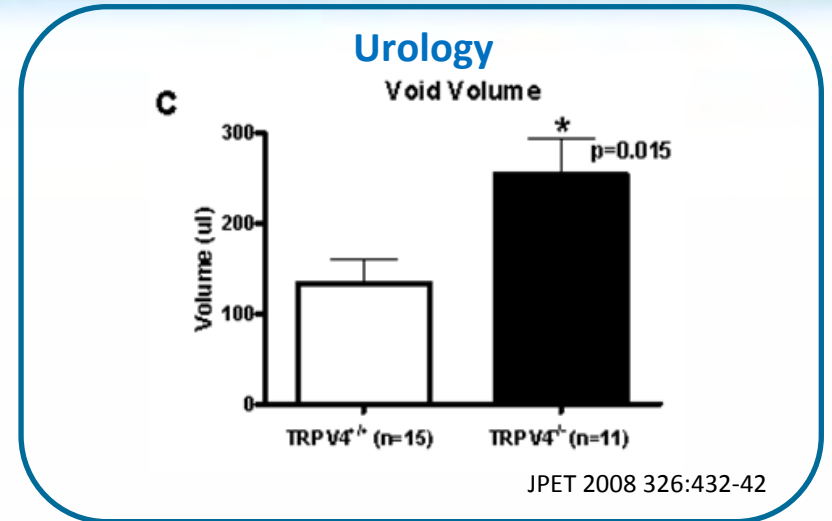
Increased bladder capacity, reduced stretch evoked responses, reduced response to inflammatory agents

→ TRPV4: potential for urology therapeutics?

- Altered pain phenotype against a number of stimuli:

Painful pressure, acetic acid, thermal, chemical mediators of inflammation (carrageenan, formalin), osmotic pain

→ TRPV4: potential for pain therapeutics?



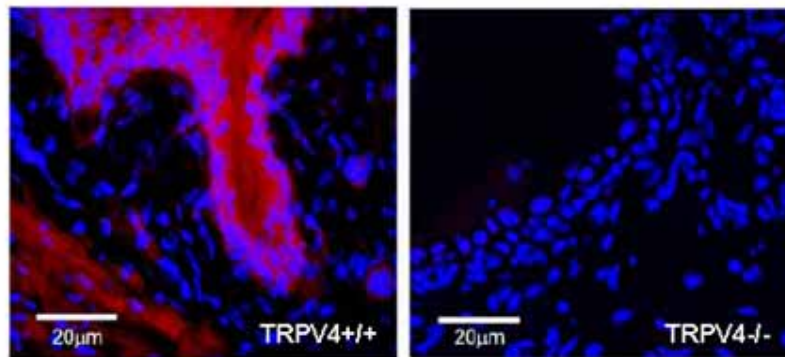
# TRPV4: A therapeutically relevant target?



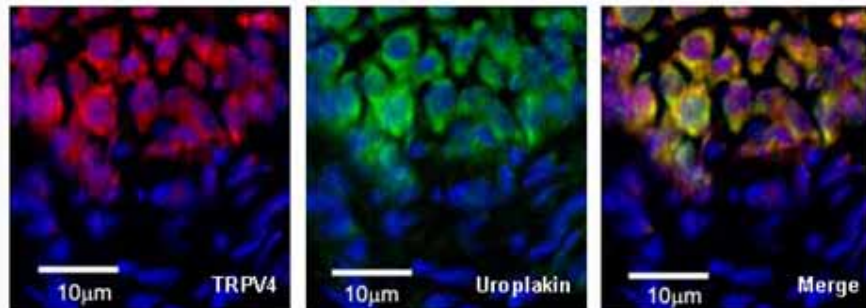
## UROLOGY

### ☐ TRPV4 – Bladder Expression

Literature data shows TRPV4 is expressed in bladder urothelium. In-house experiments confirm TRPV4 expression in mouse, rat, guinea-pig and human bladder urothelial cells

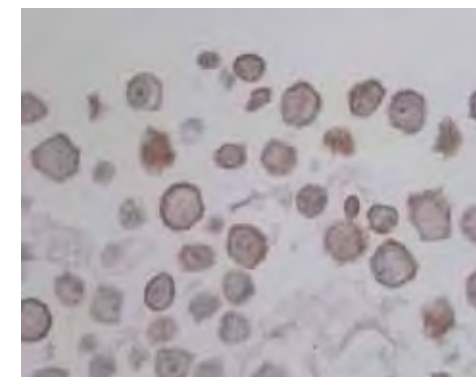


TRPV4 expression in WT and KO mouse urothelium



Co-localisation of TRPV4 and uroplakin III in mouse bladder

JPET 2008 326:432-42



TRPV4 expression in human bladder and isolated human urothelial cells (Pfizer)

# TRPV4: A therapeutically relevant target?

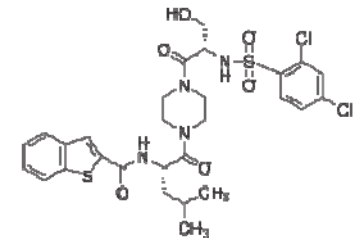
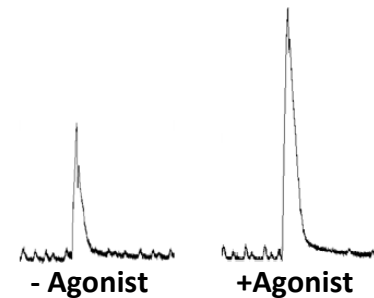
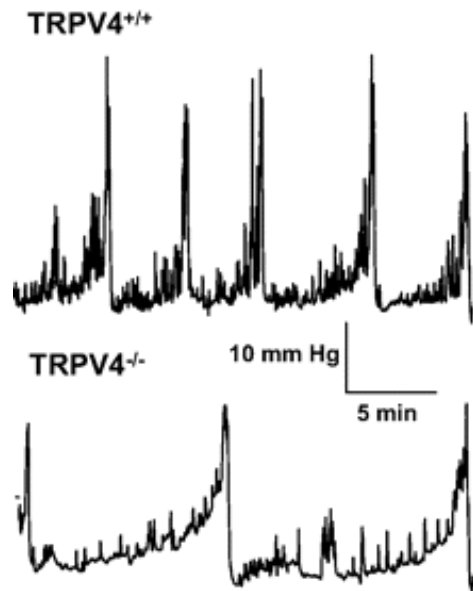


## UROLOGY

### □ TRPV4 – Role in Bladder Function

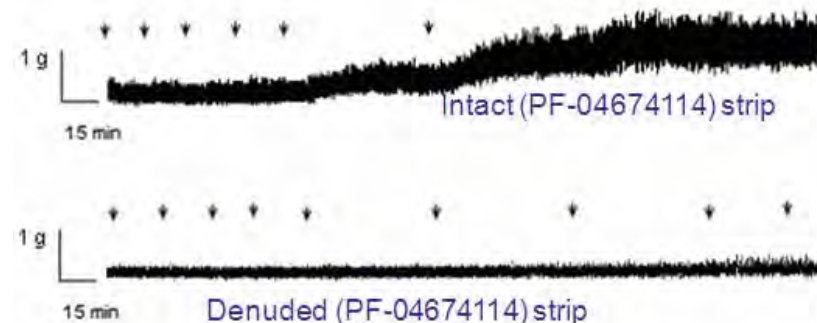
TRPV4 KO mice show an increased bladder capacity prior to voiding in conscious cystometry experiments (literature)

Selective TRPV4 agonist (GSK1016790A/PF4674114) causes various contractile effects in rat bladder strips, including increased electrically induced nerve contractions and urothelium dependent agonist induced contractions (Pfizer)



GSK1016790A/PF4674114

▪ Agonist responses in bladder strips during electrically induced nerve contractions (Pfizer)



- Conscious cystometry in TRPV4 KO and WT mice (literature)
- Agonist responses in intact and urothelium denuded bladder strips (Pfizer)

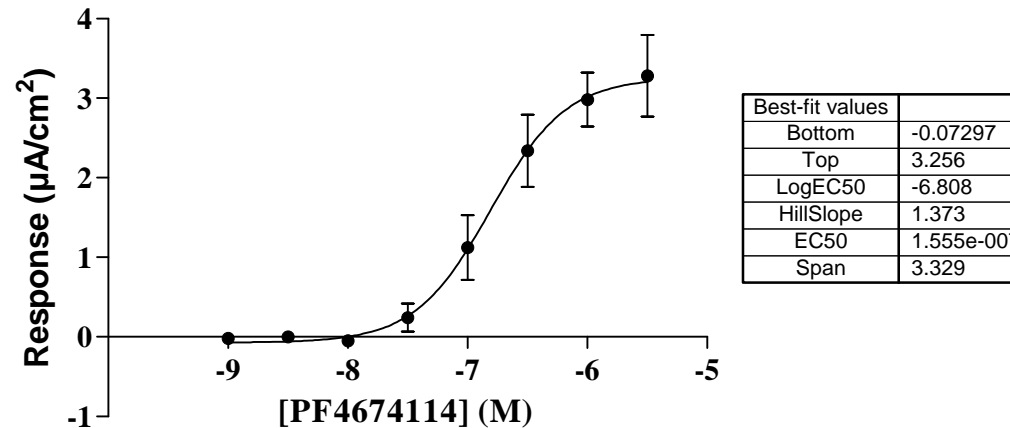
# TRPV4: A therapeutically relevant target?



## UROLOGY

### □ Effects of TRPV4 in human bladder urothelium

Effect of PF4674114 on human bladder urothelium  
(mucosal challenge, n=6)



Conductive response seen in human urothelium with TRPV4 agonist challenge (dose dependant)

Response abolished by antagonism with Ruthenium Red and TRPV4 selective compounds –  
Functional consequence of this?

# TRPV4: A safe target?



## ❑ Role in kidney function

TRPV4 expression high within kidney tubules – role in osmoregulation

TRPV4 KO mice show altered osmotic regulation and fluid intake (contradicted in some papers)

## ❑ Role in Vascular permeability

Administration of TRPV4 agonist leads to circulatory collapse in rodents → linked to vascular leakage and haemorrhage. Effects not seen when TRPV4 agonist administered to TRPV4 KO's

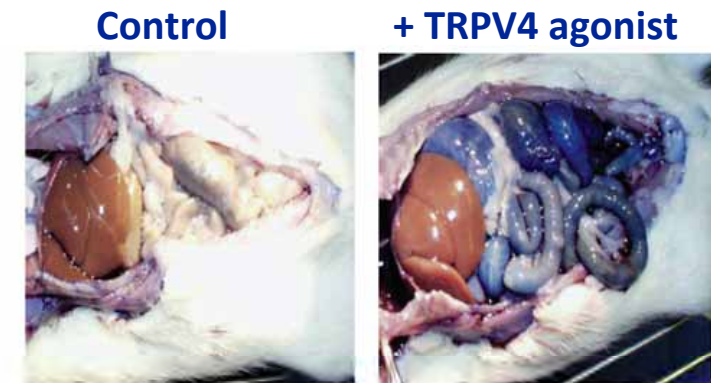
## ❑ Human TRPV4 Mutation Phenotypes

Gain-of-function mutations in TRPV4 cause autosomal dominant Brachyolmia → short trunk, short limbs, scoliosis of the spine

## ❑ Age and noise induced hearing loss in TRPV4 KO's

## ❑ Thermal selection

TRPV4 KO's prefer 34 °C over 30 °C in preference paradigm  
Slight delayed response to tail immersion in water bath



Evan's Blue extravasation in the intestine

[J PET. 2008 326\(2\), 443-52](#)



[Nature Genetics 40, 999 - 1003 \(2008\)](#)



# TRPV4 HTS: The search for selective TRPV4 antagonists



- ❑ Ruthenium Red only known TRPV4 blocker at the time (unselective pore blocker)

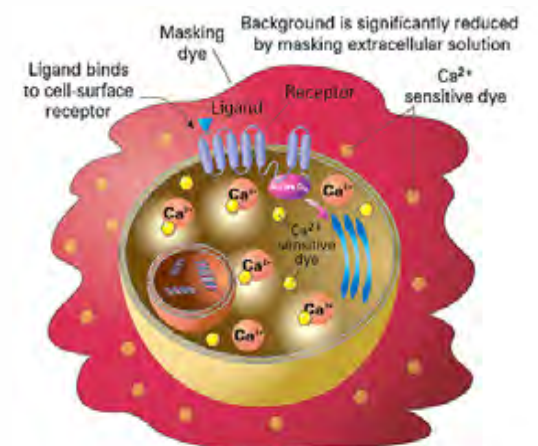
→ Require rapid delivery of selective TRPV4 small molecule tool to increase confidence in the therapeutic potential and safety of the mechanism

- ❑ High throughput screening (HTS) initiated on the Pfizer compound file

- ❑ TRPV4 calcium flux assay (FLIPR Tetra technology) utilised to meet through put requirements (~ 3 million compounds)

- ❑ Compressed file screening generated a large number of hits (~2.5% of compounds had >40% inhibition of TRPV4 at 10 $\mu$ M)

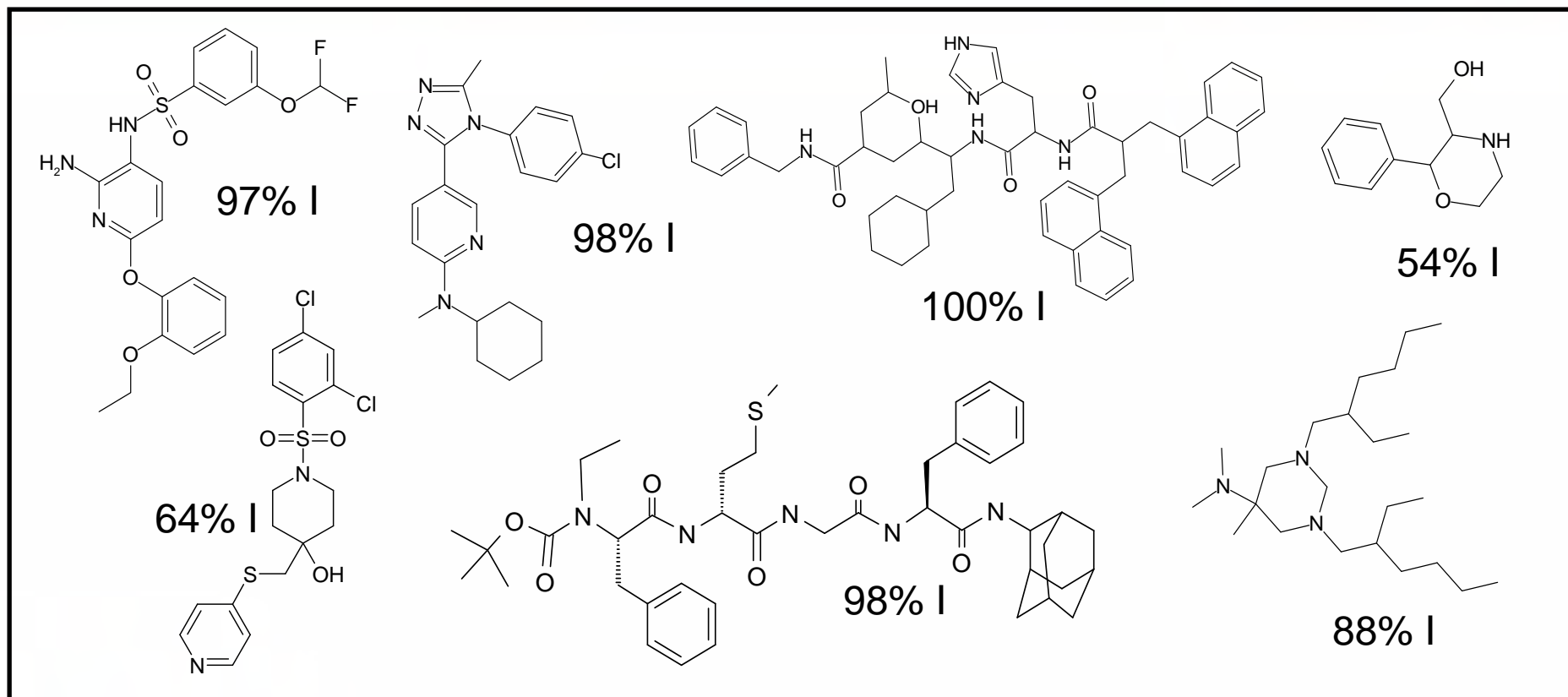
- ❑ Single-well, single-point follow-up confirmed ~11K compounds to be active (>40% inhibition at 10 $\mu$ M) → 14% confirmation rate



# TRPV4 HTS: The search for selective TRPV4 antagonists



- Diverse array of compounds look active in the assay



- Lots of actives! Good news? Which to take on to full dose response? All of them???

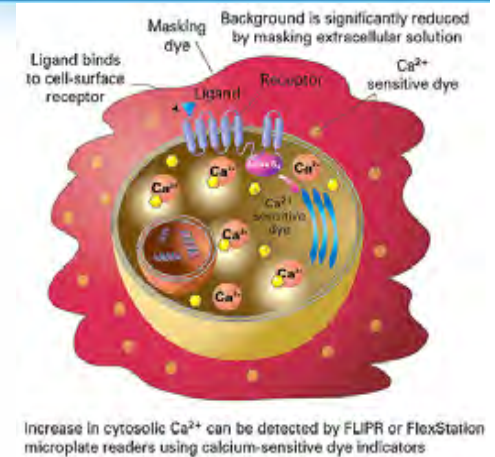


# TRPV4 HTS: The search for selective TRPV4 antagonists



❑ Calcium flux assays can deliver false positives or ‘frequent hitters’ due to a compound being reactive, autofluorescent, cytotoxic or active at another target in the signal transduction pathway

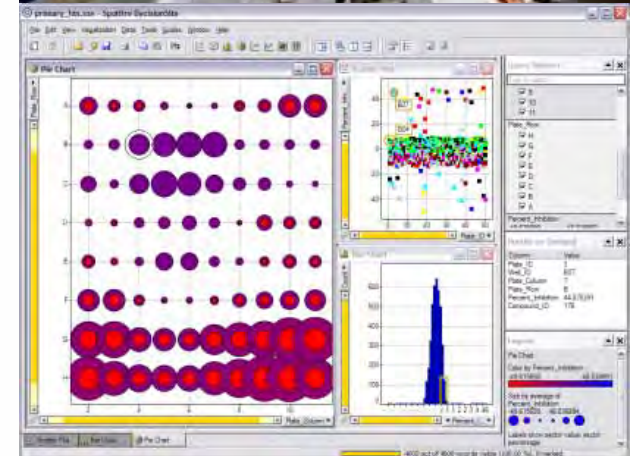
❑ Need a quick and efficient way to triage out the frequent hitters  
→ allow efforts to be focussed on true TRPV4 antagonists



❑ Could go through every compound by eye and guess



❑ .....or use a more sophisticated approach?



# TRPV4 HTS: Removing the “frequent hitters”



## □ Approach 1 : *In vitro* data

All Pfizer pharmacology data is stored in a searchable database

If a compound from 11K set was active (>75% inhibition at 10 $\mu$ M) in >1 (non-TRP) FLIPR assay it was deemed a “frequent hitter” and removed

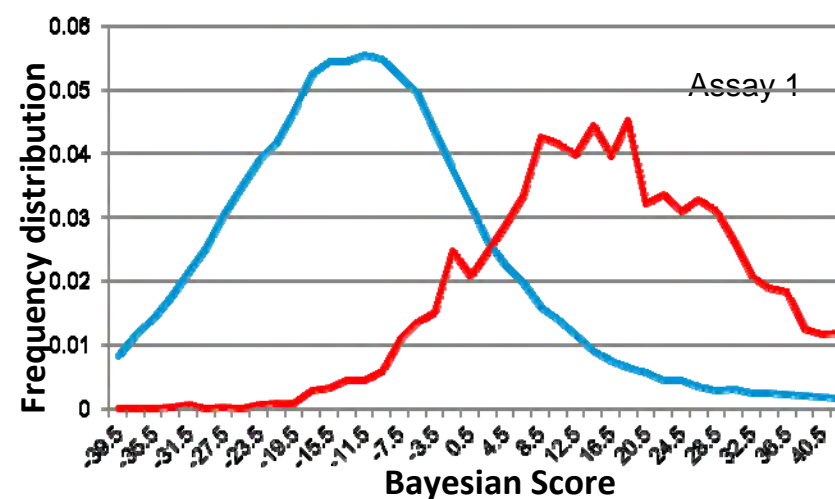
## □ Approach 2 : *In silico* data

Built Bayesian activity models for each (non-TRP) FLIPR assay. Compounds scoring highly in >1 model were deemed likely “frequent hitters” and removed

Bayesian model predicts likelihood of activity, not activity itself

Model identifies chemical fingerprints more prevalent in **actives** or in **inactives**

Model adds up all contributions to give a Bayesian score to predict “**active** or **inactive**”



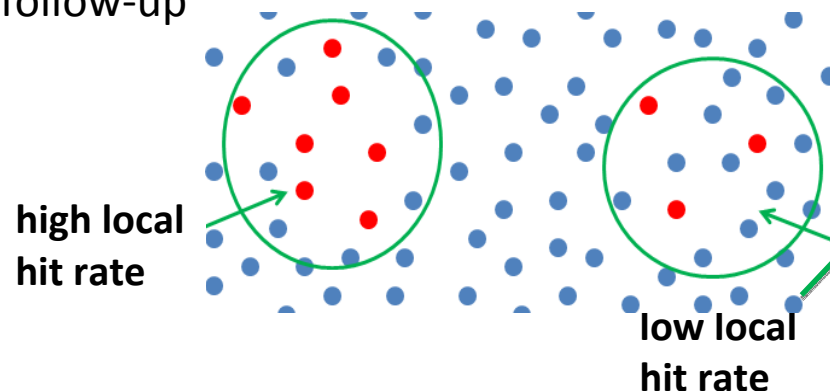
Advantage of *in silico* approach is a compound doesn't need FLIPR data in a particular assay → evidence of activity of its near neighbours is sufficient to suggest it could be active

# TRPV4 HTS: Prioritising compounds for IC50



- ❑ Removal of “frequent hitters” and “uglies” ( $\text{clogP} > 6$ ,  $\text{MW} > 600$ , reactivities etc.) → 6K compds
- ❑ Require rapid delivery of TRPV4 small molecule tool → positive filters were applied to prioritise “best” compounds in 6K set for TRPV4 IC50 follow-up
- ❖  $> 75\%$  TRPV4 inhibition at  $10\mu\text{M}$

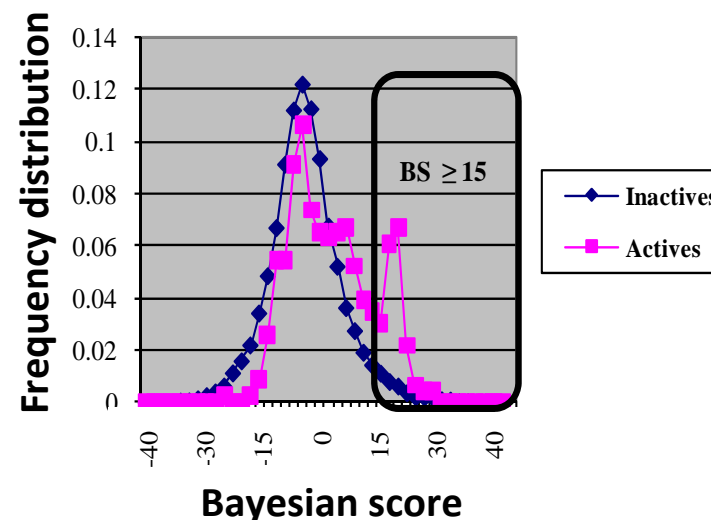
❖ High local hit rate  
(proportion of near neighbours that are active)



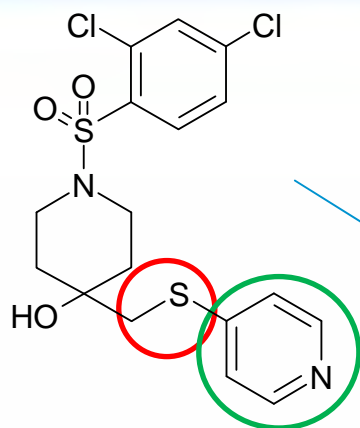
❖ High score in TRPV4 Bayesian model built on TRPV4 data

❑ ~1K compounds selected for IC50 due to their likelihood of being active TRPV4 antagonists

❑ 1 follow-up round of IC50 screening → 5 X TRPV4 series!

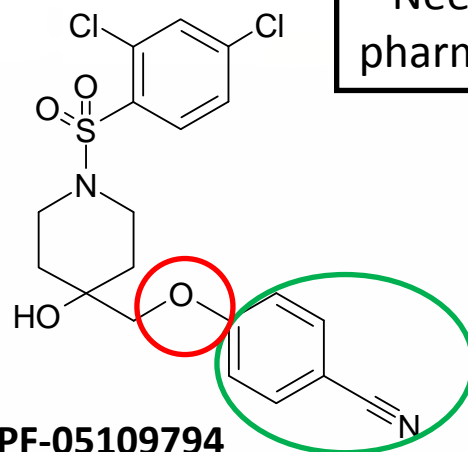


# Hit to Lead: Optimising the piperidine series



**PF-0164813** (HTS hit)

hTRPV4 IC<sub>50</sub> 2,200 nM  
LIPE/LE 3.3/0.3



**PF-05109794**

hTRPV4 IC<sub>50</sub> 49nM  
LIPE/LE 4.03/0.37  
rTRPV4 IC<sub>50</sub> 1,150nM  
HLM/RLM 43/465  
logD >3.7

- ❖ Improved potency and LIPE/LE
- ❖ Removed structural alert
- ❖ Removed metabolically labile S-linker
- ❖ Confirmed rat TRPV4 pharmacology

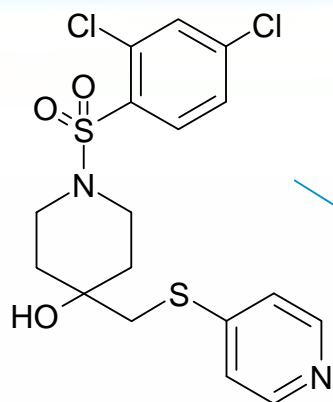
- Focus on ↑TRPV4 potency & ↑efficiency (LIPE, LE)
- Need appropriate ADME profile and rat pharmacology

LIPE = pKi – cLogP (or logD)

LE = 1.3643 \* pKi / number of heavy atoms

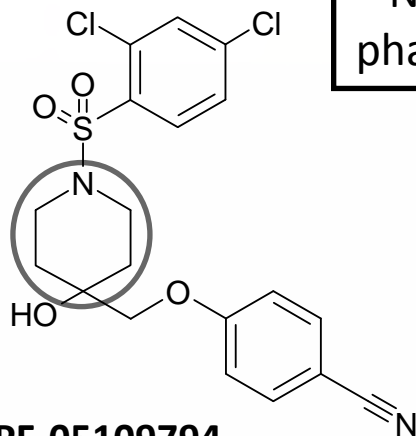
Med. Chem. Commun., 2013, 4, 244-251  
Med. Chem. Commun., 2012, 3, 174-178

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HLM/RLM 43/465  
logD >3.7

- Focus on ↑TRPV4 potency & ↑efficiency (LIPE, LE)
- Need appropriate ADME profile and rat pharmacology

- Matched Molecular Pair (MMP) analysis of Pfizer pharmacology data used to guide linker optimisation
- Azetidine replacement → ~70% chance of retaining or improving potency (& ↓logD)

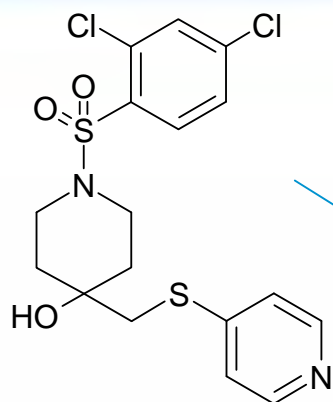
LIPE = pK<sub>i</sub> – cLogP (or logD)

LE = 1.3643 \* pK<sub>i</sub> / number of heavy atoms

Med. Chem. Commun., 2013, 4, 244-251

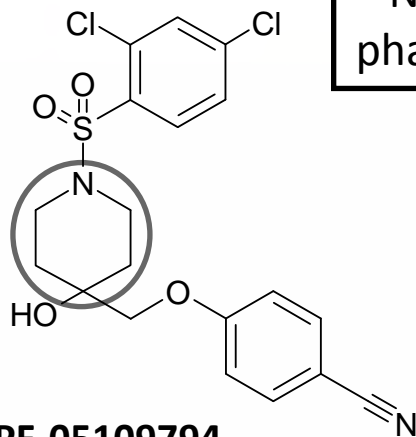
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# Hit to Lead: Optimising the piperidine series



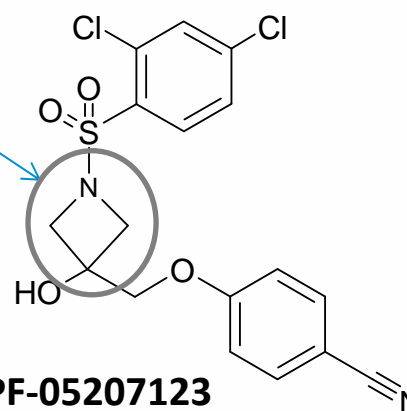
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LIPE/LE 3.3/0.3



**PF-05109794**

hTRPV4 IC<sub>50</sub> 49nM  
LIPE/LE 4.03/0.37  
rTRPV4 IC<sub>50</sub> 1,150nM  
HLM/RLM 43/465  
logD >3.7



**PF-05207123**

hTRPV4 IC<sub>50</sub> 21nM  
LIPE/LE 5.25/0.45  
rTRPV IC<sub>50</sub> 106nM  
HLM/RLM 12/113  
logD 2.9

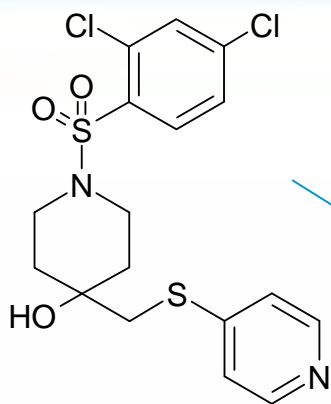
- Focus on ↑TRPV4 potency & ↑efficiency (LIPE, LE)
- Need appropriate ADME profile and rat pharmacology

LIPE = pKi – cLogP (or logD)  
LE = 1.3643 \* pKi / number of heavy atoms

Med. Chem. Commun., 2013, 4, 244-251  
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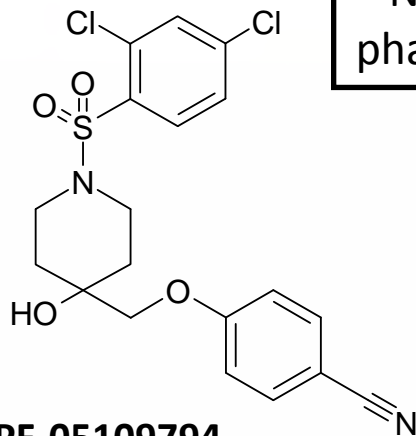


# Hit to Lead: Optimising the piperidine series



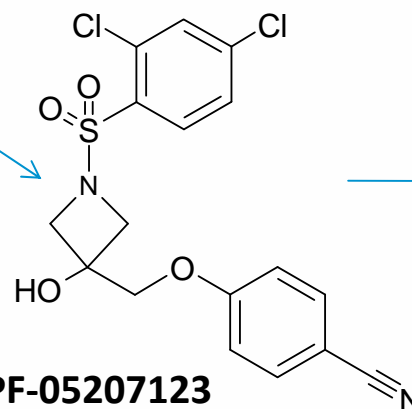
**PF-0164813** (HTS hit)

hTRPV4 IC<sub>50</sub> 2,200 nM  
LIPE/LE 3.3/0.3



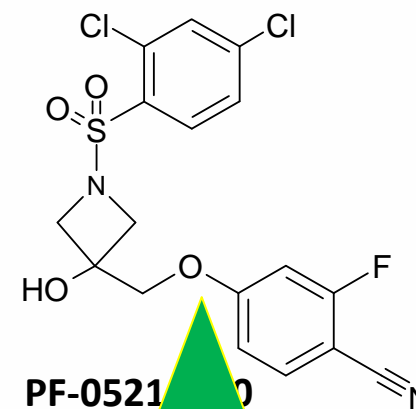
**PF-05109794**

hTRPV4 IC<sub>50</sub> 49nM  
LIPE/LE 4.03/0.37  
rTRPV4 IC<sub>50</sub> 1,150nM  
HLM/RLM 43/465  
logD >3.7



**PF-05207123**

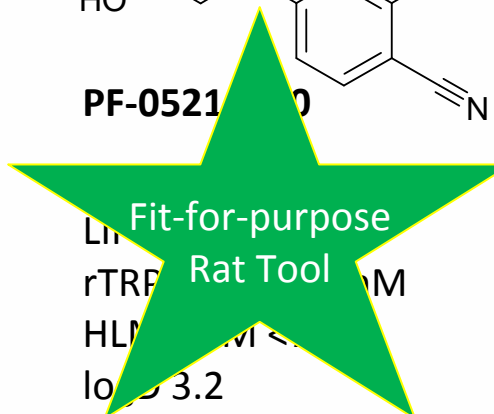
hTRPV4 IC<sub>50</sub> 21nM  
LIPE/LE 5.25/0.45  
rTRPV IC<sub>50</sub> 106nM  
HLM/RLM 12/113  
logD 2.9



**PF-05211100**

hTRPV4 IC<sub>50</sub> 10nM  
rTRPV IC<sub>50</sub> 106nM  
HLM/RLM 12/113  
logD 3.2

- Focus on ↑TRPV4 potency & ↑efficiency (LIPE, LE)
- Need appropriate ADME profile and rat pharmacology



LIPE = pK<sub>i</sub> - cLogP (or logD)  
LE = 1.3643 \* pK<sub>i</sub> / number of heavy atoms

# PF-05214030: Additional characterisation



## ☐ PF-05214030

h/r TRPV4 IC50 4/27nM (FLIPR assay)

TRP selectivity >100 fold

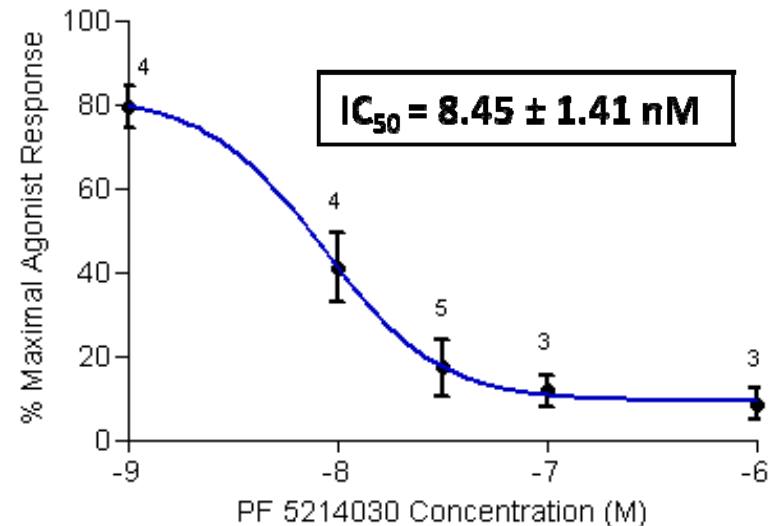
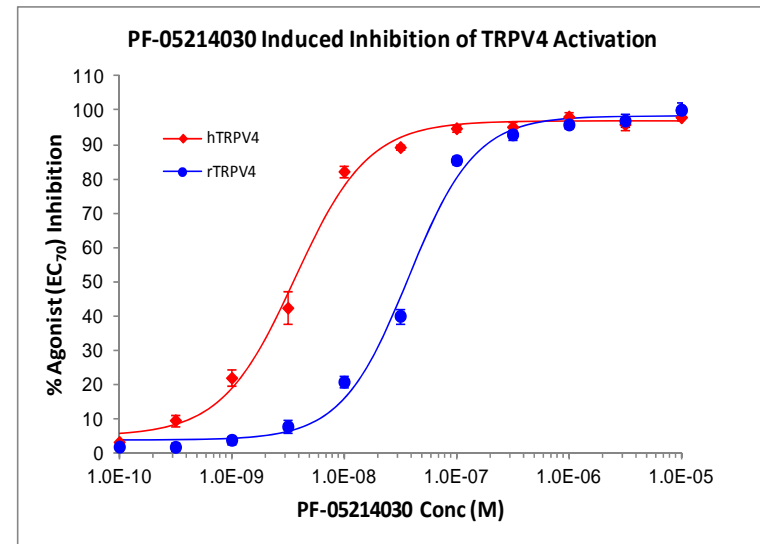
Wide-ligand profile >100 fold

Dofetilide/Herg 6.6µM/4.3µM

Rat PK  
T<sub>1/2</sub> 2.2hrs  
Cl 23 (1/3 LBF)  
F 73%

☐ Antagonist potency confirmed in patch clamp (IC50 = 8.5nM) & hypotonicity assays (IC50 = 6.7nM)

☐ Antagonist potency confirmed in tissue:  
rBladder K<sub>b</sub> 40 nM  
mBladder K<sub>b</sub> 16 nM

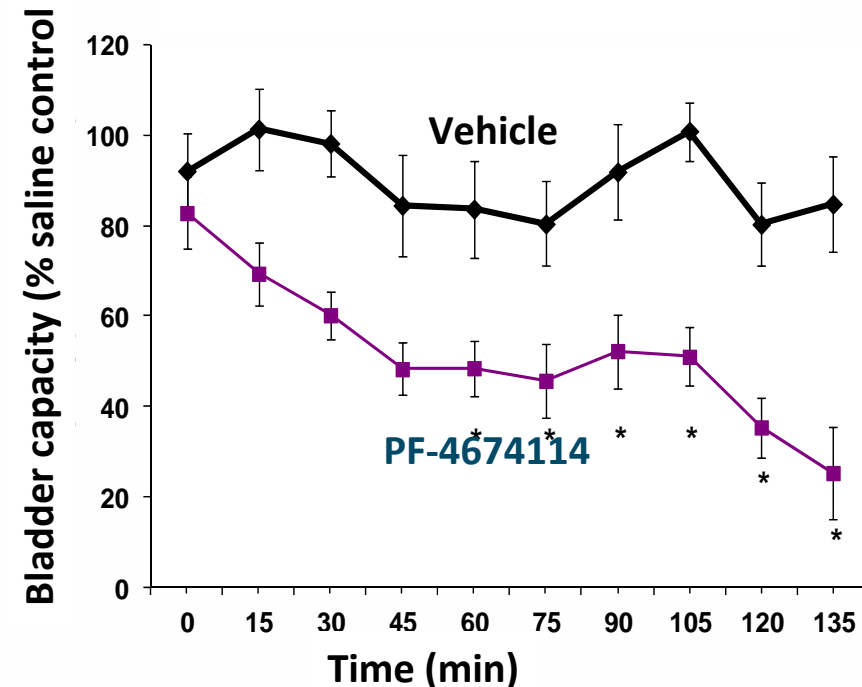
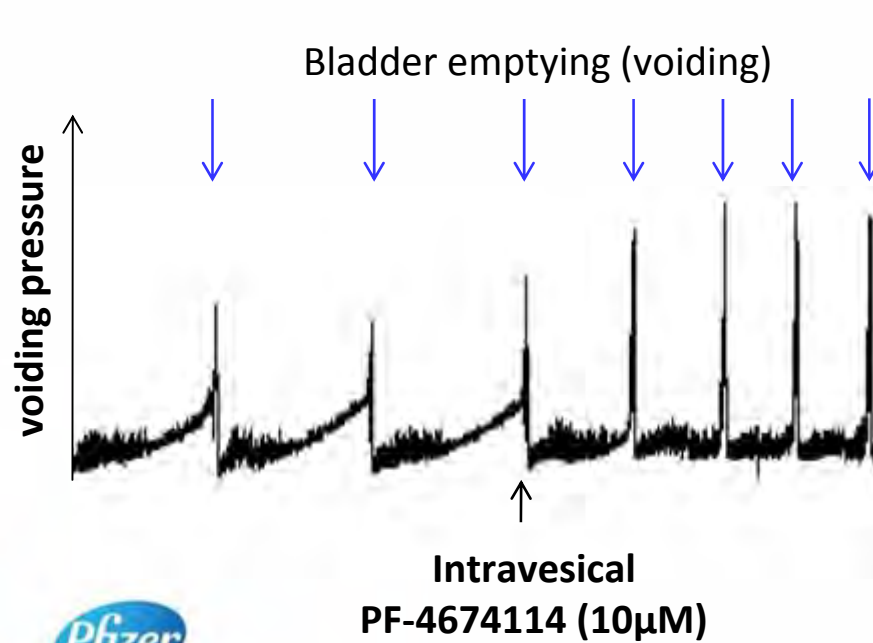


# PF-05214030: Proof of *in vivo* Pharmacology



- TRPV4 agonist given intravesically caused increased frequency of bladder emptying, increased voiding pressure and reduced bladder capacity in anaesthetised rats during bladder cystometry

## Effect of PF-4674114 on bladder capacity and voiding behaviour during continuous cystometry

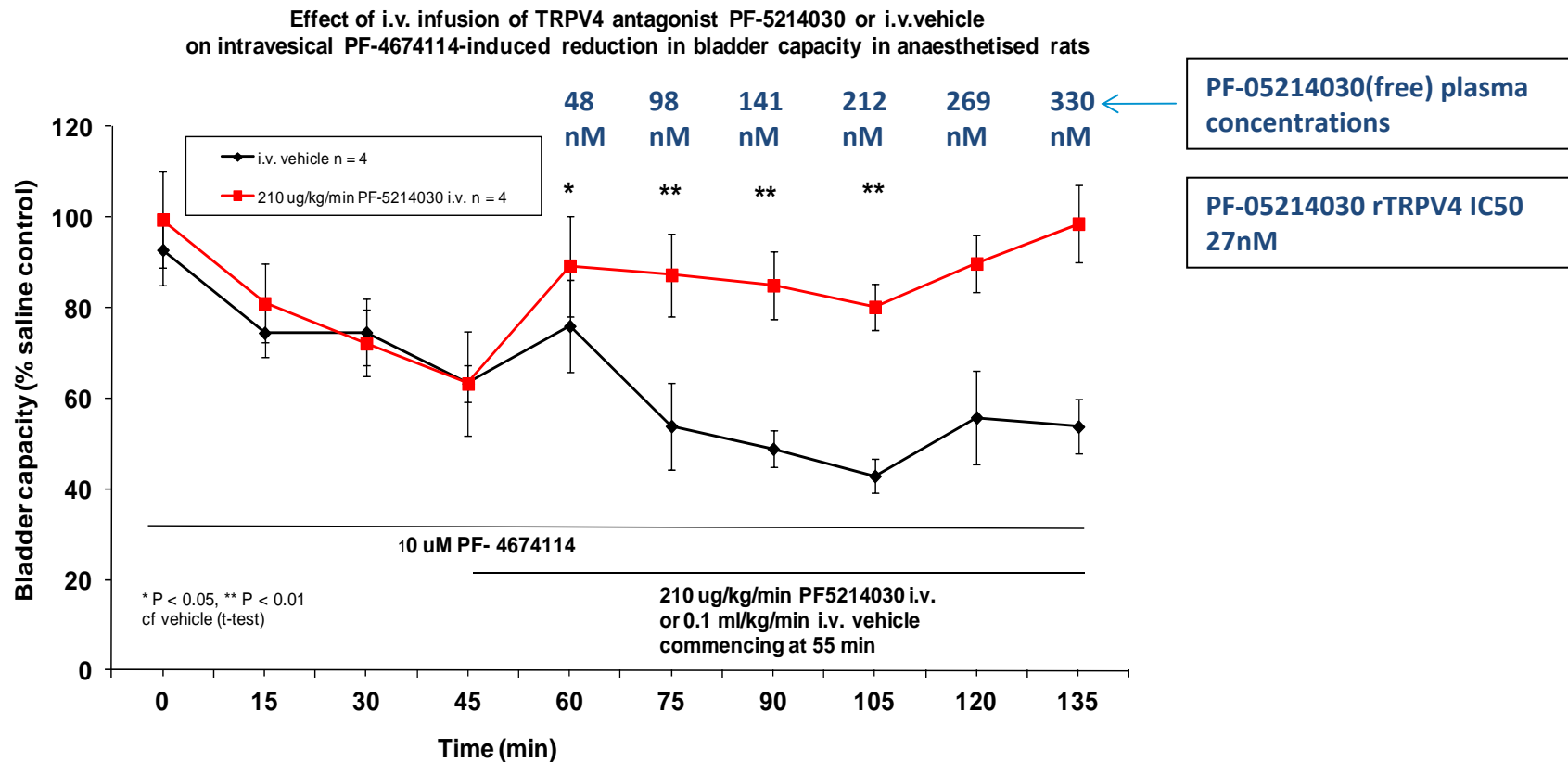


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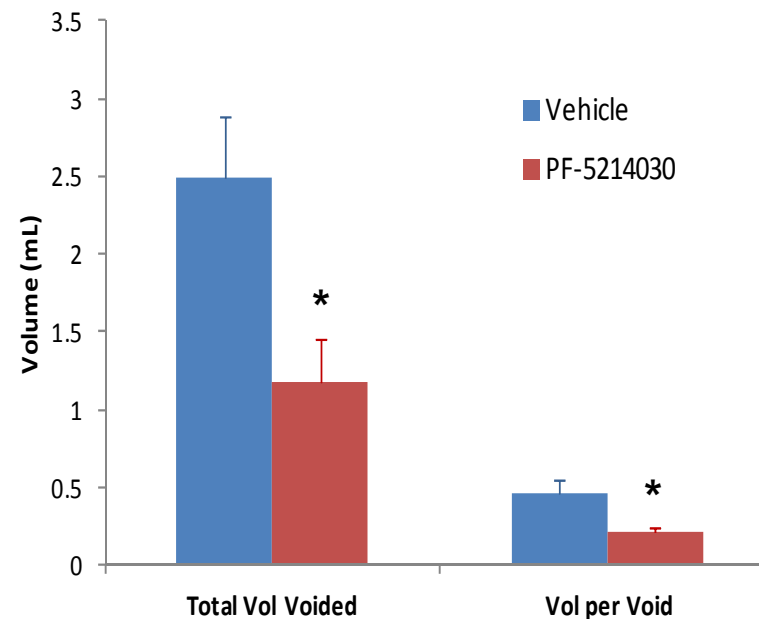
☐ i.v. infusion of PF-5214030 reversed the reduction in bladder capacity caused by intravesical infusion of a TRPV4 agonist (GSK1016790A/ PF-4674114) → **proof of *in vivo* pharmacology**



# PF-05214030: Efficacy studies



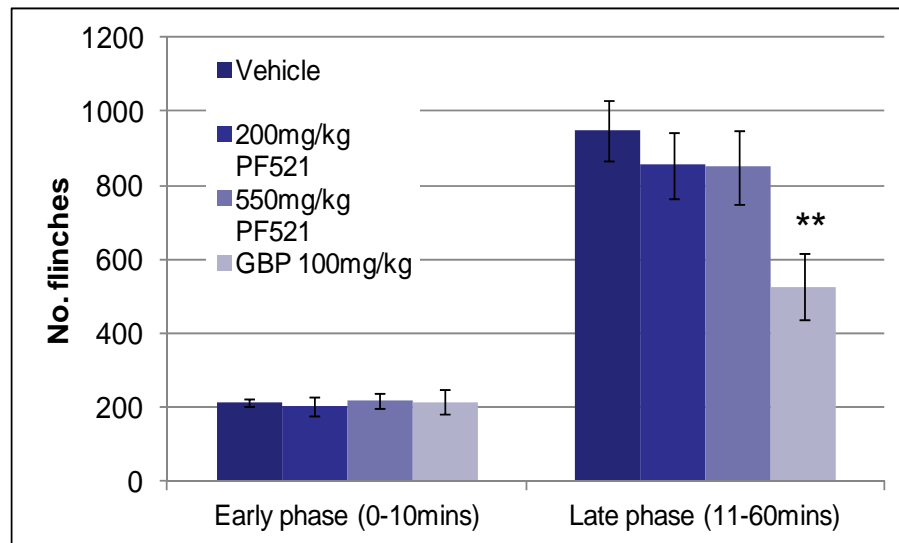
- ❑ i.v. infusion of PF-5214030 (~8 x IC50) had **no effect** on bladder capacity when filling bladder with saline (anaesthetised rat cystometry model)
- ❑ **No effect** in rat bladder distension model
- ❑ Subcutaneous PF-5214030 (~8 x IC50) caused significant reduction in total volume voided and volume per void in conscious rats (metabolic cages)
  - suggests a direct effect of the antagonist on kidney function
  - measurement of urine constituents indicated urinary concentration effects



# PF-05214030: Efficacy studies

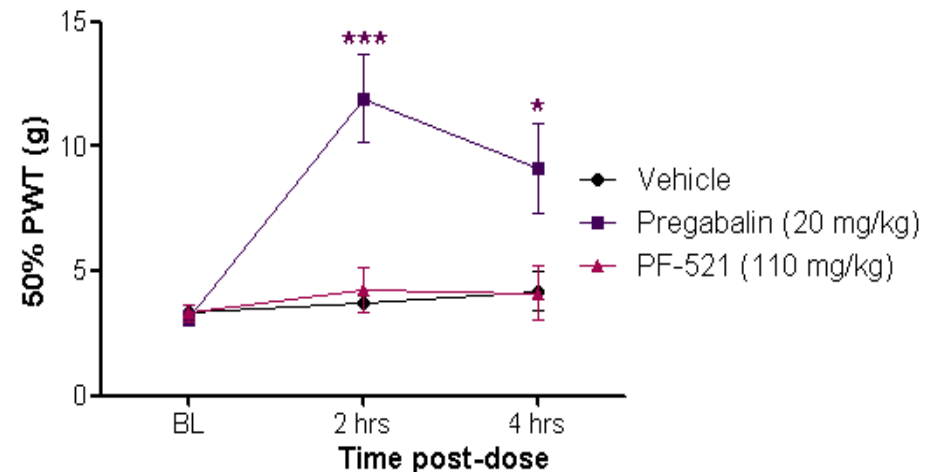


□ PF-5213040 caused **no effect** (at 8-10 x IC50) on pain behaviours in either formalin induced flinching or CFA induced hypersensitivity in rats



**Effect of PF-5214030 and Gabapentin on formalin induced flinching in rats**

**Stats: ANOVA between treatment vs. vehicle (\*\*p<0.01)**



**Effect of PF-5214030 and Pregabalin on static allodynia (48hrs post CFA injection) in rats**

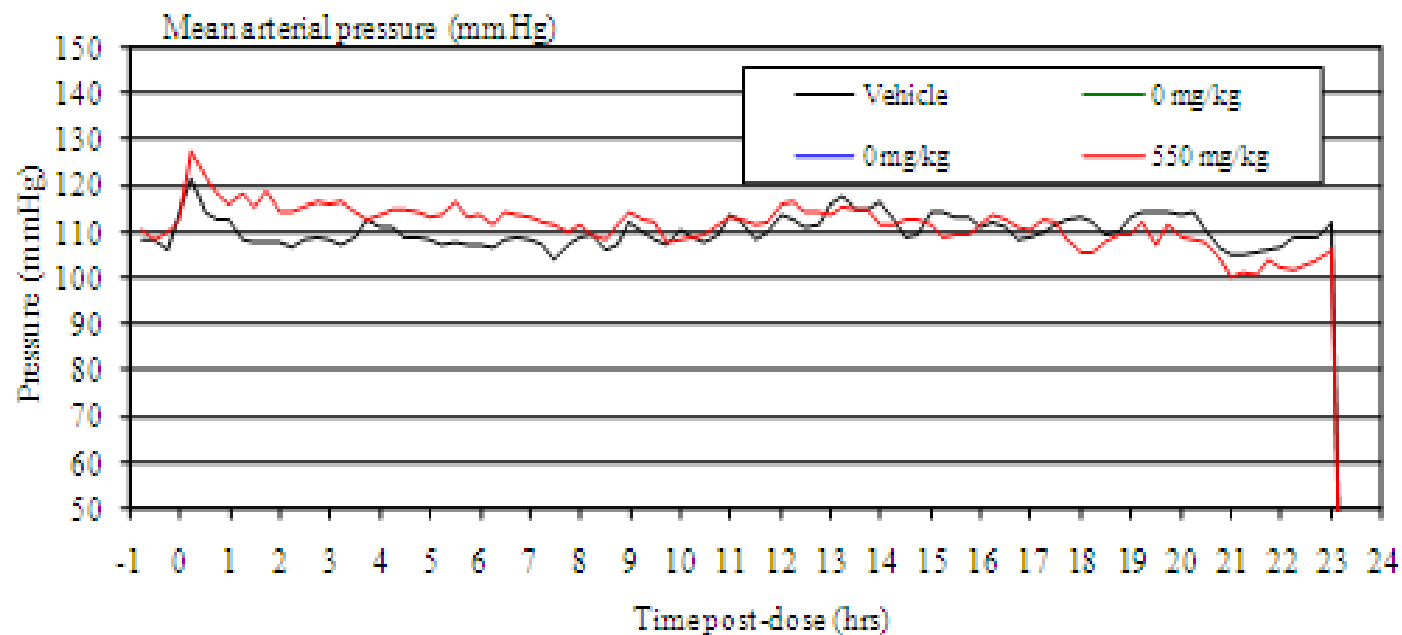
**Stats: ANOVA vs. vehicle at each time point in ipsilateral hindpaw (blocked by day)  
\* P < 0.05, \*\*\* P < 0.001**

**All data are expressed as mean ± SEM (n=8)**

## PF-05214030: CV studies



- ❑ Potential CV liabilities of TRPV4 antagonism investigated with PF-5214030 in conscious, freely moving telemetered rats (cross-over study)
- ❑ PF-5214030 caused a small but significant increase in blood pressure (~10X IC50) but no effect on heart rate, body temperature or activity (PD effect correlated with PK)



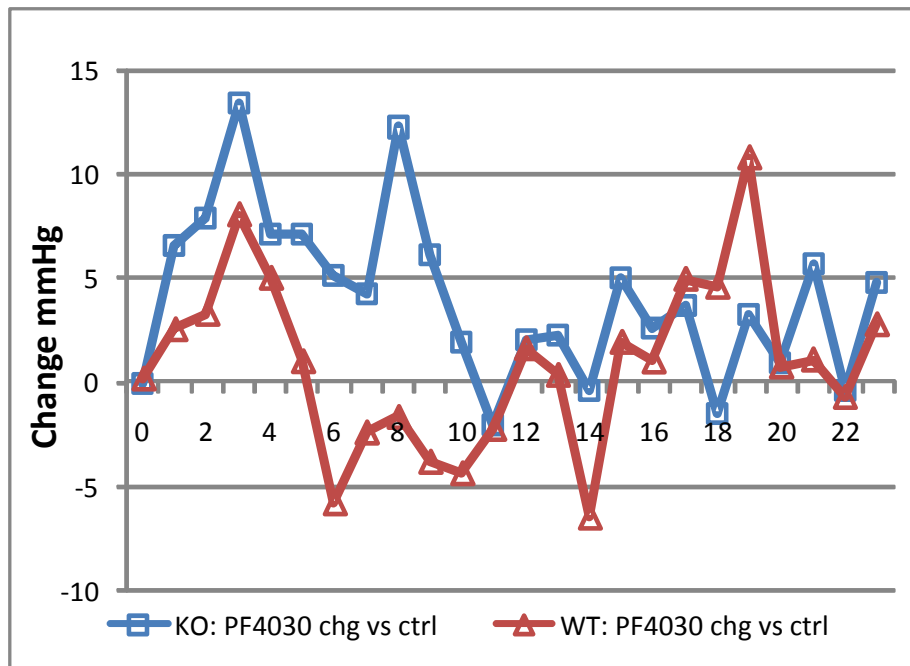
- ❑ Compound or mechanism effect?

# PF-05214030: CV studies

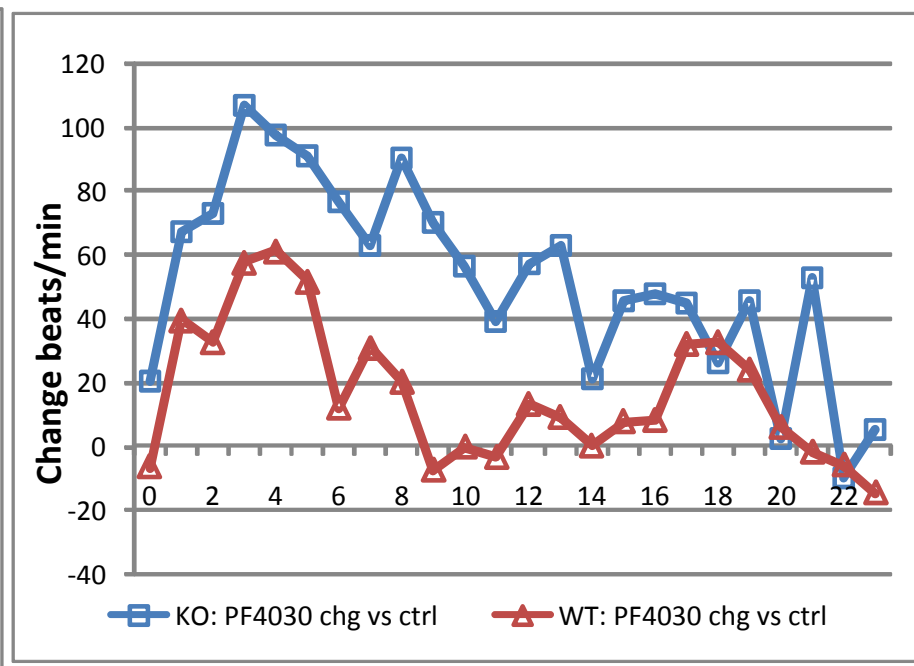


- ❑ Role of TRPV4 in the CV effect of PF-5214030 further assessed in telemetered wild type and TRPV4 KO mice
- ❑ Both KO and wild type mice showed increased blood pressure and tachycardia in response to PF-5214030 – potential off-target effect of PF-5214030 or metabolite of PF-5214030?

Day 1 change from vehicle control in systolic pressure with PF'4030: KO & WT mice



Day 1 change from vehicle control in heart rate with PF'4030: KO & WT mice

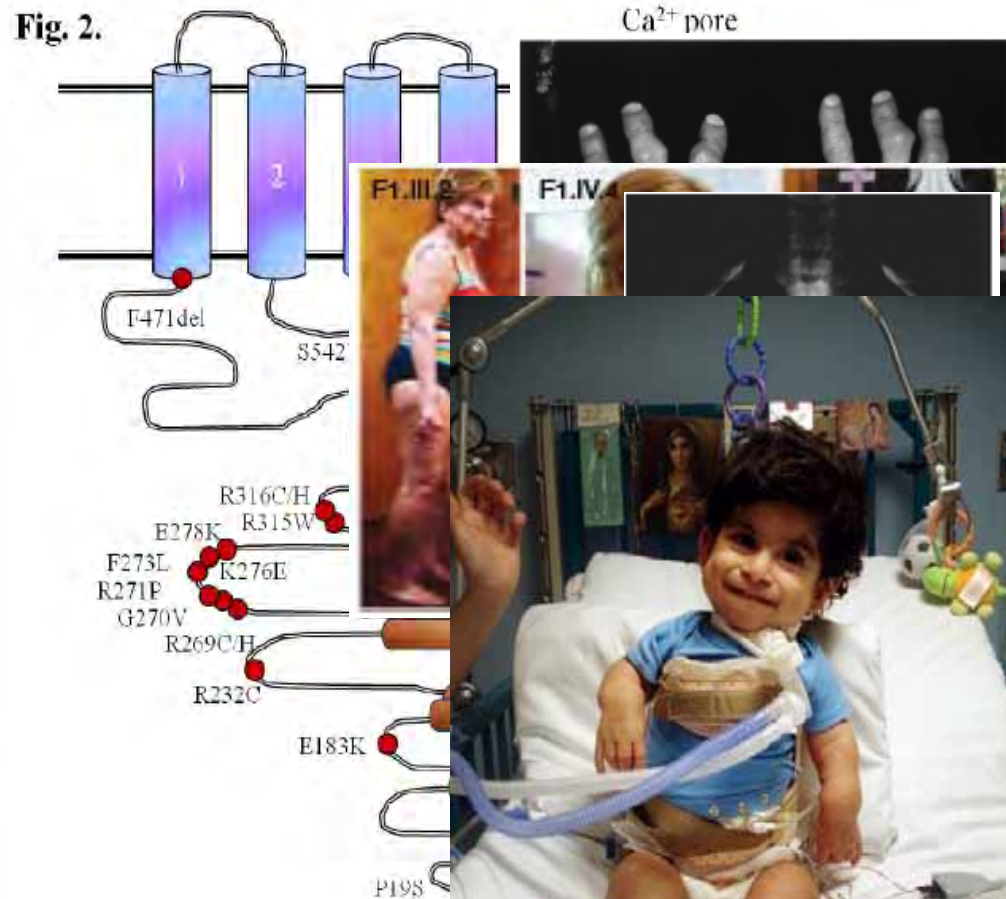




# More human TRPV4 mutant data: Time to walk away from TRPV4



❑ More evidence of TRPV4 mutations causing severe abnormalities in humans.....



Exp Mol Med. 2012, 44(12), 707-722

## **Fetal Akinesia**

Bone and nervous system impairment  
Complete absence of movement

## **Familial digital arthropathy-brachydactyly**

Aggressive osteoarthropathy of the fingers and toes

## **Charcot-Marie-Tooths Disease type 2C**

Nervous system disorder leading to progressive weakness and atrophy

## **Brachyolmia**

Characterized by a short trunk, scoliosis, and mild short stature

## **Metatropic Dysplasia**

Characterized by short extremities, a short trunk with progressive kyphoscoliosis, and craniofacial abnormalities

## **Scapulo-peroneal spinal muscular atrophy**

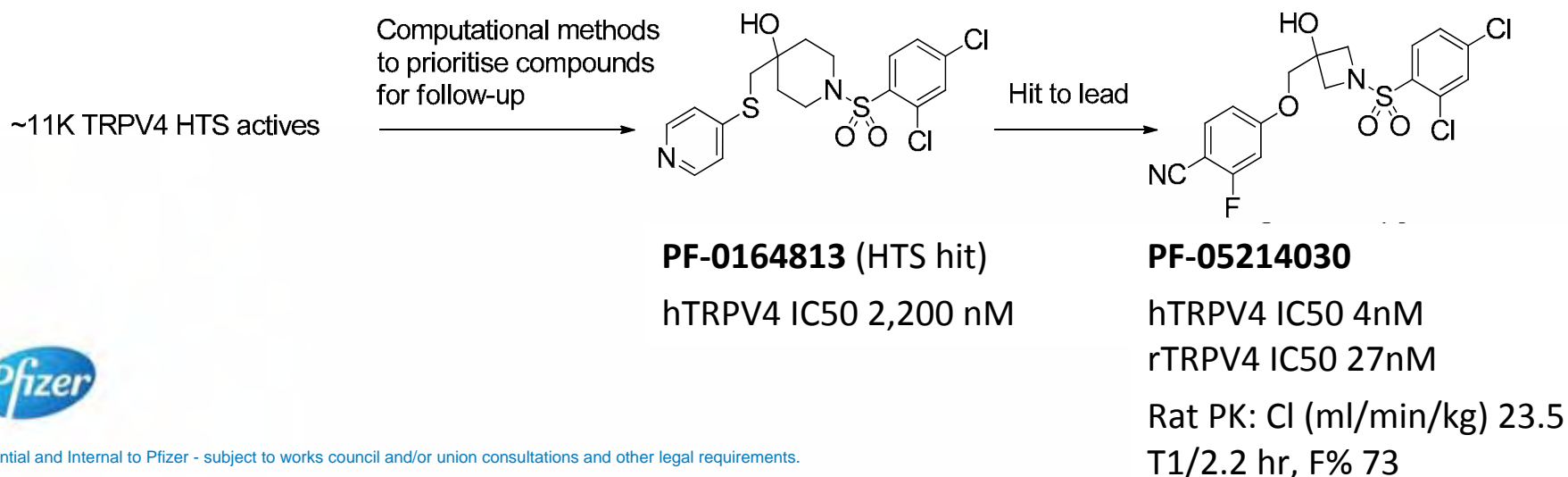
weakness of the scapularis muscle and bone abnormalities

❑ Failure of PF-05213040 in efficacy studies plus additional hTRPV4 mutation data → stop TRPV4 projects for non-life threatening indications requiring peripheral compound exposure

# Conclusions: Part 1



- ❑ TRPV4 project required rapid delivery of a TRPV4 antagonist to establish confidence in biological rationale
- ❑ HTS delivered numerous chemical “actives”. The application of computational techniques (including Bayesian modelling) in the triage of TRPV4 HTS data enabled rapid deprioritisation of “frequent hitters” and the prioritisation of likely actives
- ❑ Two rounds of IC50 screening → 5 chemical series → focus on hydroxypiperidines
- ❑ Pairwise analysis in Hit-to-Lead work delivered hydroxyazetidines series
- ❑ Full utilisation of available data facilitated rapid delivery of potent chemical tool → HTS screen to delivery of rat tool (PF-05214030) in < 7 months



## Conclusions: Part 2



- ❑ PF-05214030 antagonist potency confirmed in patch clamp, hypotonicity assays and tissue experiments
- ❑ *In vivo* proof of pharmacology (reverses the effects of TRPV4 agonist in bladder)
- ❑ **BUT** no effect in any pain or genitourinary efficacy models
- ❑ **AND** altered rat micturition profile likely driven from effect on kidney
- ❑ PF-5214030 caused a small but significant increase in blood pressure → KO data suggests an off-target cf. on target effect
- ❑ Failure of PF-05213040 in efficacy studies plus additional hTRPV4 mutation data → STOP!
- ❑ **BUT** we did get to the STOP decision quickly → HTS to STOP in ~9 months. Rapid NO GO decisions as important as GO decisions in Drug Discovery

**Thank you for your kind attention**