Small Molecule TLR7 Agonists for the Treatment of HCV Infection

David Pryde

Pfizer Labs, Sandwich, UK
Pfizer Neusentis, Granta Park, Cambridge, UK

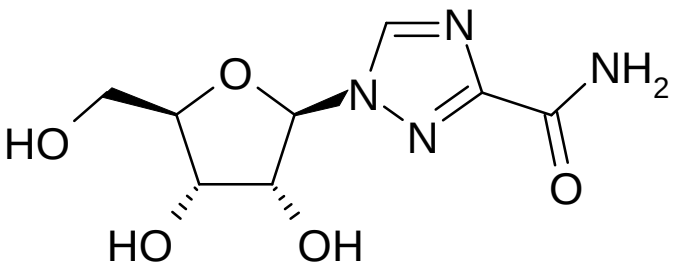
David.Pryde@pfizer.com

Outline

- **HCV**
  - Current standard of care
  - Where might a TLR7 agonist fit in and why?
- **TLR7 agonists**
  - Mechanistic details of immune stimulation
  - Chemistry starting points
  - Challenges
- **Identification of a development candidate**
  - Potency
  - Solubility
  - Solving an aldehyde oxidase liability
- **Clinical data with PF-4878691**
  - A question of therapeutic index

Hepatitis C virus

- **HCV is a *flaviviridae* +ve stranded RNA virus**
  - >190 million individuals infected worldwide
  - Virus replicates primarily in the liver
  - 85% of patients develop a chronic infection
  - 20% of these go on to develop cirrhosis in 10-30 years
- **Current standard of care is PEG-IFNα2a + ribavirin**
  - 48% SVR in genotype 1 patients with12 month therapy
  - Flu-like symptoms, depression, anemia, high discontinuation rate



Ribavirin

http://www.pegasys.com/about-pegasys/default.aspx

Developing new treatments for HCV

- **Direct acting antivirals**
  - HCV Protease (NS3), HCV RNA polymerase (NS5B)
  - Other non-structural proteins (NS5A, NS4B, NS4A)
  - Structural proteins (Core, p7 channel)
  - Combination of IFNα and selective inhibitor limited by IFNα side-effects
- **Indirect antivirals**
  - Using the innate immune response to boost production of endogenous IFN's, mediated by Toll-like receptors (TLR's)
  - Induction of the whole suite of IFN's, not just one

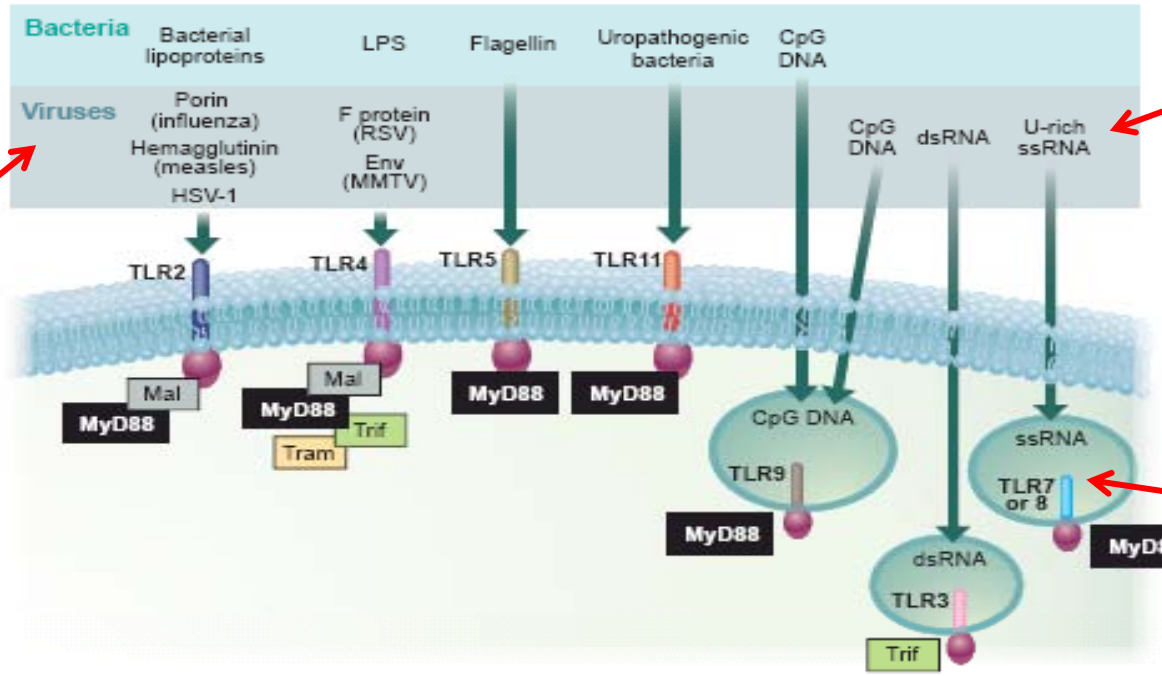
Seeking an oral TLR7 agonist to be used in IFN-sparing regimens with improved efficacy and reduced side effects relative to IFNα2a

Ex.Op.Drug Disc., **2009**, 4(3), 293-314

Toll-like receptors and TLR7

- **Large family of type 1 trans-membrane proteins**
  - Expressed on immune cells, especially dendritic cells
  - Recognise pathogenic components and *via* signalling cascade, induce inflammatory response
  - Induces cytokines, including interferons

TLRs recognise a range of pathogens



TLR7 recognises viral nucleic acid

TLR7 is located in endosomal vesicles

Science, **2004**, 303, 1481

Small molecule TLR7 agonists

- **Precedented agents**

Imiquimod (3M)

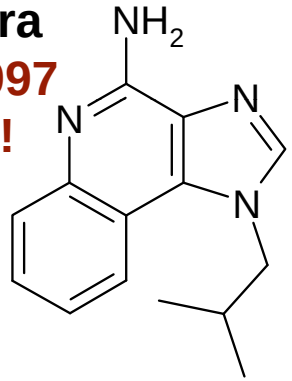
Topically administered as a 5% cream, Aldara

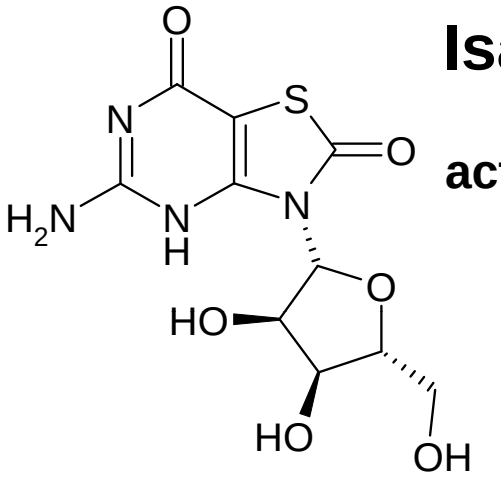
Launched for HPV 1997

MOA found in 2002!

EC_{50} 1μM

Low solubility





Isatoribine (Anadys)

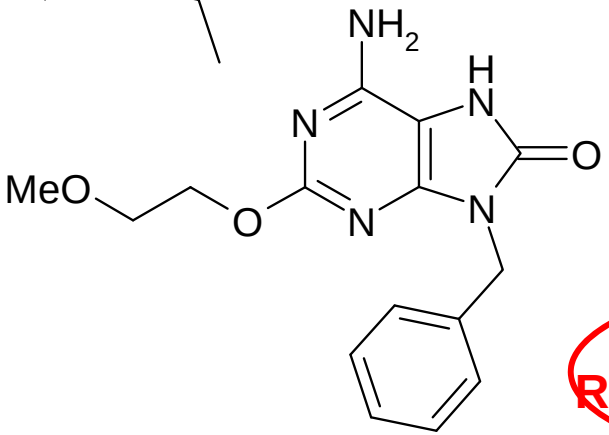
Anti- HCV activity with IV formulation

Oral prodrug pursued

EC_{50} 50μM

Low solubility

Poor absorption



Sumitomo (AZ)

Pre-clinical IFN response data

Highy potent IFN stimulator

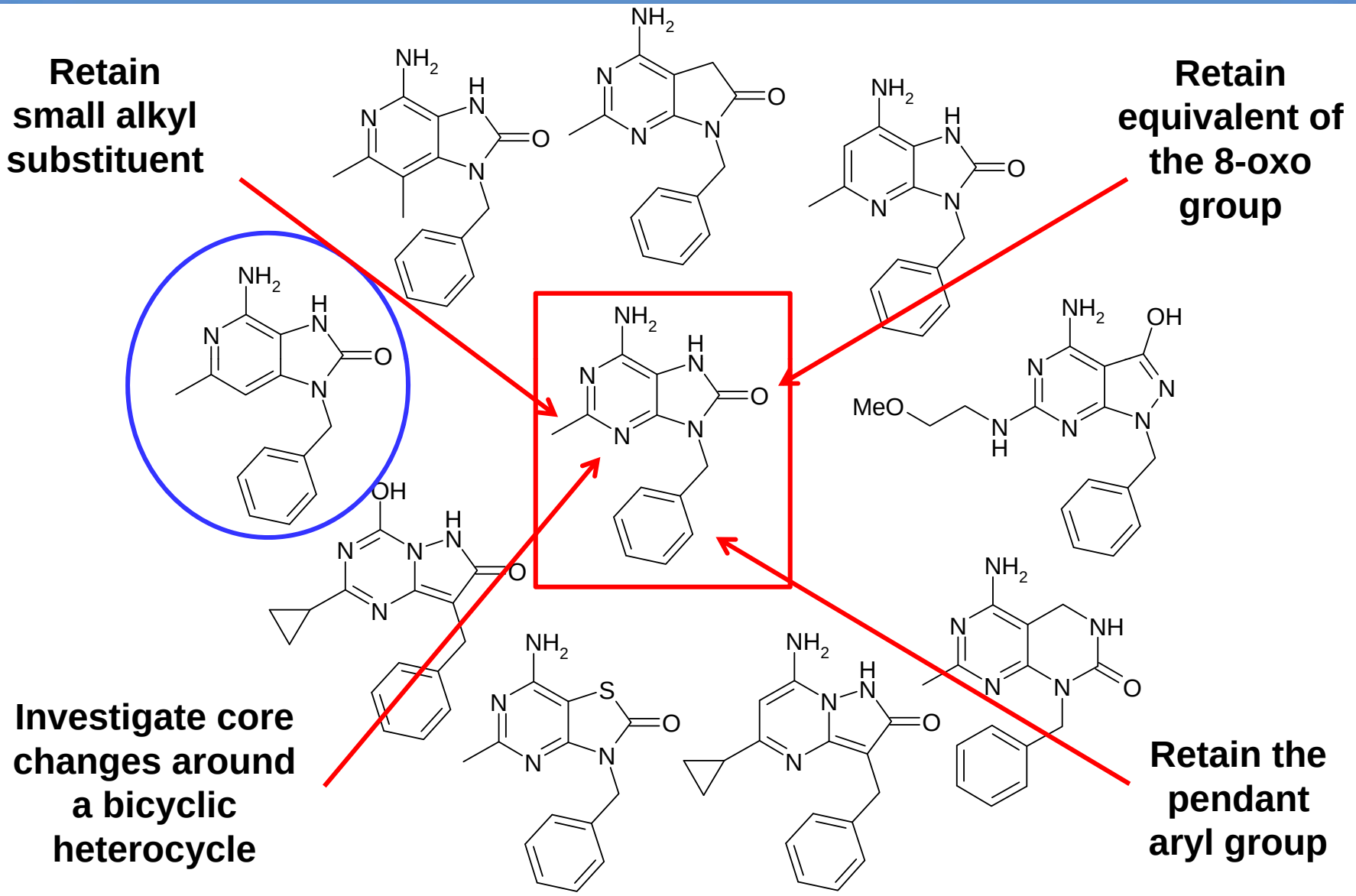
Inhaled indications

EC_{50} 20nM

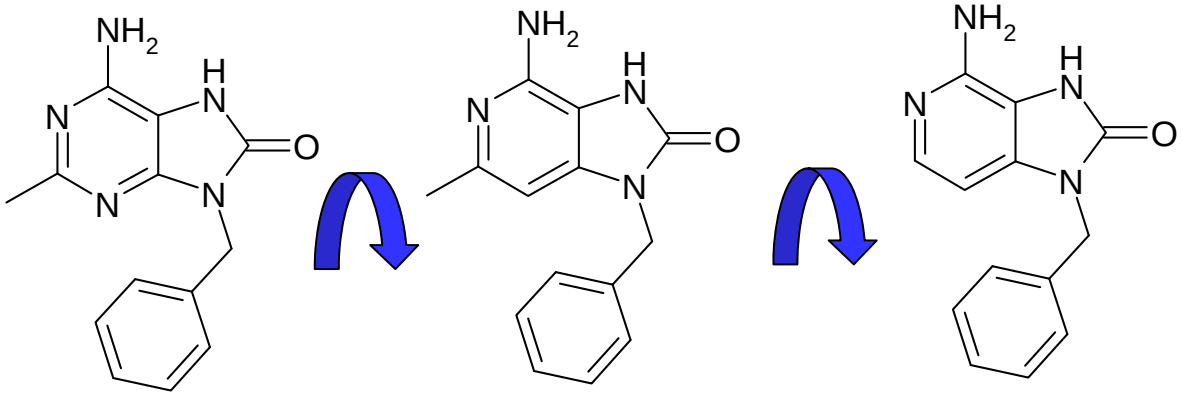
Reasonable solubility + absorption

J Med Chem. **2002**, 45(25), 5419

Purine analogues



3-Deazapurines



EC_{50} 829nM
MWt. 255
LogD 2.3
HLM <7μL/min/mg

EC_{50} 1540nM
MWt. 254
LogD 2.3
HLM <7μL/min/mg

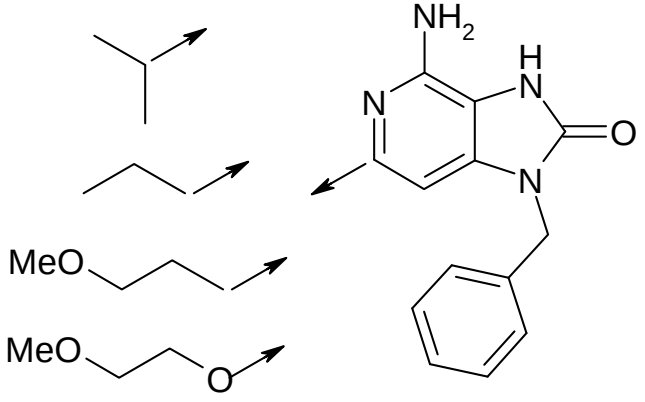
EC_{50} >4000nM
MWt. 240
LogD 1.9
HLM <7μL/min/mg

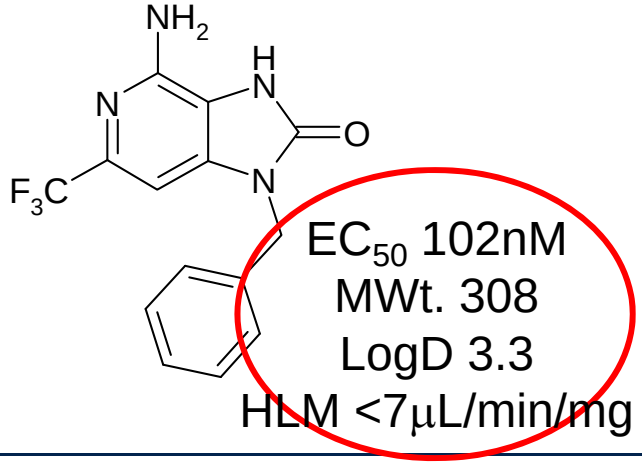
EC_{50} 304nM

EC_{50} 480nM

EC_{50} >4000nM

EC_{50} 870nM

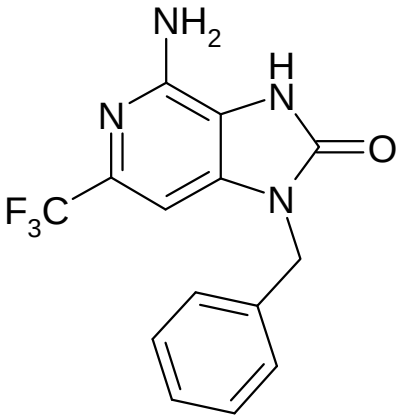




EC_{50} 102nM
MWt. 308
LogD 3.3
HLM <7μL/min/mg

A solubility issue

- **The first set of compact deazapurines were very low solubility**
  - Initial potency was good
  - Pharmacokinetics were encouraging



Rat PK
Cl 33mL/min/kg
V_{dss} 2.7L/kg
$T_{1/2}$ 1h

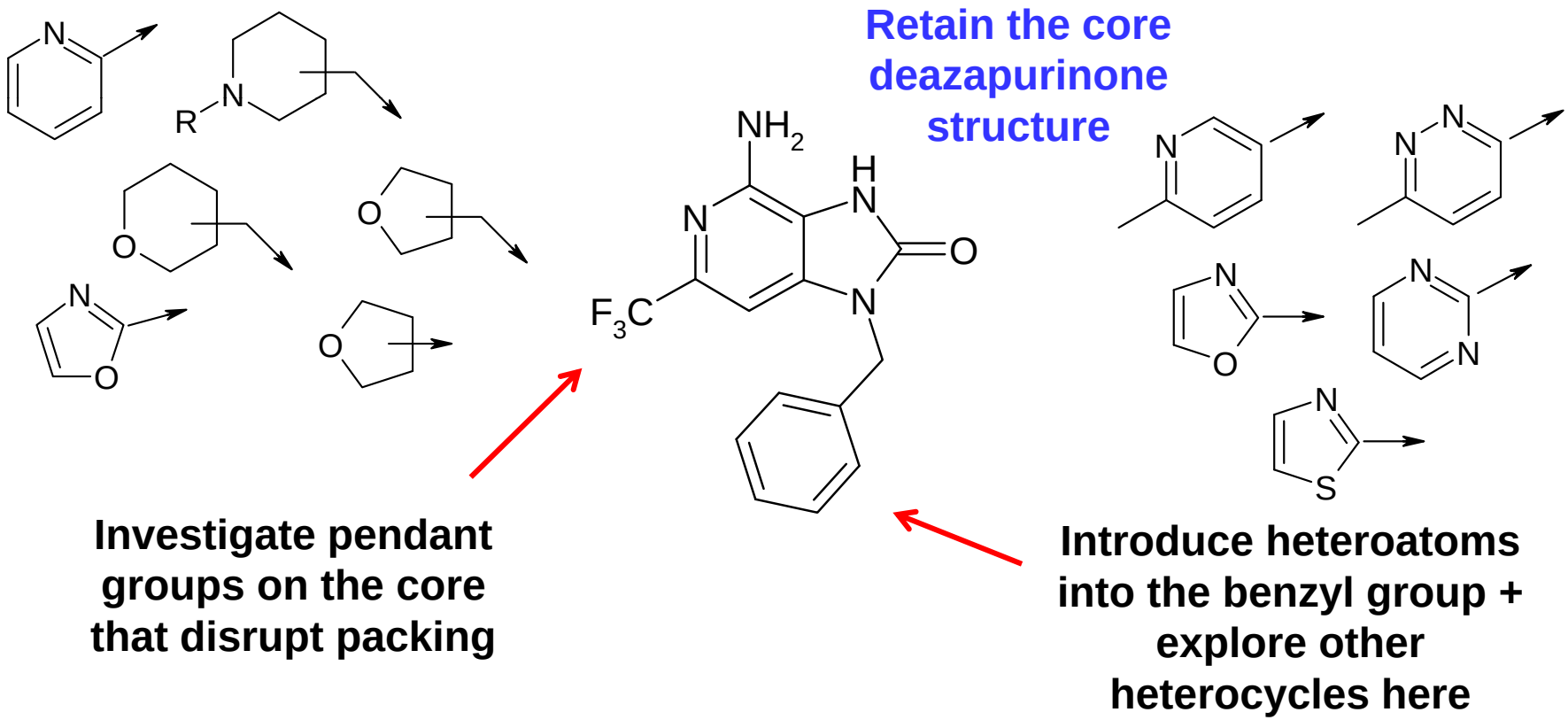
Dog PK
Cl 6mL/min/kg
V_{dss} 2.7L/kg
$T_{1/2}$ 5.5h

Predicted human PK
Cl 8mL/min/kg
V_{dss} 2.4L/kg
$T_{1/2}$ 3h

EC_{50} 102nM
MWt. 308
LogD 3.3
HLM <7μL/min/mg
Aq. sol. ~1μg/mL
T_m ~365°C

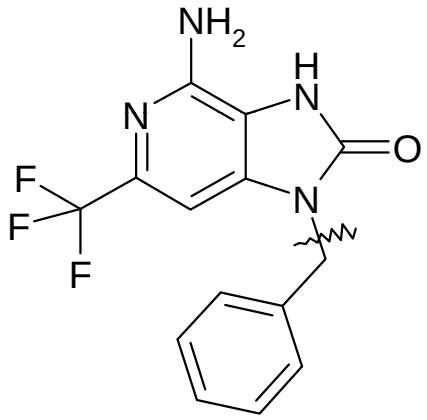
Solubilizing SAR

- **Increasing solubility investigated on three fronts**
  - *Basic groups (potent but permeability + clearance issues)*
  - Introduction of heteroatoms to disrupt hydrocarbon packing
  - Introducing 3D shape to disrupt packing and facilitate solvation

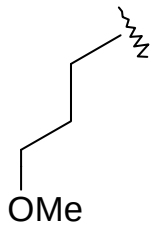


N9 SAR

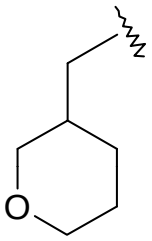
- **Methyl pyridyl substituent was one of the more potent, with reasonable solubility**



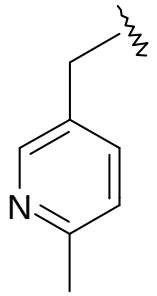
EC_{50} 100nM
Aq. sol. 1μg/mL



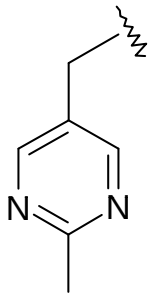
EC_{50} 833nM
Sol. 23μg/mL



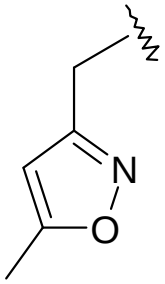
EC_{50} 1440nM
Sol. 37μg/mL



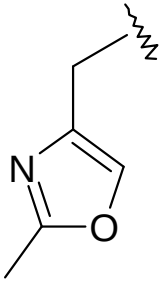
EC_{50} 50nM
Sol. 22μg/mL



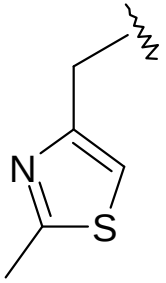
EC_{50} 366nM
Sol. 152μg/mL



EC_{50} 103nM
Sol. 4μg/mL



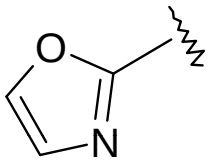
EC_{50} 193nM
Sol. 35μg/mL



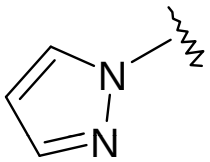
EC_{50} 79nM
Sol. 8μg/mL

C2 SAR

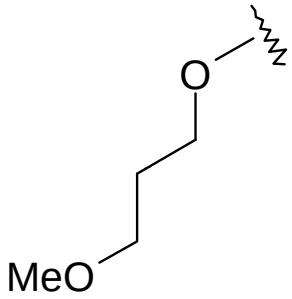
- **C2 heterocycles looked the most promising for both potency and solubility**
  - Subtle ether SAR provided some back-up options



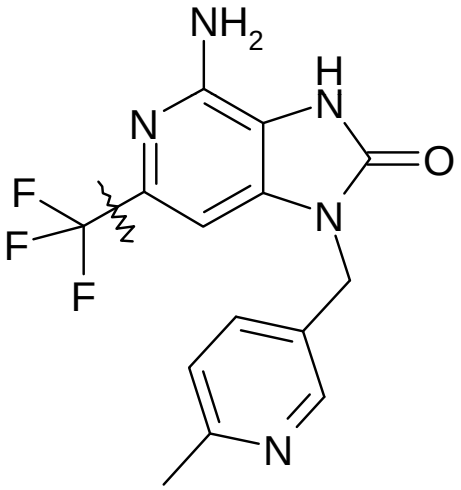
EC_{50} 102nM
Sol. 66μg/mL



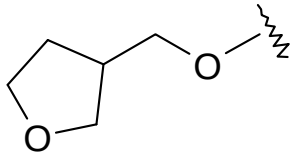
EC_{50} 633nM
Sol. 45μg/mL



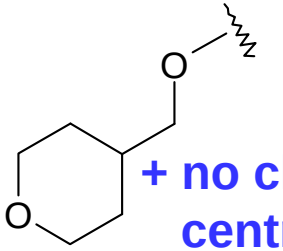
EC_{50} >4000nM
Sol. ND



EC_{50} 50nM
Aq. sol. 22μg/mL

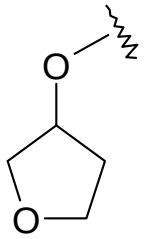


EC_{50} 474nM
Sol. 1μg/mL



+ no chiral centre!

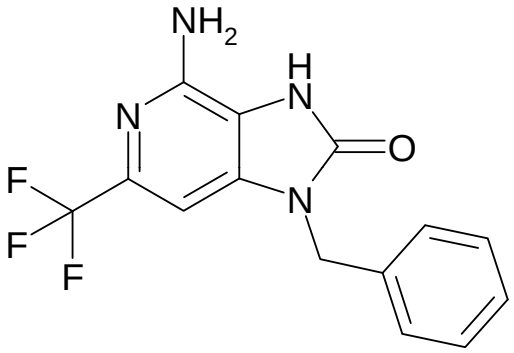
EC_{50} 99nM
Sol. 17μg/mL



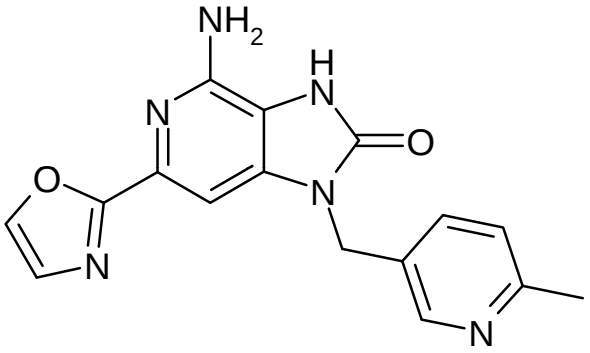
EC_{50} >4000nM
Sol. ND

An unexpected in vivo profile

- **The 2nd generation agents had better solubility, physchem properties, *in vitro* ADME and retained potency**



↑ solubility



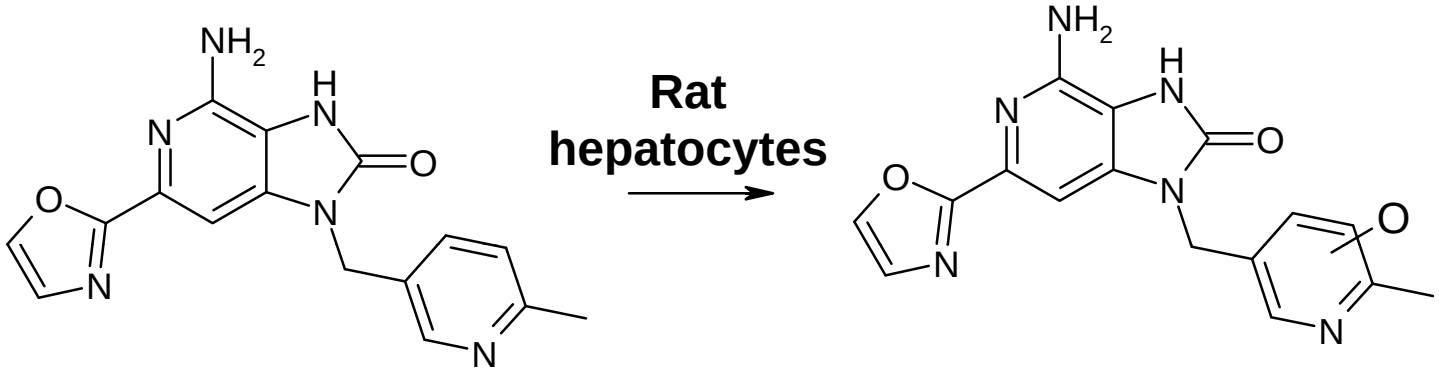
EC_{50} 102nM
Sol. 1μg/mL
MWt. 308,LogD 3.3
HLM <7.0; RLM <8.5
HGluc 5.2
HHep 8.0
Rat Cl < LBF

EC_{50} 102nM
Sol. 66μg/mL
MWt. 322, LogD 1.6
HLM 9; RLM <8.5
HGluc < 3.5
HHep < 5
Rat Cl >> LBF

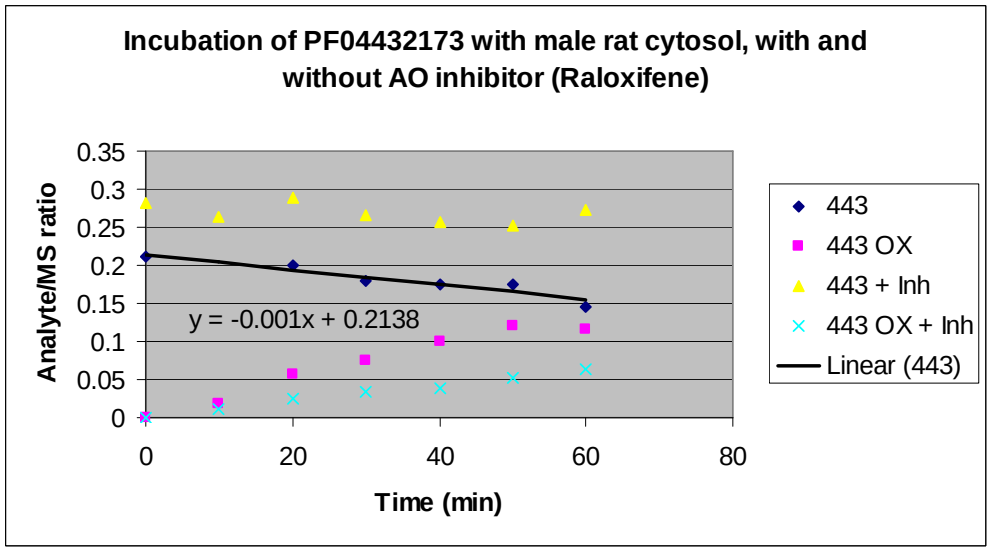
So whats gone wrong?

Aldehyde oxidase activity

- **Heterocyclic analogues from within this series were substrates for AO, especially rat AO**



Major metabolite

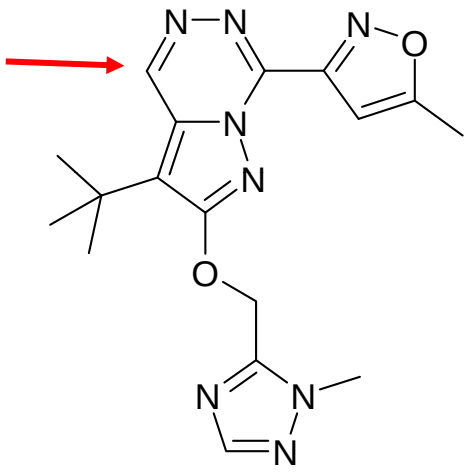


Oxidised metabolite not picked up in microsomes or in human hepatocyte systems

Generation of metabolite by AO confirmed using a specific AO inhibitor, Raloxifene

Aldehyde oxidase

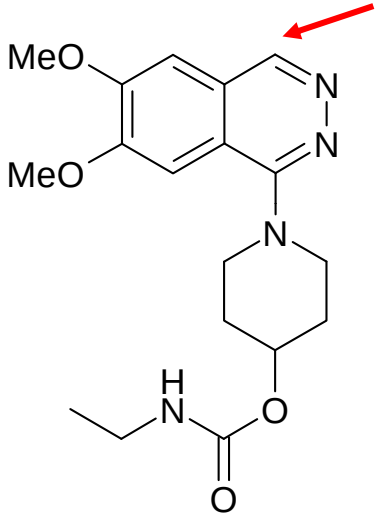
- **AO is a Mo-cofactor containing enzyme found mainly in cytosol; known to oxidise heterocycles**



Merck $GABA_A$ inverse agonist

No AO turnover in dog + rat cytosol

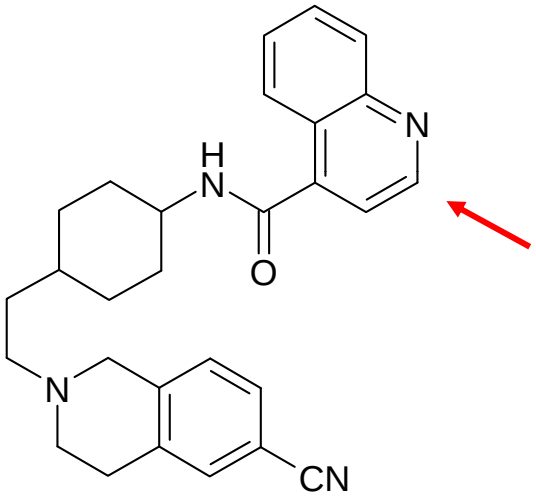
Major route in rhesus and human



Carbazeran

No AO turnover in dog cytosol

Major route in human



Gsk D3 antagonist

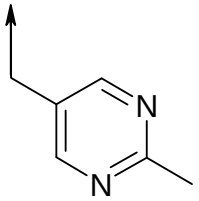
No AO turnover in dog + rat cytosol

Major route in rhesus and human

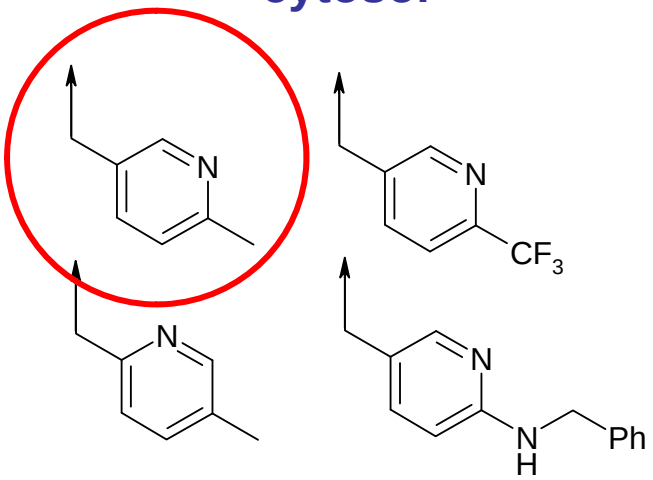
Direct AO-susceptibility is predictable

- **AO liability is very substitution + electronics dependent**
- **Beware species differences with AO**
  - Usually more prevalent in human than preclinical species but not with our TLR7 systems!

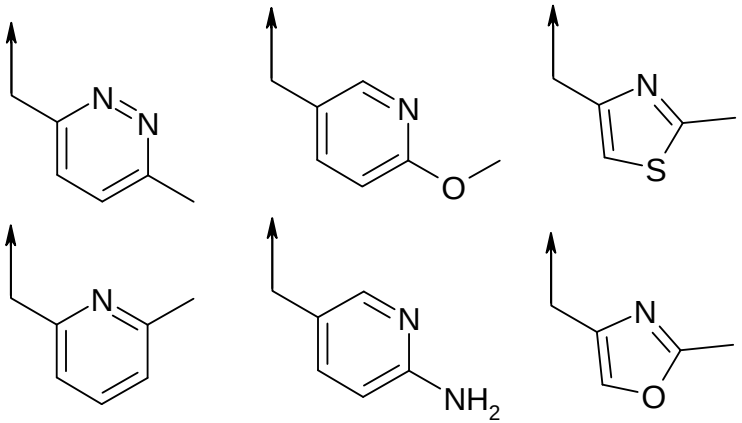
Rapid turnover in rat cytosol



Rapid to moderate turnover in rat cytosol

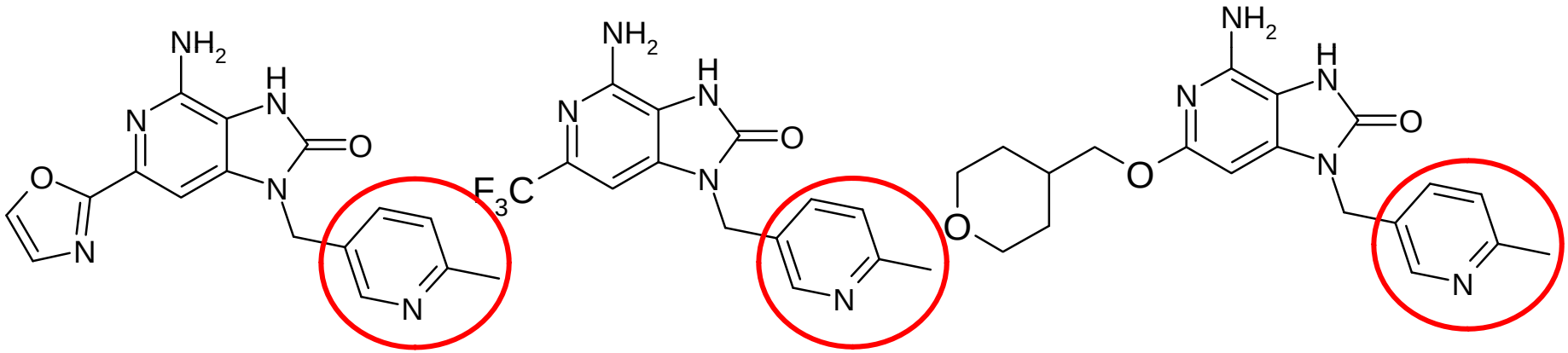


Stable in rat cytosol



But AO liability can be tuned remotely

- **Recognition by AO can be tuned by making remote alterations away from the susceptible group**
- **Can be rationalised through modelling**



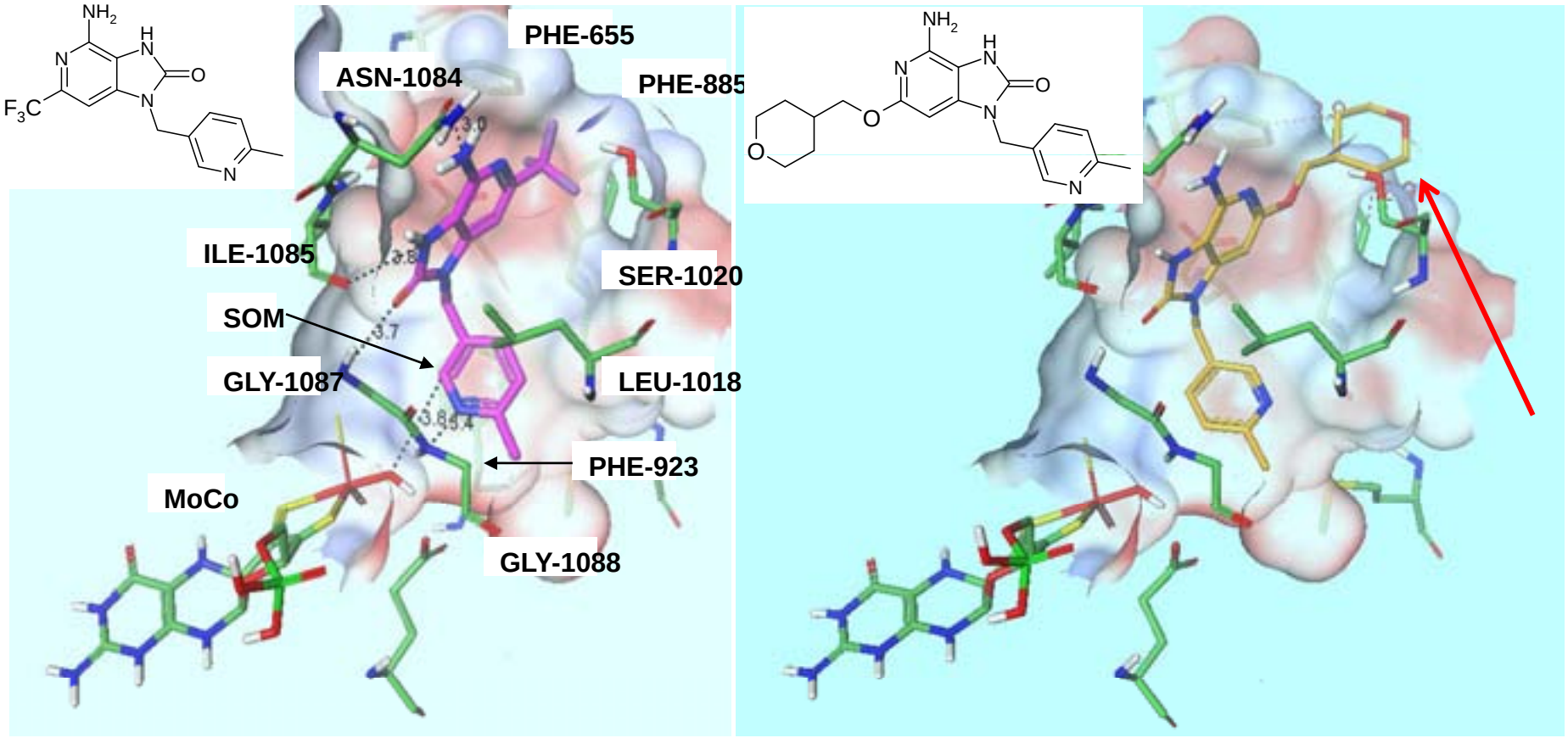
Rapid to moderate turnover in rat cytosol

Rapid to moderate turnover in rat cytosol

Stable in rat cytosol

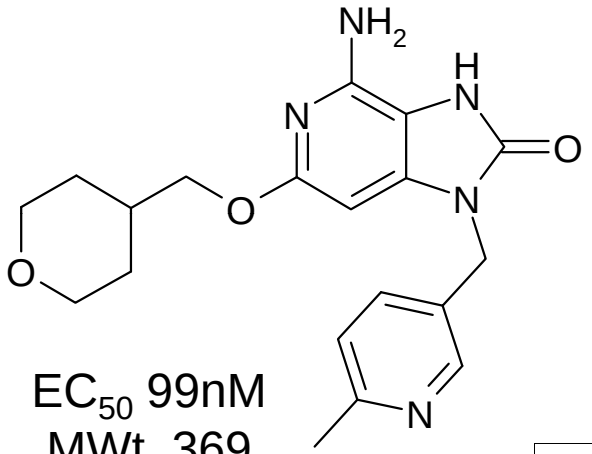
Structural rationale

- **Docked structure rationalises turnover of CF_3 agent**
- **THP analogue clashes with Ser1020**



PF-4601218

- **A development candidate with fit for purpose potency, pharmacokinetic and physchem credentials**



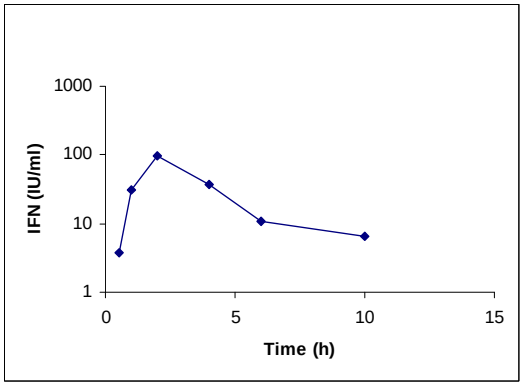
EC_{50} 99nM
MWt. 369
LogD 2.1
HLM <7μL/min/mg
Aq. sol. ~20μg/mL

>100 x selective in a broad panel of kinases, receptors, ion channels and enzymes

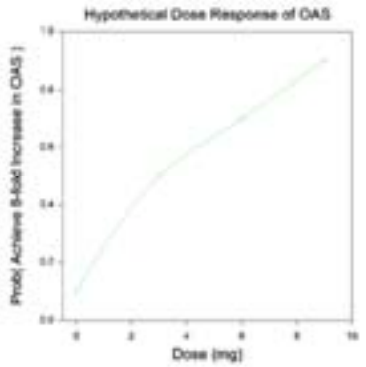
Rat PK
Cl 36mL/min/kg
V_{dss} 1.3L/kg
$T_{1/2}$ 0.4h

Predicted human PK
Cl 16mL/min/kg
V_{dss} 2.1L/kg
$T_{1/2}$ 1.5h

Measure IFN levels in mouse



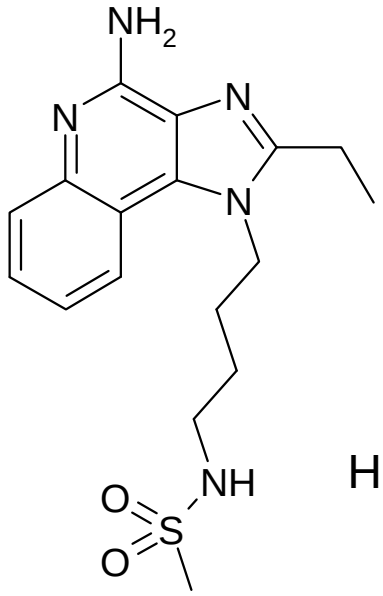
Modelled PK-PD in human



30mg human dose predicted to match exogenous IFN

A new player enters

- **Pfizer acquires Coley Pharmaceuticals and with it PF-4878691**
  - Already investigated at single doses from 2-20mg in healthy volunteers with transient, dose-dependent increases in white blood cells
  - Most expeditious route to POM with this compound
- **Healthy volunteer study**
  - Twice-weekly oral dosing
  - 2 weeks
    - days 1, 4, 8 and 11
  - 3, 6 and 9mg doses
  - Biomarkers monitored
    - 2,5-oligo adenylate synthetase
    - direct response to IFN's



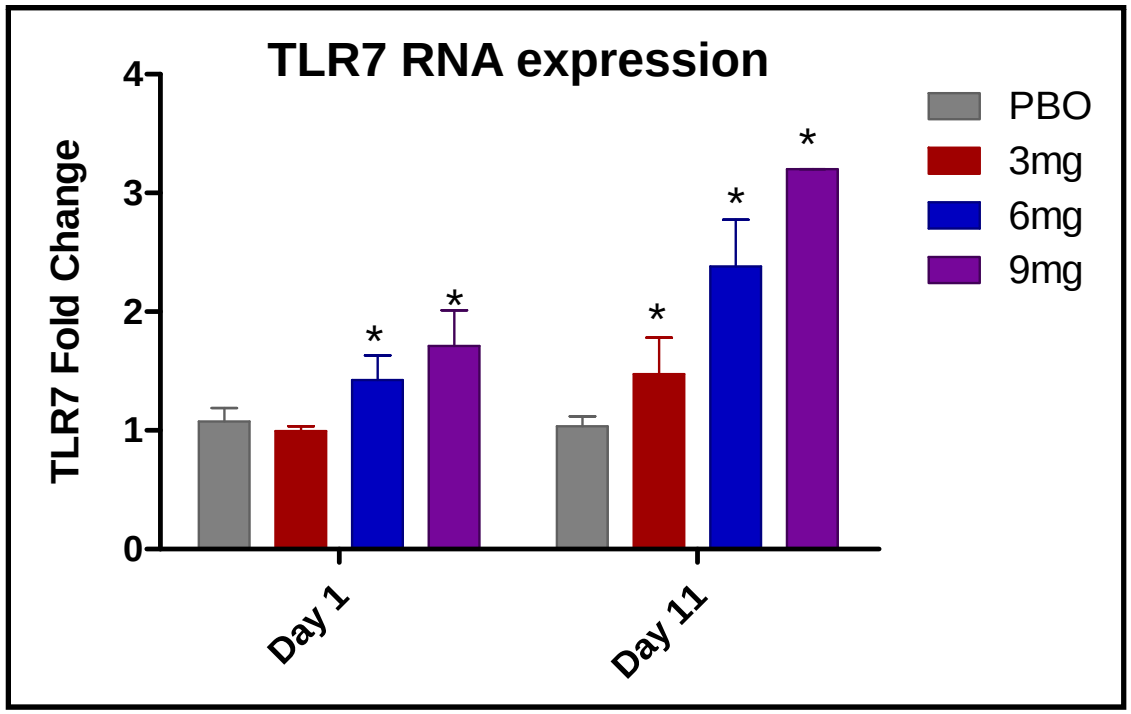
EC_{50} ~100nM
MWt. 361
LogD 1.2
HLM <7μL/min/mg

Adverse events observed

- **Biomarker induction in a dose-dependent and dose-frequency related manner**
  - Consistent with anticipated TLR7 agonism expected to provide an antiviral response
- **Two subjects in the 9mg dose group had serious adverse events**
  - Flu like symptoms, hypotension, lymphopenia
  - Study was discontinued
- **TLR7 stimulation at doses predicted to be efficacious were associated with adverse events with this compound**
- **A single non-responder was identified in the study**
  - Single polymorphism in the IFNα receptor 1 subunit; V168L

Effects of repeat TLR7 agonist dosing

- **TLR7 expression increases in response to TLR7 agonism in a dose-dependent manner**
- **Consequently, IFN-stimulated biomarkers + cytokine levels also increase in a dose-dependent manner**
  - Positive feedback loop, very low TI for systemic applications



Summary

- **Seeking a novel, oral, potent TLR7 agonist for HCV**
- **Initial investigations into novel core structures identified the 3-deazapurinone template**
  - Encouraging potency and pharmacokinetics
  - Solubility issues
- **Attempts to improve solubility uncovered aldehyde oxidase liabilities**
  - Recognition by rat AO in particular, however a simple preclinical prediction path was preferred
  - SAR identified direct and remote strategies to avoid AO activity
- **Quality CAN identified**
- **Clinical data obtained with an acquired compound**
  - Very narrow TI over pro-inflammatory cytokines
  - Dose -dependent auto-induction of the TLR7 receptor in vivo
  - Programme halted

Acknowledgements

with thanks to…

Thien Duc Tran
Peter Jones
Gerwyn Bish
Michael Paradowski
David Fox
Gemma Parsons
Jerry Hu
Mark Fidock
Mike Westby
Carl Laxton
Nigel Horscroft
Tanya Parkinson
Rob Webster
Amy Thomas