HTS and non-HTS approaches towards TRPV1 antagonists

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SCI - Hot topics in drug discovery: finding the next lead

November 2009

Schering-Plough

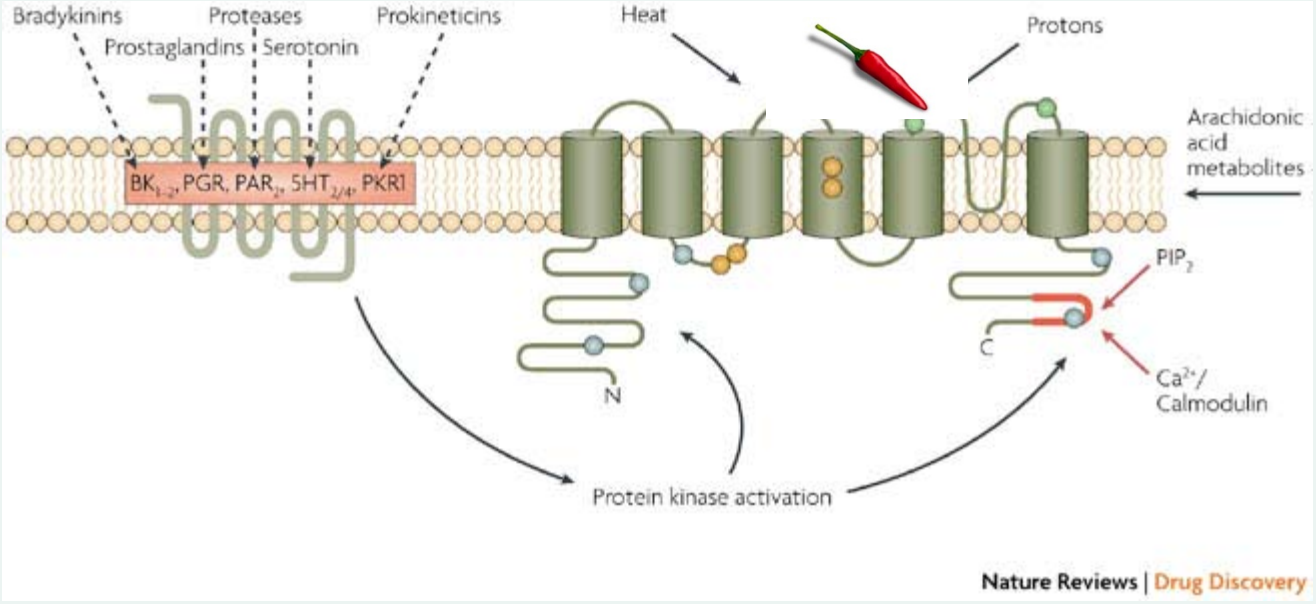
Overview of presentation

- Background to TRPV1
- HTS approach
- Pharmacophore design
- Pharmacopiea HTS
- Summary

VR1 (TRPV1) receptor

Transient Receptor Potential:

- Member of the TRP channel super family
- Ligand gated cation channel (Ca^{2+}/Na^{+})
- Cloning and characterisation by Julius and co-workers in 1997
- Distribution
  - Widespread throughout CNS (cortex, hippocampus, amygdala) and periphery (airway, skin, tongue, bladder, pancreas, GI tract)



Exploiting the TRPV1 channel

- The premise behind *agonists* is that nociceptive fibres would be desensitised and would therefore not be able to transmit pain signals, rather like the effects of local anaesthetics.

  - Topical CAP creams developed for treatment of neuropathic pain
  - NeurogesX Inc. developing high conc. (5-10%) CAP patch (Transdolor) for neuropathic pain
  - Anesiva developing highly purified form of capsaicin, Adlea™ for Osteoarthritis
  - Civamide (Zucapsaicin) intranasal application for cluster headaches
  - Concerns over the initial pain, potential for severe irritation

- The premise behind *antagonists* is that you would be able to block the effects of lowered pH on the sensitisation of nociceptors to prevent the development of hyperalgesia and allodynia.

  - TRPV1 knockout mice show a clear attenuation of thermal hyperalgesia
  - Small moleculeTRPV1 antagonists are active in rat/mouse pain models
  - Centrally penetrant antagonists show superior efficacy over peripherally restricted agents

Key points

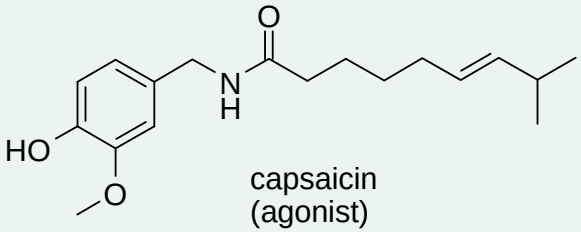
- Literature suggests TRPV1 may have potential for the treatment of chronic pain and/or post-op pain
- S/Es of NSAIDs/COX2 inhibitors have created an opportunity for new analgesics with novel mechanisms demonstrating improved tolerability

Aims: Develop orally active TRPV1 antagonists, suitable for once or twice daily dosing for the treatment of both acute post-operative and chronic inflammatory pain

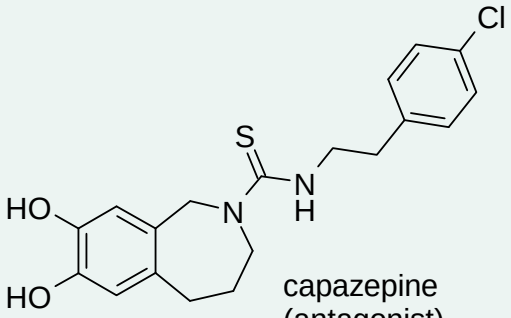
Patent status

- Program initiated in 2004
- 11 patents in 2004 (430 in 2009)
- Number of Pharma involved
- Similar chemotypes
  - First generation focussed on CAP/CAZ analogues

- Urea/Isostere present
- Solubility looks like an issue
- Amgen and SB entering clinic

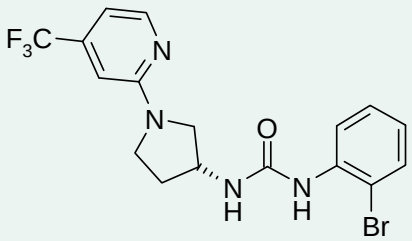


capsaicin
(agonist)

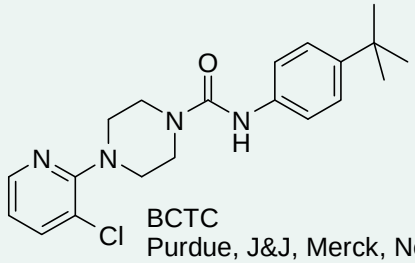


capazepine
(antagonist)

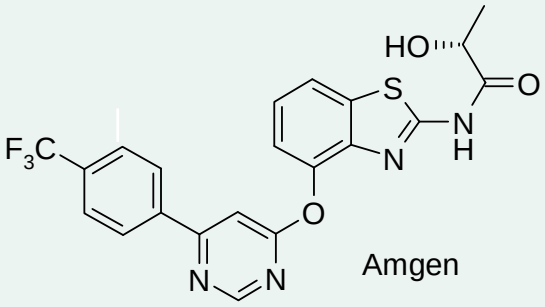
Novartis



Smithkline Beecham



BCTC
Purdue, J&J, Merck, Neurogen



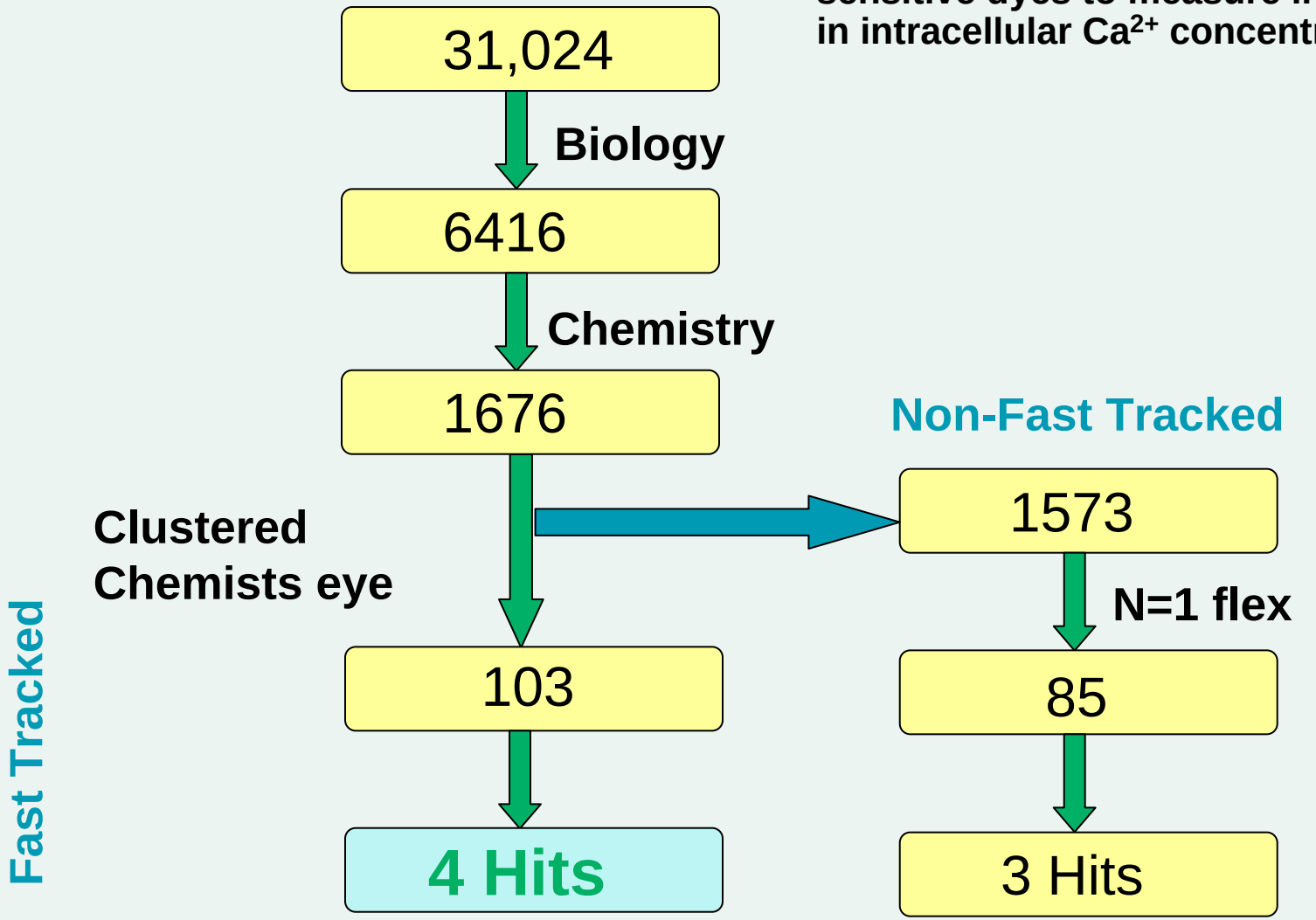
Amgen

Strategy

- Competitive area
  - Clear need for good starting IP position
- Initiated multiple hit-finding approaches
  - Screening of Newhouse collection
  - Collaboration with Pharmacopiea (external screening)
  - Computational approaches
    - No rational drug design possible (No relevant crystal structures)
    - Ligand based similarity approach possible - use known TRPV1 ligands

HTS: 304,000
>75% Inh. @10μM

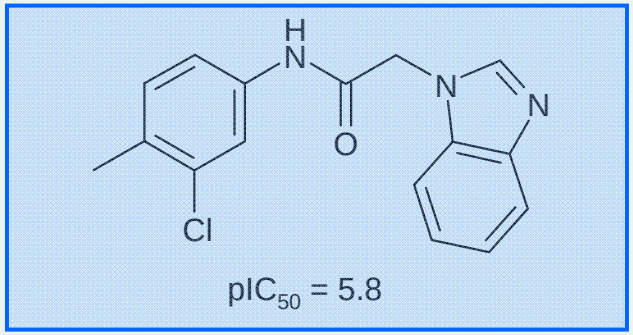
hVR1 Ca^{2+} influx assay
Use fluorescence based calcium sensitive dyes to measure increase in intracellular Ca^{2+} concentration

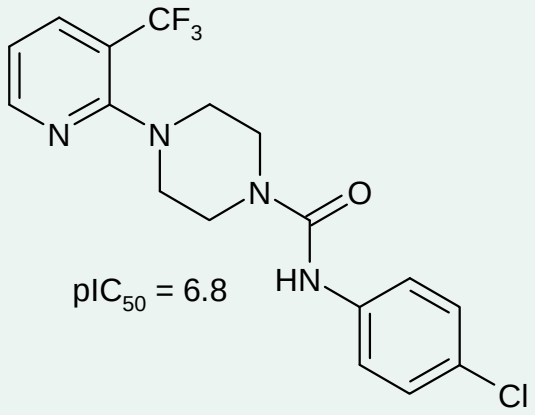


Spotfire used to rapidly filter compounds

TRPV1 confirmed hits

- Benzimidazole taken forward

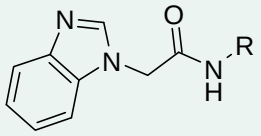


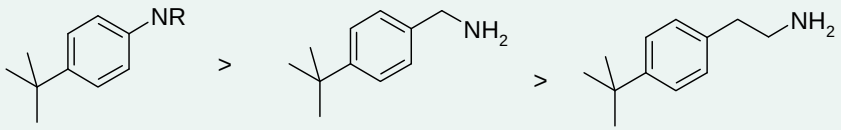


Hit commercially available from multiple suppliers

BCTC-like compounds confirm assay validity

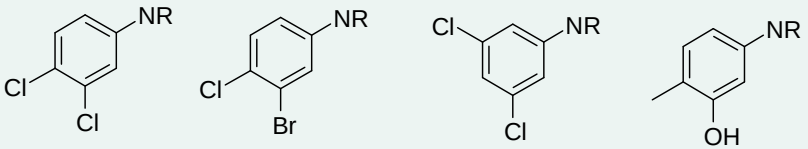
SAR: Aromatic region



- 

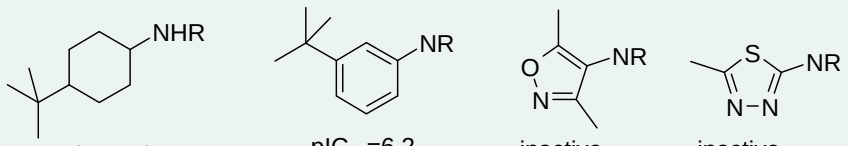
pIC$_{50}$=5.9 > pIC$_{50}$=7.0 > pEC$_{50}$=6.9

  ^{t}Bu>^{i}Pr>Me
  lipophilic

- 

pIC$_{50}$=5.9 pIC$_{50}$=5.9 pIC$_{50}$=5.9 inactive

  Lipophilic

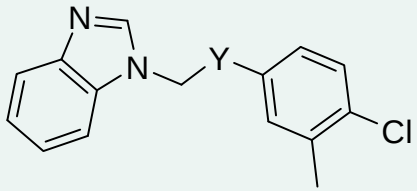
- 

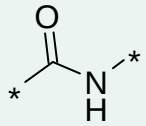
pIC$_{50}$=5.6 pIC$_{50}$=6.2 inactive inactive

  Polar groups poor
  Cycloalkyl active

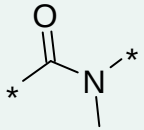
SAR: Amide linker

- Limited SAR in this region
- Donor and acceptor impart activity
  - Similar findings in the literature

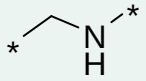




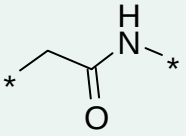
$pIC_{50} = 5.8$



$pIC_{50} = 4.9$



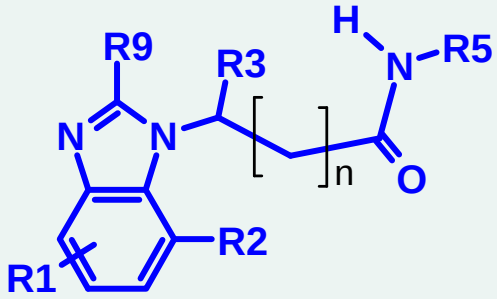
$pIC_{50} = 4.3$

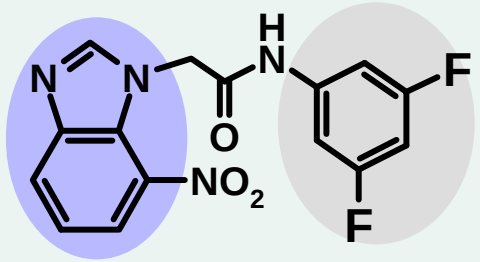


inactive

A change in priority

- Nov. 04 Astra Zeneca published WO 04100865

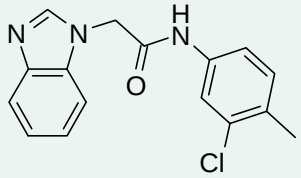




IC_{50} = 50nM

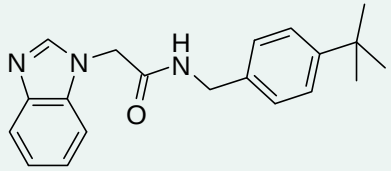
Astra Zeneca

Alkyl, Aryl, Heteroaryl
Heteroaryl or cycloalkyl fused with aryl



in-house HIT

pIC_{50} = 5.8

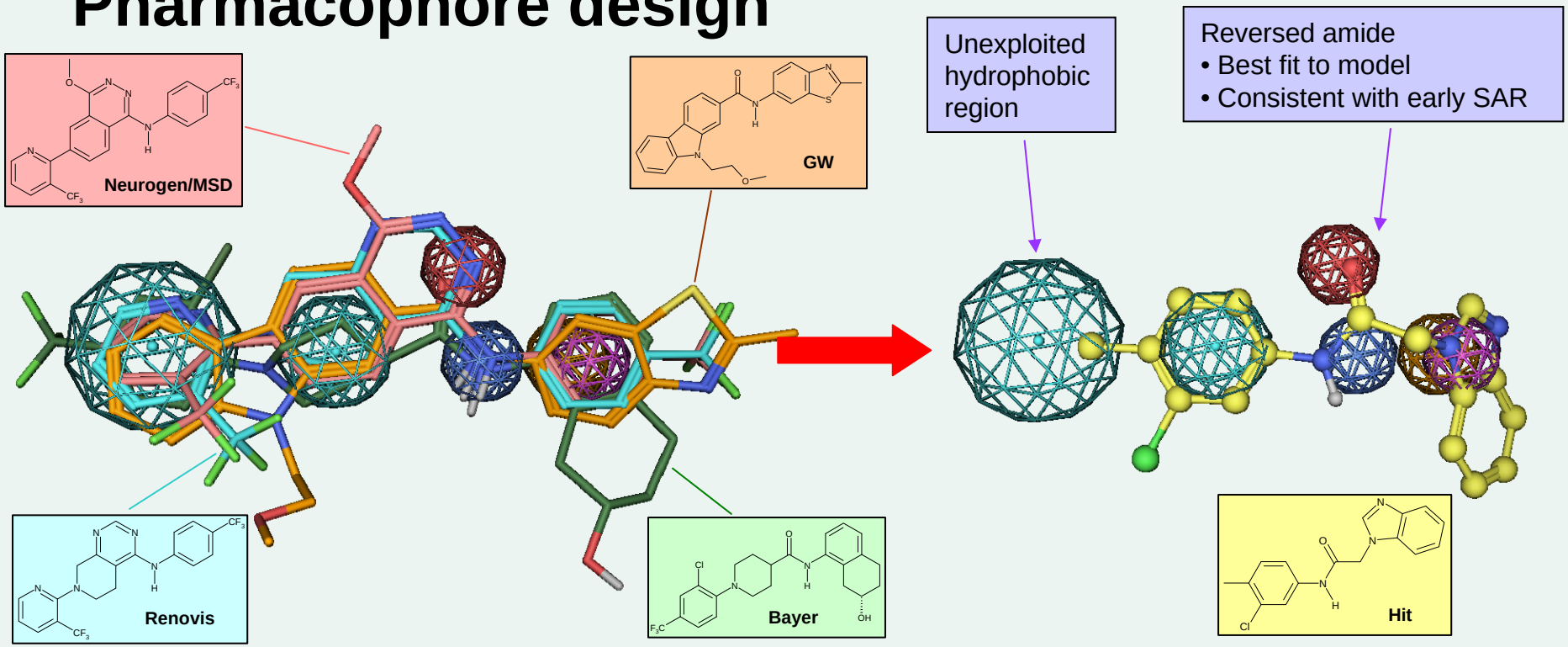


pIC_{50} = 7.04

Covered by AZ patent

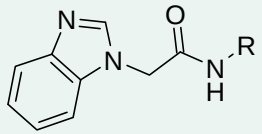
- Where do we go from here?

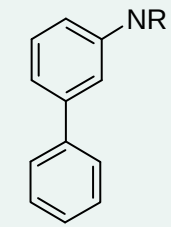
Pharmacophore design



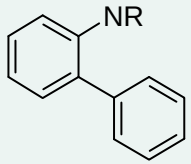
- Pharmacophore constructed using >30 compounds from patent literature (examples above)
  - Refined using internal ligands and HTS hits
- Aim: to develop a structural rationale for activity
  - Identify pharmacophoric elements of literature compounds, and of HTS hits
- To steer and/or prioritise chemistry
  - To identify new proprietary compounds
  - Address key optimisation goal: introduce solubilising functionality

Exploiting the pharmacophore





pIC_{50}=6.8



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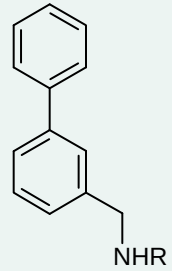
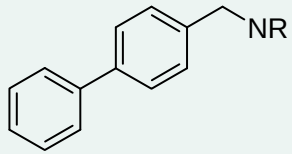
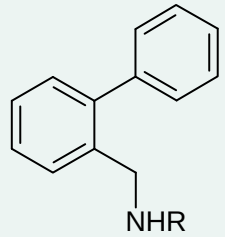


pIC_{50}=5.7



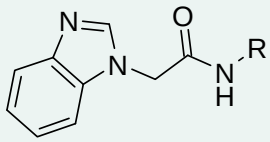
pIC_{50}=6.1

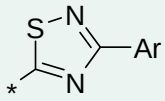


pIC_{50}=6.0

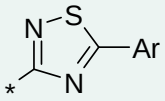
Non-specific lipophilic region?

SAR: Central ring optimisation

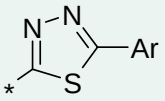




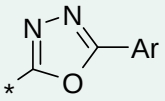
pIC_{50} = 5.5
Solkin = 1



pIC_{50} = 5.2
Solkin = 1



inactive
Solkin = 1



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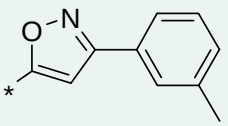
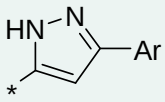
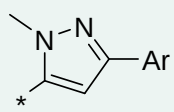
Solkin = 84.2



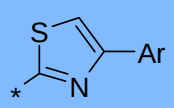
pIC_{50} = 6.0
Solkin = 1



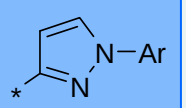
inactive
Solkin ND



pIC_{50} = 4.6
Solkin = 60.8



pIC_{50} = 5.9
Solkin = 1



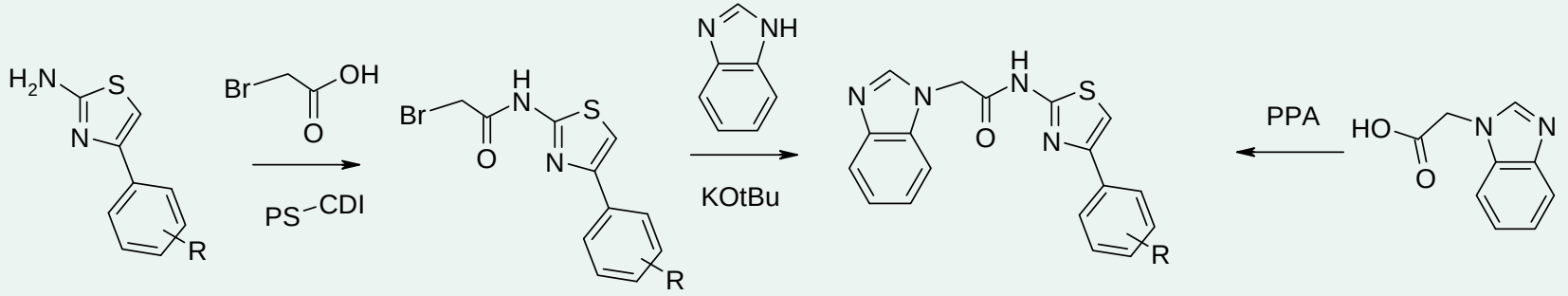
pIC_{50} = 5.7
Solkin = 18.5

Ar = phenyl

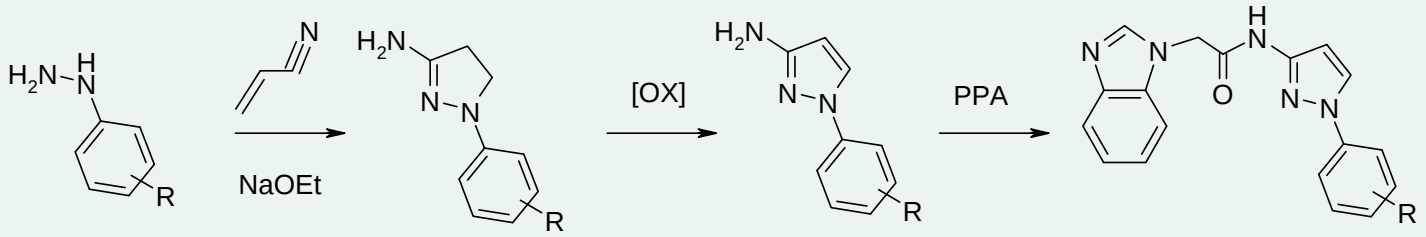
- Not all heterocycles active
- Promise with N-substituted pyrazole and aminothiazole

Chemistry

- Need to avoid column chromatography
  - Acid bromide / chloride give side reactions, HBTU no product isolated
  - Resin bound CDI quantitative, filtered and taken onto next step

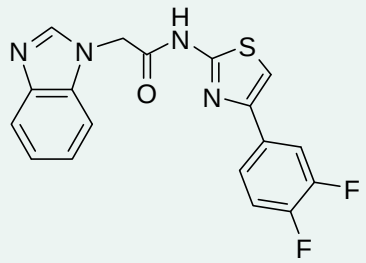


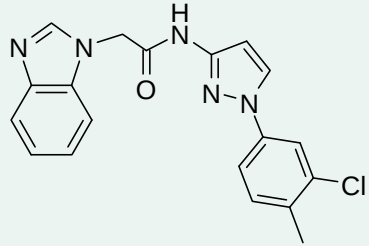
  - benzimidazole isosteres can be introduced in a similar way (above)



Thiazoles and pyrazoles

Study	Thiazole	Pyrazole
pIC_{50} (CAP)	7.0	7.2
Inh. CAP rVR1 DRG	7.8	9.0
Inh. Heat rVR1 DRG	87.4% @1mM	NT
R mic. t1/2(CLint)	46 (<34)	83
H mic. t1/2(CLint)	>120 (<12)	42
hERG (pKi)	<4	<4

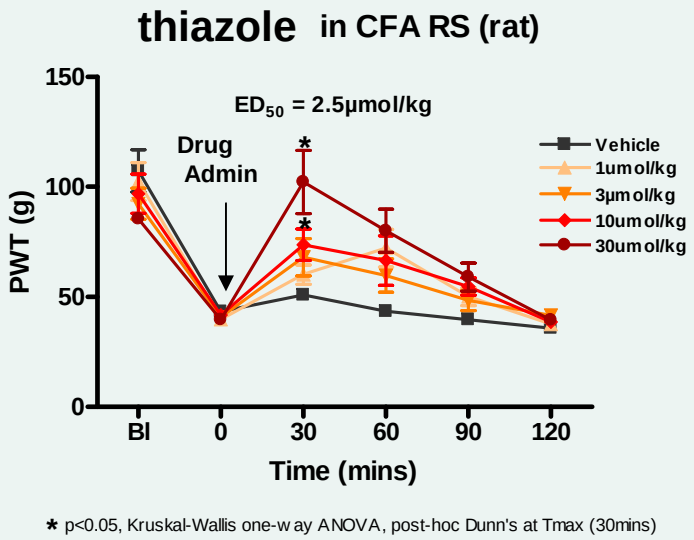




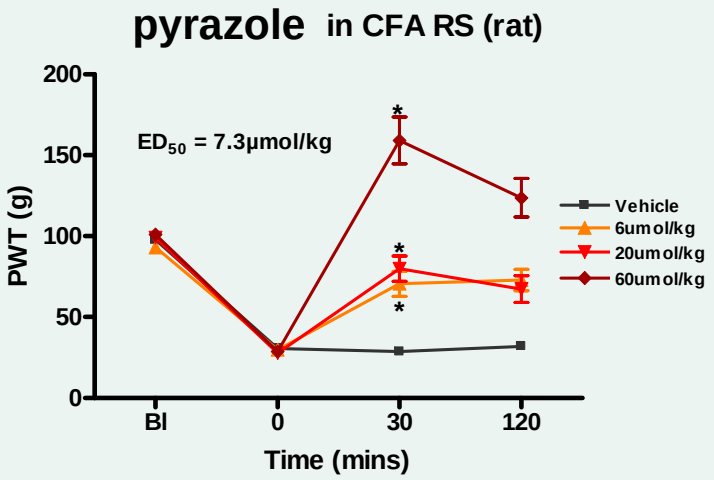
	i.v dose (mg/kg)	Cl (mL/min/kg)	V_{ss} (L/kg)	$T_{½}$(h)	p.o dose (mg/kg)	$AUC_{0\text{-}in}$ (ng.h/mL)	T_{max} (h)	C_{max} (ng/mL)	F (%)
Thiazole	1.0	3.9	0.3	1.1	10	66029	2	9955	>100
Pyrazole	2.0	11.4	0.35	0.47	10	4162	3	545	28

Good oral bioavailability for in vivo use

In vivo efficacy



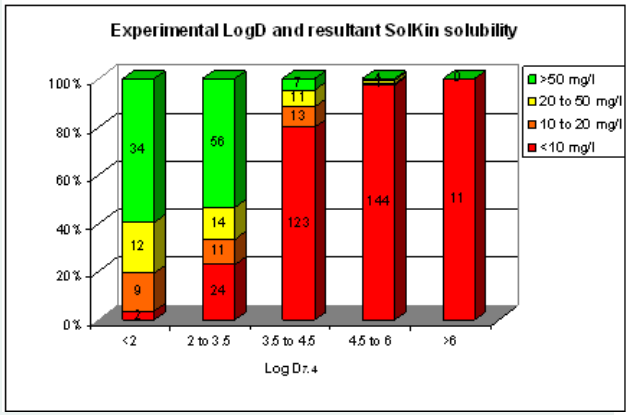
* p<0.05, Kruskal-Wallis one-way ANOVA, post-hoc Dunn's at Tmax (30mins)

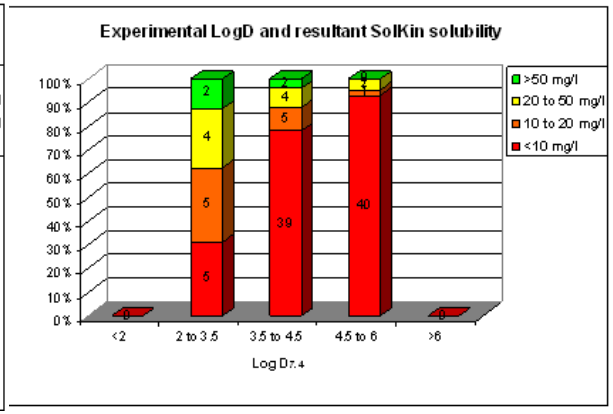


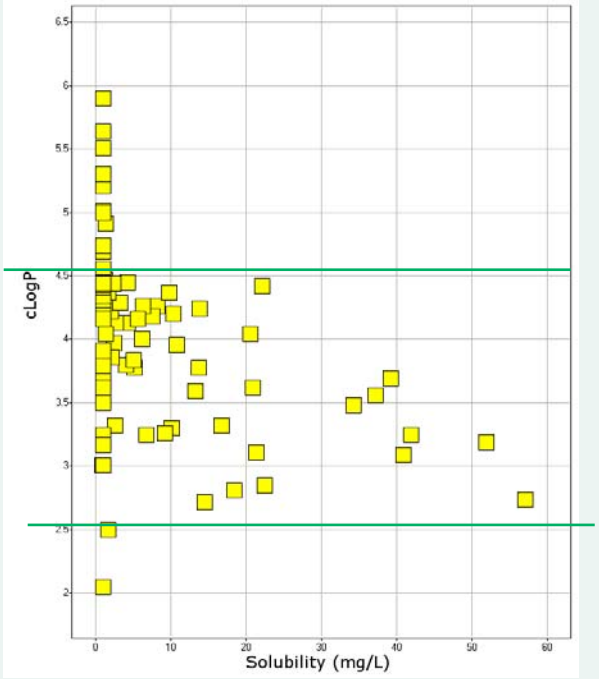
* p<0.05, Kruskal-Wallis one-way ANOVA, post-hoc Dunn's at Tmax (30mins)

- In vivo efficacy shown with thiazole and pyrazole

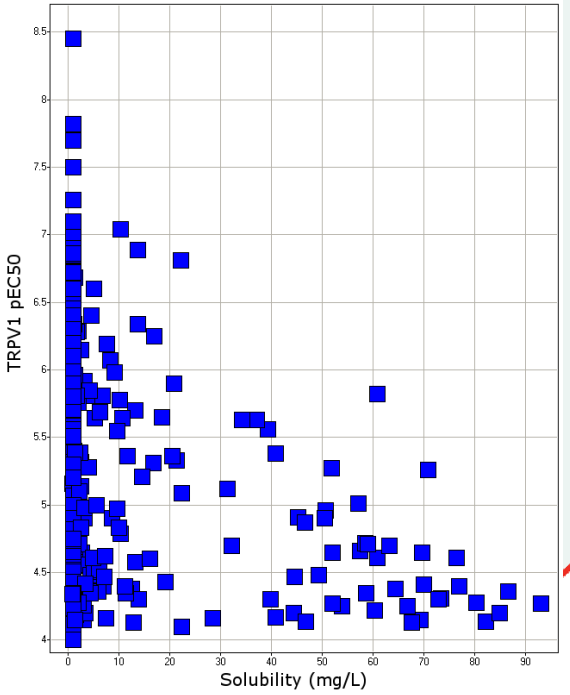
Solubility







- Solubility >10mg/L; elogD<3.5 (clogP 2.5-4.5)
- Trend of Increase Solubility = Less activity
- Solubility not governed by logP
  - Probably due to conformation
- Pharmacophore to be used to identify regions for introducing solubilising features

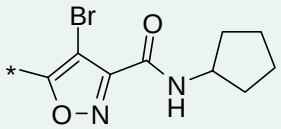


Hit identification

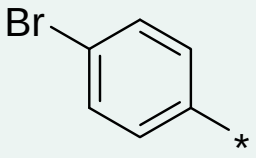
- Screened at Pharmacopeia
- Hit-to-Lead objectives
- Increase potency
- Improve solubility

Hit
pIC_{50} = 6.6
Solkin <1mg/L

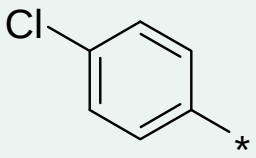
Hit-to-Lead SAR



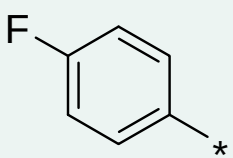
Isoxazole 5-substitution (aromatic region)



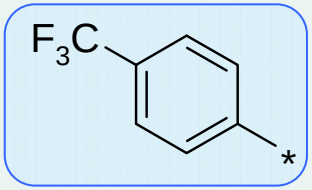
pIC_{50} = 6.4



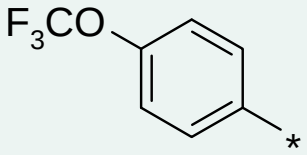
pIC_{50} = 6.6



pIC_{50} = 6.4



pIC_{50} = 7.3



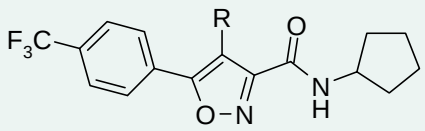
pIC_{50} = 5.9

para-CF_3 optimal

Hit-to-Lead SAR

Isoxazole 4-substitution (halogen region)

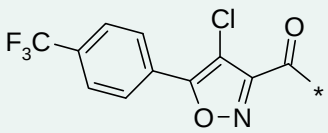
R	
H	pIC_{50} = 6.7
Me	pIC_{50} = 7.0
F	pIC_{50} = 6.9
Cl	pIC_{50} = 7.8
Br	pIC_{50} = 7.3
CN	pIC_{50} = 7.0

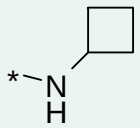


Cl > Br > F but Me and CN tolerated

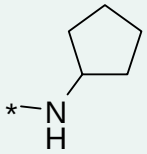
Hit-to-Lead SAR

Amide substitution (amine region)

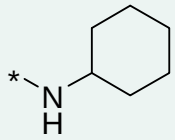




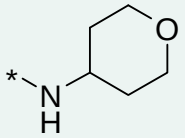
pIC_{50} = 7.1



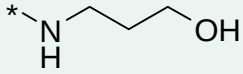
pIC_{50} = 7.8



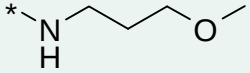
pIC_{50} = 7.0



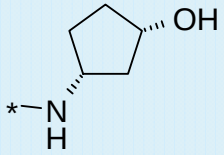
pIC_{50} = 7.4



pIC_{50} = 6.2



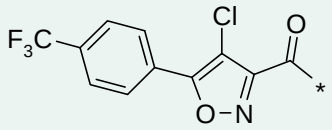
pIC_{50} = 5.7



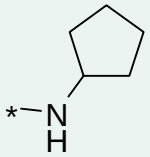
pIC_{50} = 7.7
Solkin 5mg/L

Cyclic systems more potent than acyclic
Cyclic amino alcohols potent and soluble

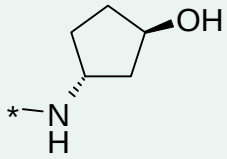
Hit-to-Lead SAR



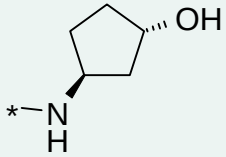
Cyclopentyl and cyclohexyl derivatives (amine region)



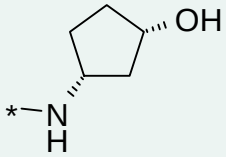
pIC_{50} = 7.8



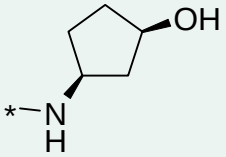
pIC_{50} = 6.7
Solkin 4.3mg/L



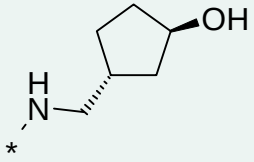
pIC_{50} =6.1



pIC_{50} = 7.7
Solkin 5.1mg/L



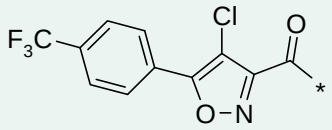
pIC_{50} =7.3
Solkin 6mg/L



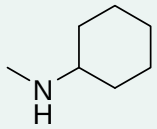
pIC_{50} = 6.6

Subtle changes in stereochemistry has a dramatic effect on potency
Cis more potent than trans

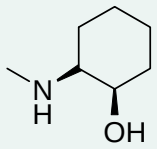
Hit-to-Lead SAR



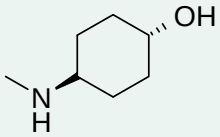
Cyclopentyl and cyclohexyl derivatives (amine region)



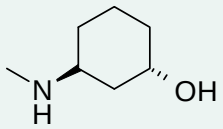
pIC_{50} = 7.0



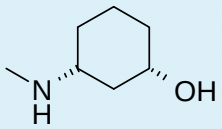
pIC_{50} = 7.2



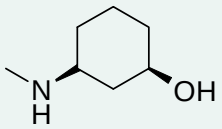
pIC_{50} = 6.4



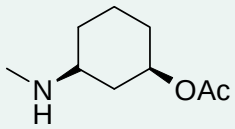
pIC_{50} =6.8



pIC_{50} = 8.5
1S, 3R



IC_{50} = 6.9

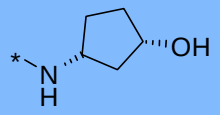
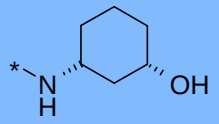
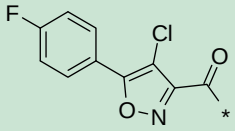
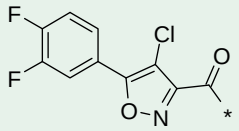
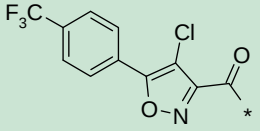


IC_{50} = 5.7

Hydroxy group imparts activity and solubility

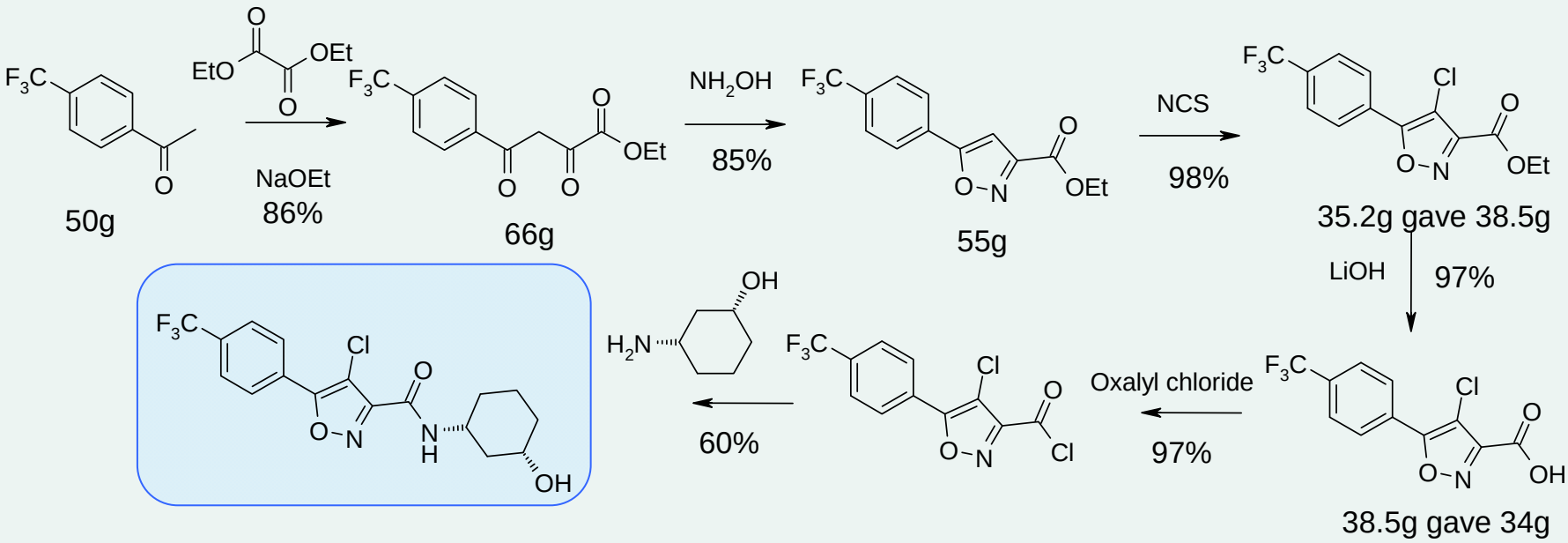
Hit-to-Lead SAR

Combinations of pendant groups

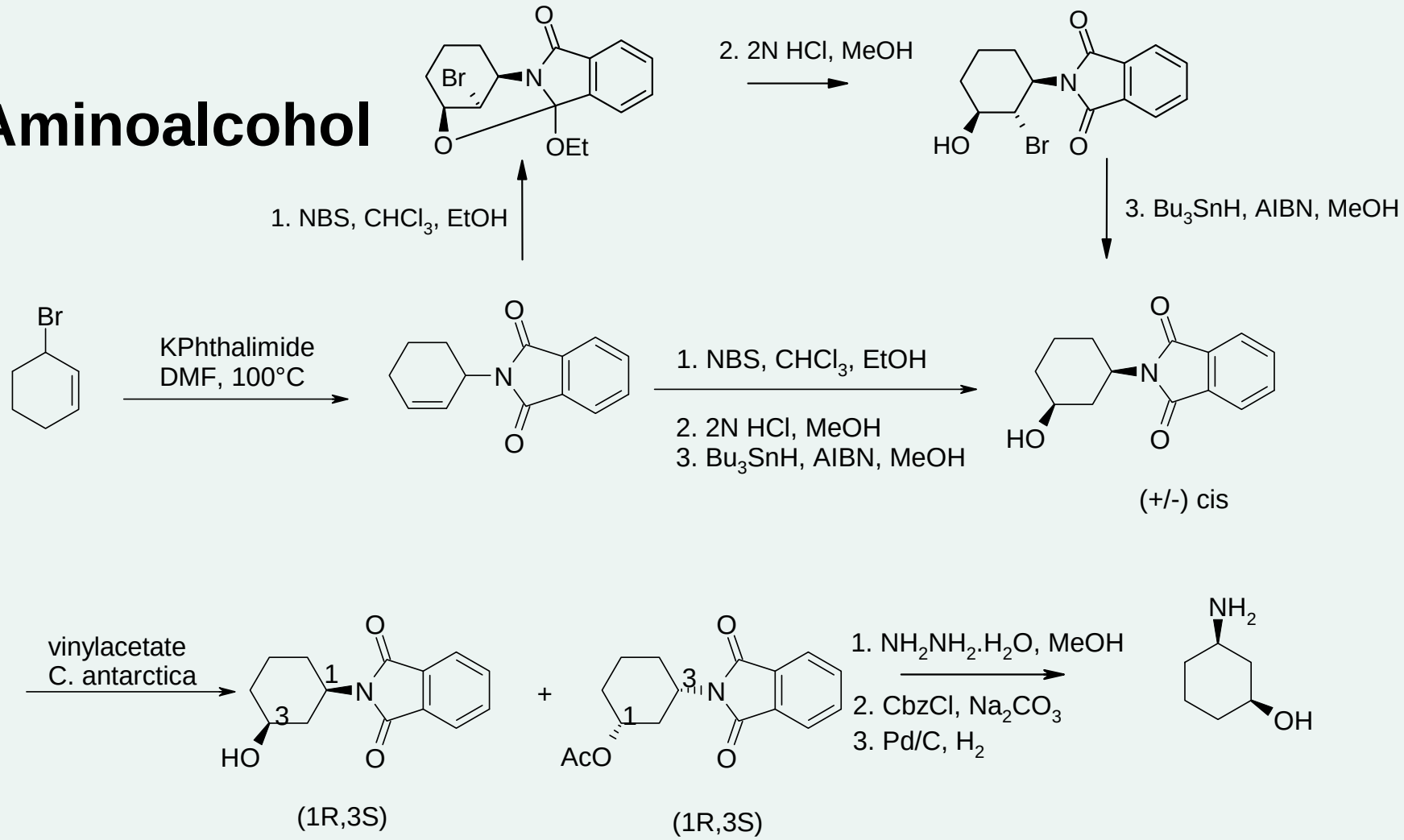
		
	**pIC_{50} =6.3** **Solkin 23mg/L**	**pIC_{50} =6.8** **Solkin 23mg/L**
	**pIC_{50} =6.4** **Solkin 28mg/L**	**pIC_{50} =7.1** **Solkin 17mg/L** — **Lead**
	**pIC_{50} =7.7** **Solkin 5mg/L**	**pIC_{50} =8.5** **Solkin 1.7mg/L**

Pendant Fluoro group improves solubility in combination with hydroxyl moiety

Synthetic route

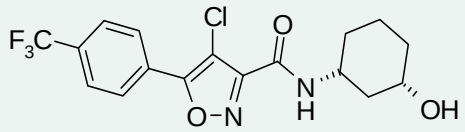


Aminoalcohol



See Tetrahedron Asymmetry 2004, 15(13), 2051-2056

Lead optimisation

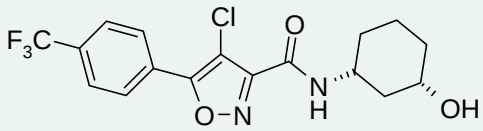


Study	Current best
hTRPV1 pIC_{50}	9.3
$eLogD_{(7.4)}$	4.53
Solkin Solubility	1.7 mg/L
hERG pKi pEC_{50}	<4 <5
Rat microsomes Cl_{int}	14 (µL/min/mg)
Human microsomes Cl_{int}	<12 (µL/min/mg)
Rat Hep. stability Cl_{int}	17 (µL/min/10^6 cells)
Human Hep. Stability Cl_{int}	<1 (µL/min/10^6 cells)
CYP450 Inhibition	All isoforms > 50µM
PPB %	98.9 (human), 99.5 (rat)

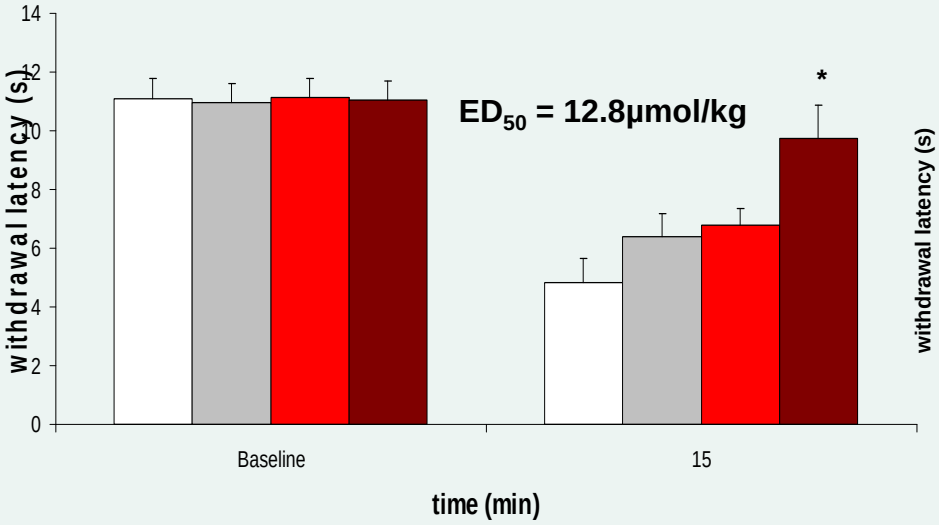
i.v. (2mg/kg)	
Cl (mL/min/kg)	5.44
Vss (L/kg)	0.77
$T_{½}$ (h)	1.90
Oral (10mg/kg) (gelatin/mannitol)	
C_{max} (ng/mL)	2787
T_{max} (h)	2.79
$T_{½}$ (h)	4.10
AUC (ng/mL.h)	31847
F%	100
Brain exposure	
Mean Brain:Plasma	1.0 – 1.2

Compound selected for further studies

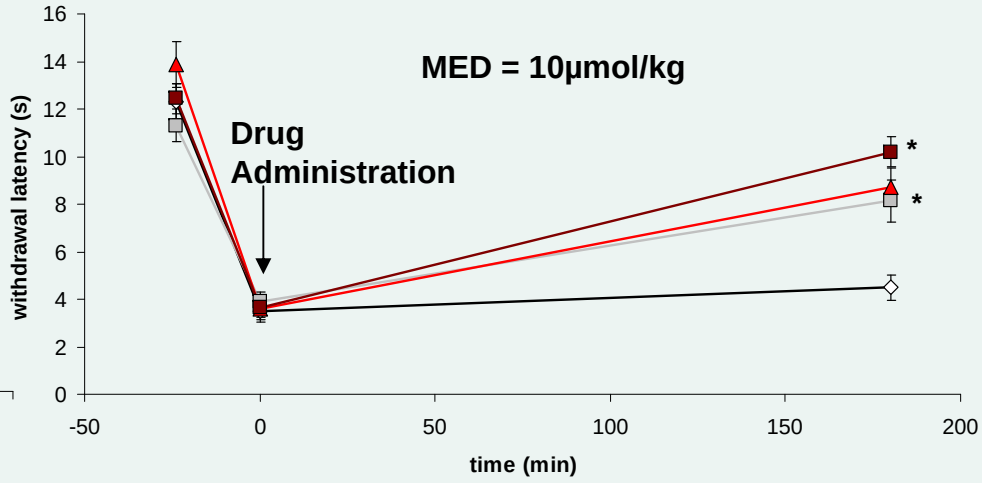
In Vivo Efficacy Models

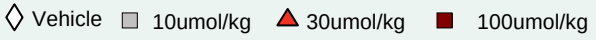


Capsaicin Thermal Hyperalgesia

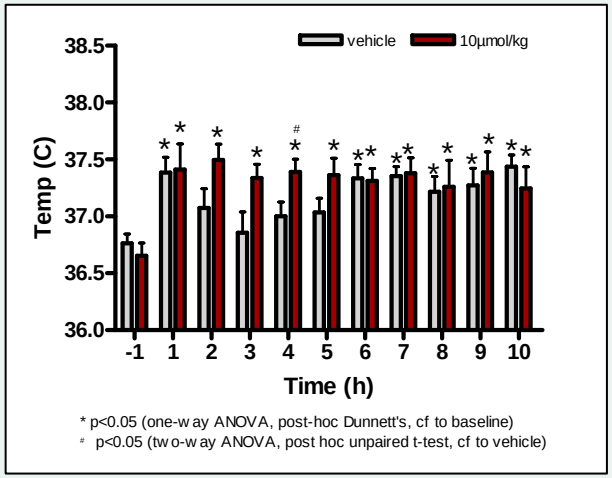


CFA Thermal Hyperalgesia

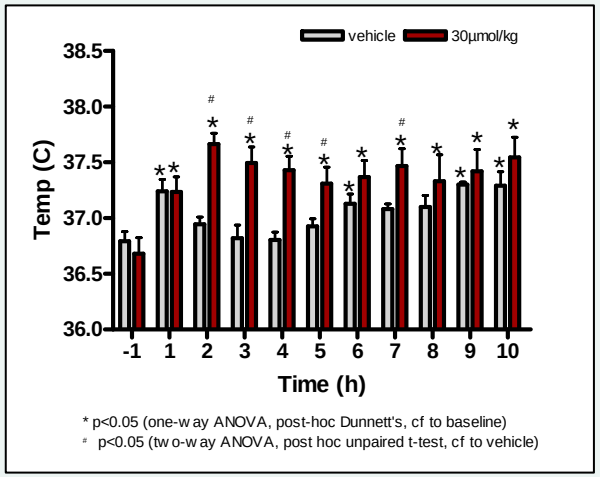




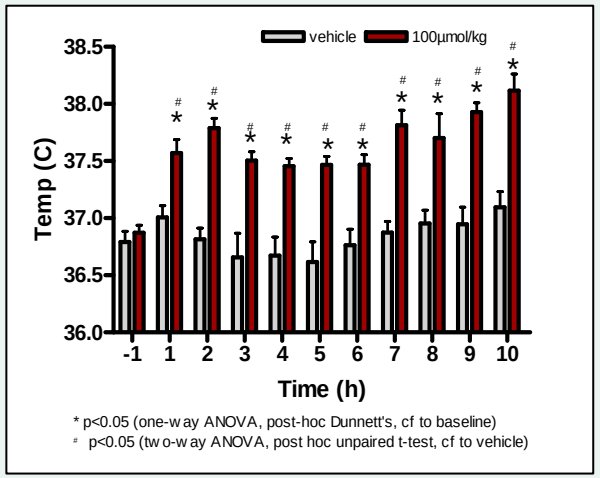
Hyperthermia in Rats



10µmol/kg



30µmol/kg

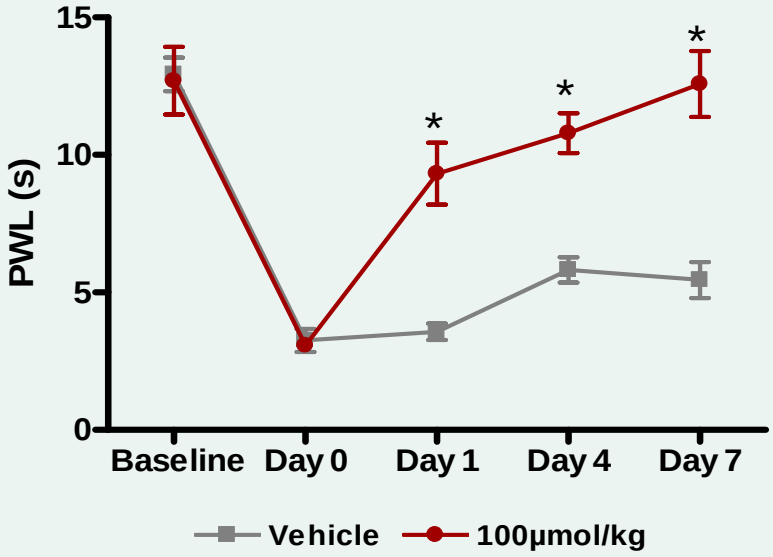


100µmol/kg

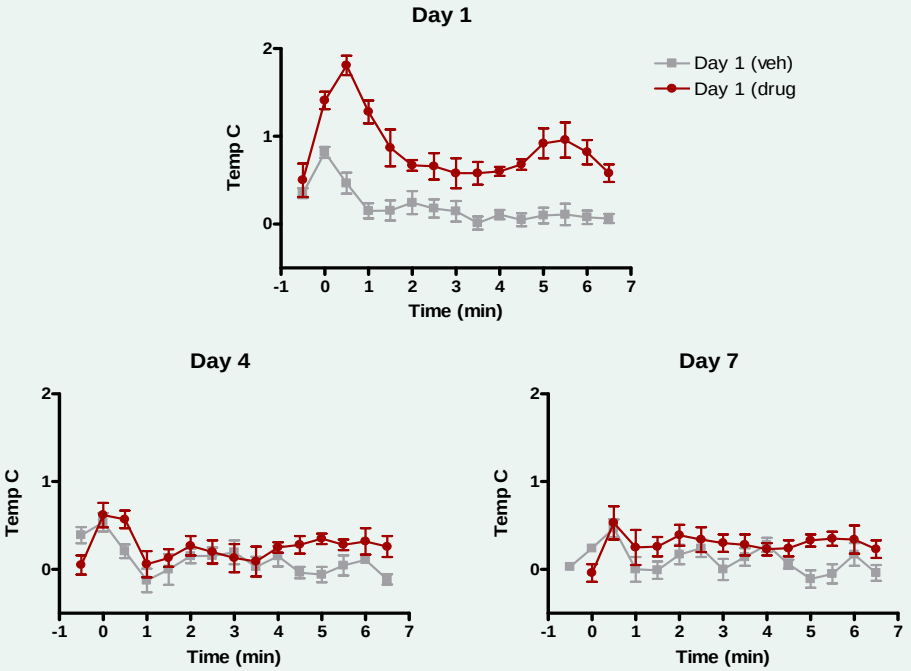
- Dose-related temperature increases at 10, 30 and 100µmol/kg
- Repeated dosing in rats (CFA thermal hyperalgesia)
  - Tolerance of hyperthermia response
  - Analgesic efficacy maintained

Tolerance

maintained analgesic efficacy



tolerance to hyperthermia

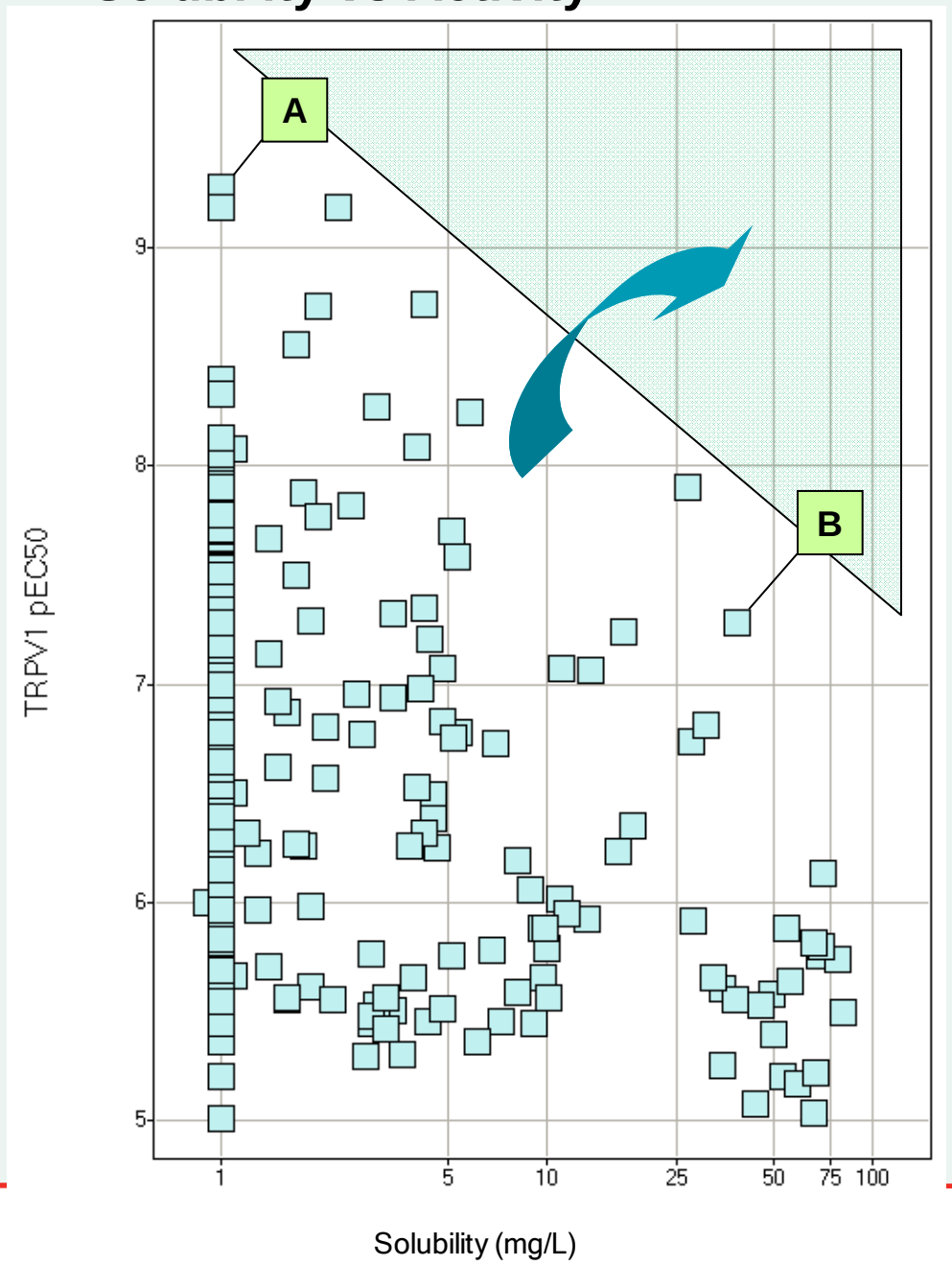


- Literature reports
  - Other TRPV1 antagonists increase temperature in several species (~1ºC max.)
  - Hyperthermia induced by AMG 517 in man (dose-related and 1 very sensitive individual)
  - Dose titration a potential management strategy

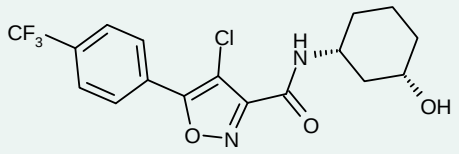
"Feasible to modulate TRPV1 in a manner that does not cause hyperthermia while maintaining efficacy in rodent pain models"

Pain 2008, 136(1-2), 3 and 202 and JPET 2008, 326, 218

Solubility vs Activity



A



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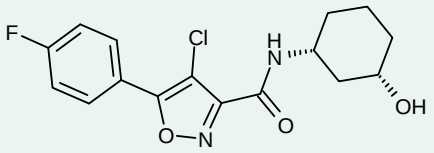


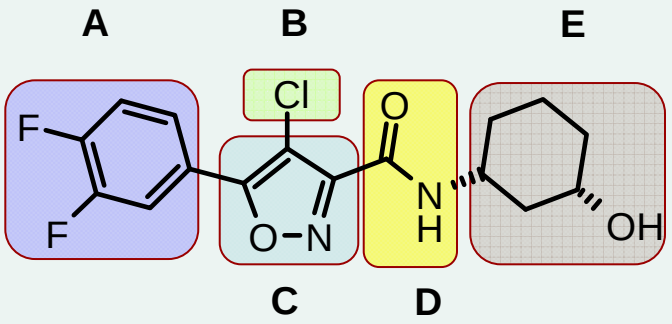
- Aim to improve solubility

Summary of SAR

Substitution boosts potency

4-CF_3 optimal for potency

3-F aids solubility

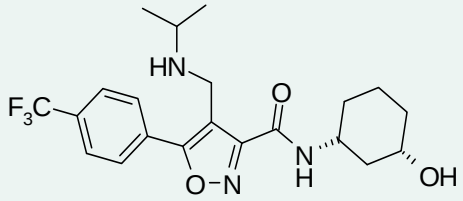


Stereochemistry key

Hydroxy aids potency and solubility

- Promising potency and solubility achieved with this chemotype
  - Region B
  - Introduce polar functionality

Lead optimisation

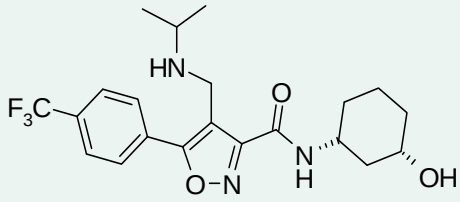


Study	compound
hTRPV1 pIC_{50}	7.2
$eLogD_{(7.4)}$	3
Solkin Solubility	82 mg/L
hERG pKi	5.2
Rat microsomes Cl_{int}	12 (µL/min/mg)
Human microsomes Cl_{int}	12 (µL/min/mg)
Rat Hep. stability Cl_{int}	6 (µL/min/10^6 cells)
Human Hep. Stability Cl_{int}	6 (µL/min/10^6 cells)
CYP450 Inhibition	All isoforms > 50µM
PPB %	74 (human), 68 (rat)

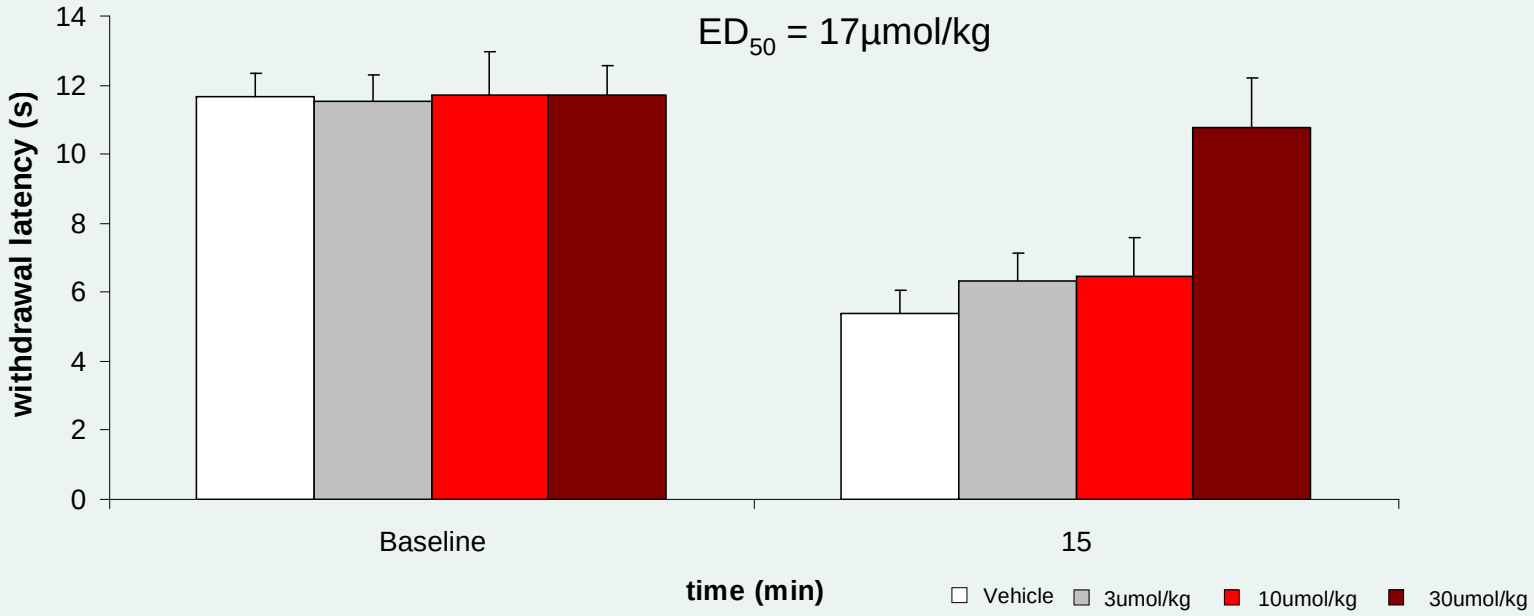
i.v. (2mg/kg)	
Cl (mL/min/kg)	12.5
Vss (L/kg)	5.9
$T_{½}$ (h)	5.4
Oral (10mg/kg) (gelatin/mannitol)	
C_{max} (ng/mL)	212.7
T_{max} (h)	2
$T_{½}$ (h)	6
AUC (ng/mL.h)	2887
F%	19.5

Compound selected for further studies

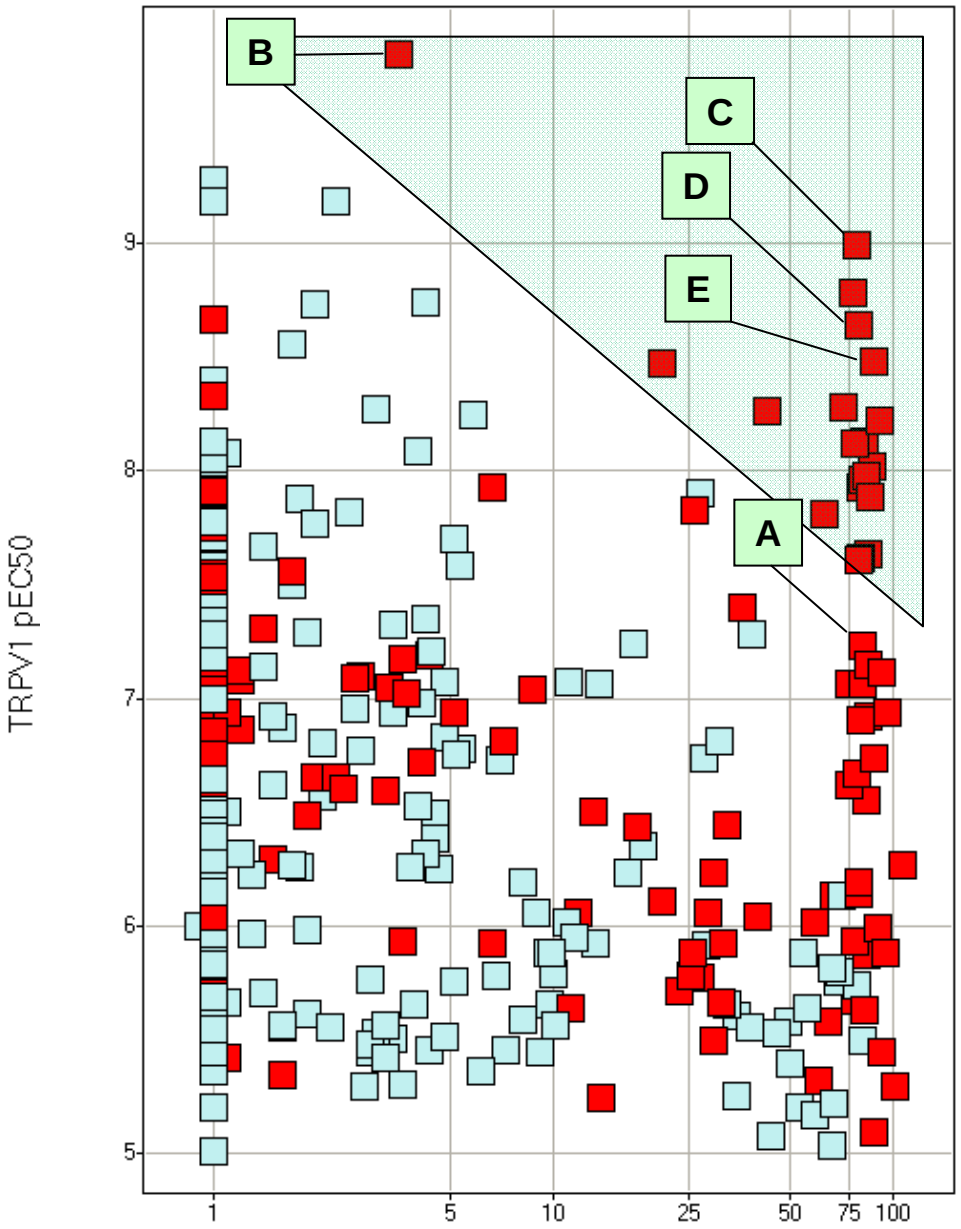
In Vivo Efficacy



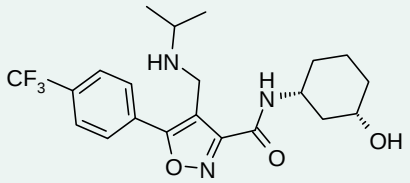
Capsaicin Thermal Hyperalgesia



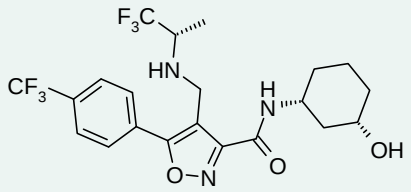
Solubility vs Activity



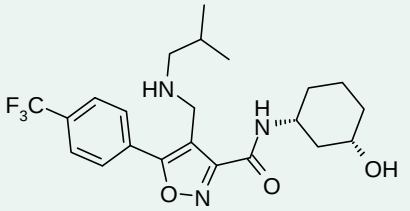
A



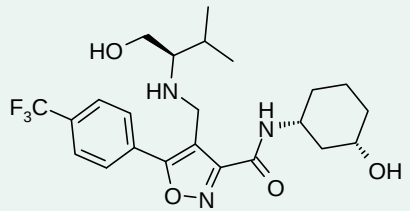
B



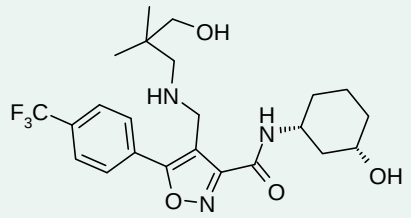
C



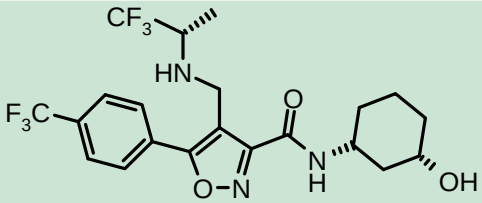
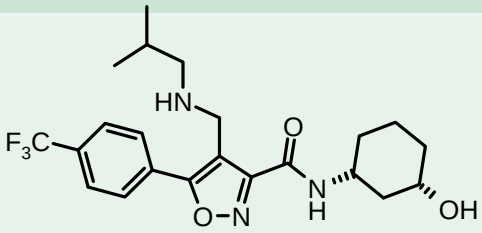
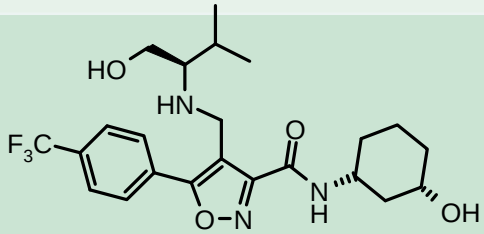
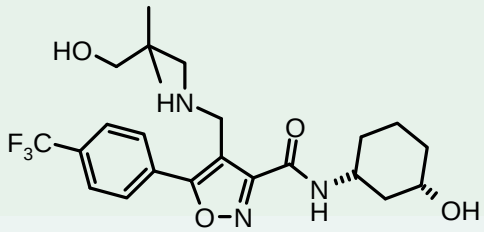
D



E



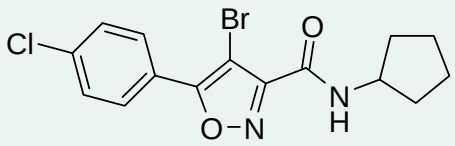
Amines

	TRPV1 pIC_{50}	Solkin (mg/L)	HLM Clint (mL/min.mg protein)	hERG % @ 100μM	PPB (% bound)
	9.8	3.5	66	59	H 95 R 96
	9.0	78	12	86	H 82 R 75
	8.6	80	12	83	H 74
	8.5	88	12	69	H 64

TRPV1 Hit-to-Selection

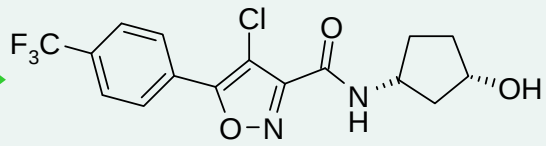
Lead Finding

Library



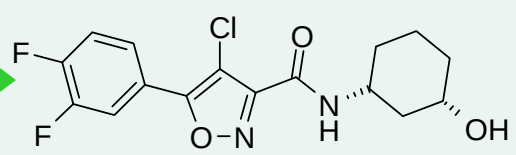
pIC_{50} = 6.6
Solkin <1mg/L

Hit-to-Lead



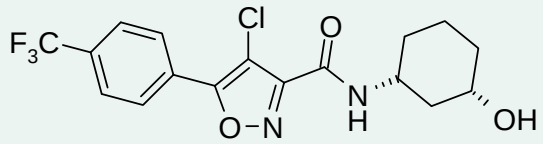
pIC_{50} = 7.6
Solkin 5mg/L

Lead

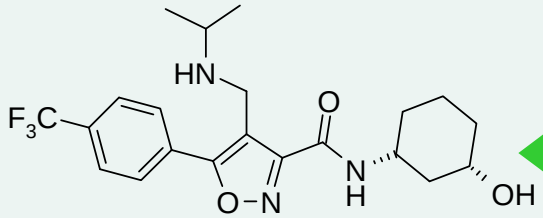


pIC_{50} = 7.1
Solkin 17mg/L

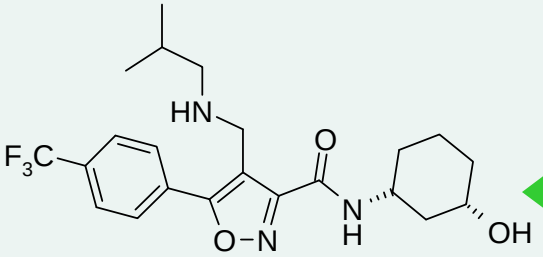
Optimisation



pIC_{50} = 9.2
Solkin 1mg/L



pIC_{50} = 7.2
Solkin 82mg/L



pIC_{50} = 9.0
Solkin 78mg/L

Improved potency and solubility

Questions remain

- Can the on-target hyperthermia be managed
  - Compounds with a shorter half-life
  - Co-dosing with anti-pyretics
  - Modality-specific profiles
  - Individual susceptibility

- We eagerly await the outcome of future clinical trials

Brain Research 2009, Gavva et al.

Summary

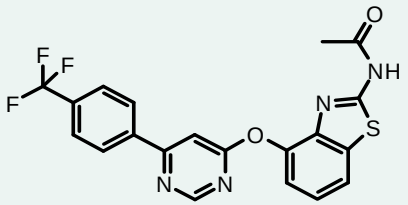
- **Difficult to progress commercially available hit**
- **Pharmacophore modelling in parallel to HTS essential for rapid progression**
- **Inherent poor solubility of series**
- **Novel lead series based on isoxazoles**
  - Potent and water-soluble
  - Efficacious in preclinical animal models of pain
  - Good ADME profile
  - No identified safety/toxicity issues
  - Hyperthermia observed in rats

- Acknowledgements

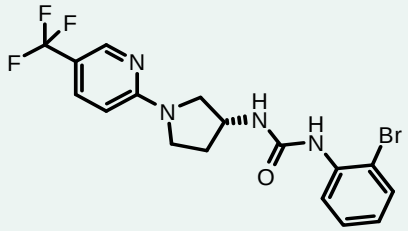
Competition

- Amgen took AMG 517 to Ph I observed hyperthermia
  - Back-up compound AMG 628 hIC_{50} = 0.9nM, active in inflammatory and neuropathic pain models
- GSK – SB 705498 reported in Ph II
  - 400mg reduced capsaicin evoked flare and heat evoked pain
  - No hyperthermia AEs reported
- Abbott ABT-102 clinical trials
- Neurogen/Merck – MK-2295/NGD-8243, Ph II for pain
- Glenmark/Lilly – GRC-6211 Ph II for pain
- Mochida licensed TRPV1 preclinical program to Wyeth (Jan. 2008)
- Japan Tobbacco Inc. JTS-653 phase I

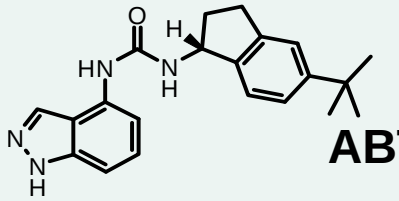
- Discovery – several candidates/originators including Renovis/Pfizer, J & J, Pacific, Sanofi-Aventis, Sangamo, Digital Biotech, DiverDrugs



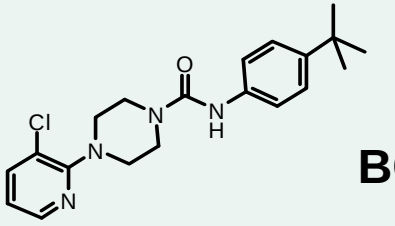
AMG-517



SB-705498



ABT-102



BCTC

Pain 2007, 132(1-2), 132