



# Finding the Gini: brain penetrant kinase inhibitors for the treatment of neurodegenerative diseases

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14 September, 2011

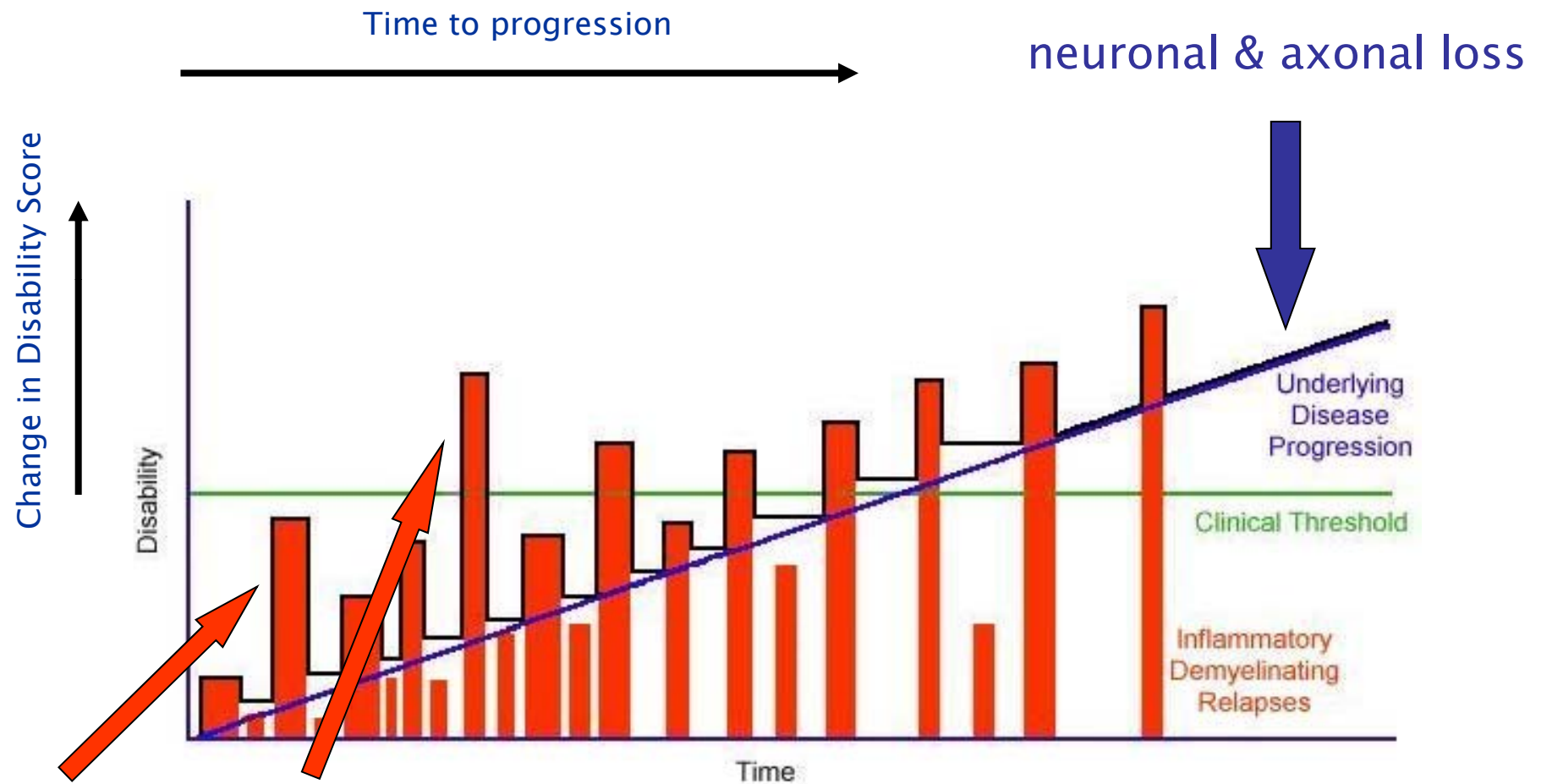
Piotr Graczyk

Eisai Ltd. Hatfield, UK

*hhe*  
human health care

1. Multiple sclerosis – brief introduction
2. Origin of the 7-azaindole series
3. SAR and chemistry of the prototype series
4. In vivo activity
5. Why selectivity may be needed
6. How to measure selectivity – Gini coefficient
7. Finding compounds with improved Gini
8. Explanation of selectivity
9. Synthesis of the best series
10. Activity *in vivo*

# Multiple sclerosis – clinical presentation



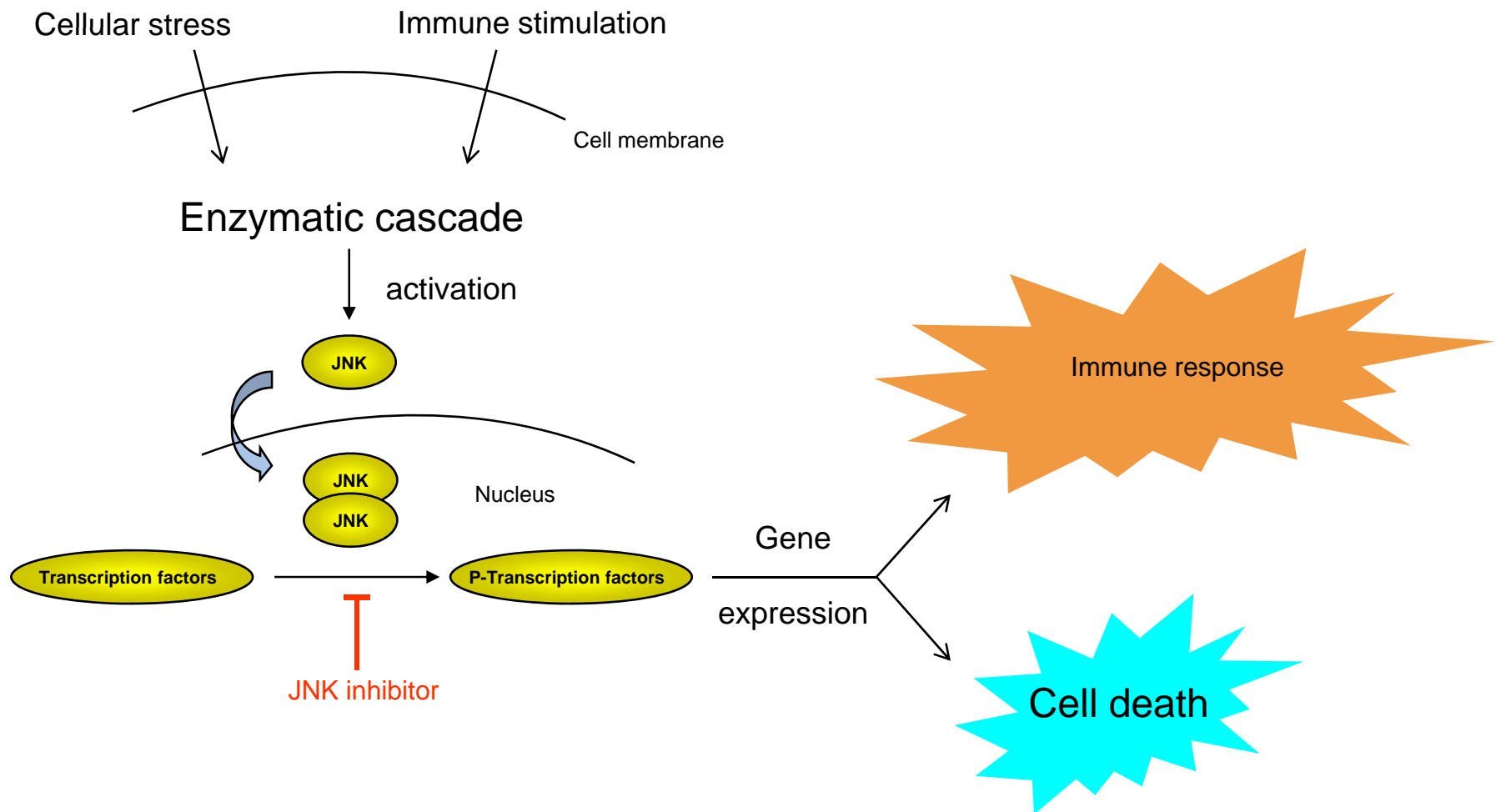
Inflammation  
IFN $\beta$ , Tysabri, Copaxone, FTY720

Compston & Coles, 2008

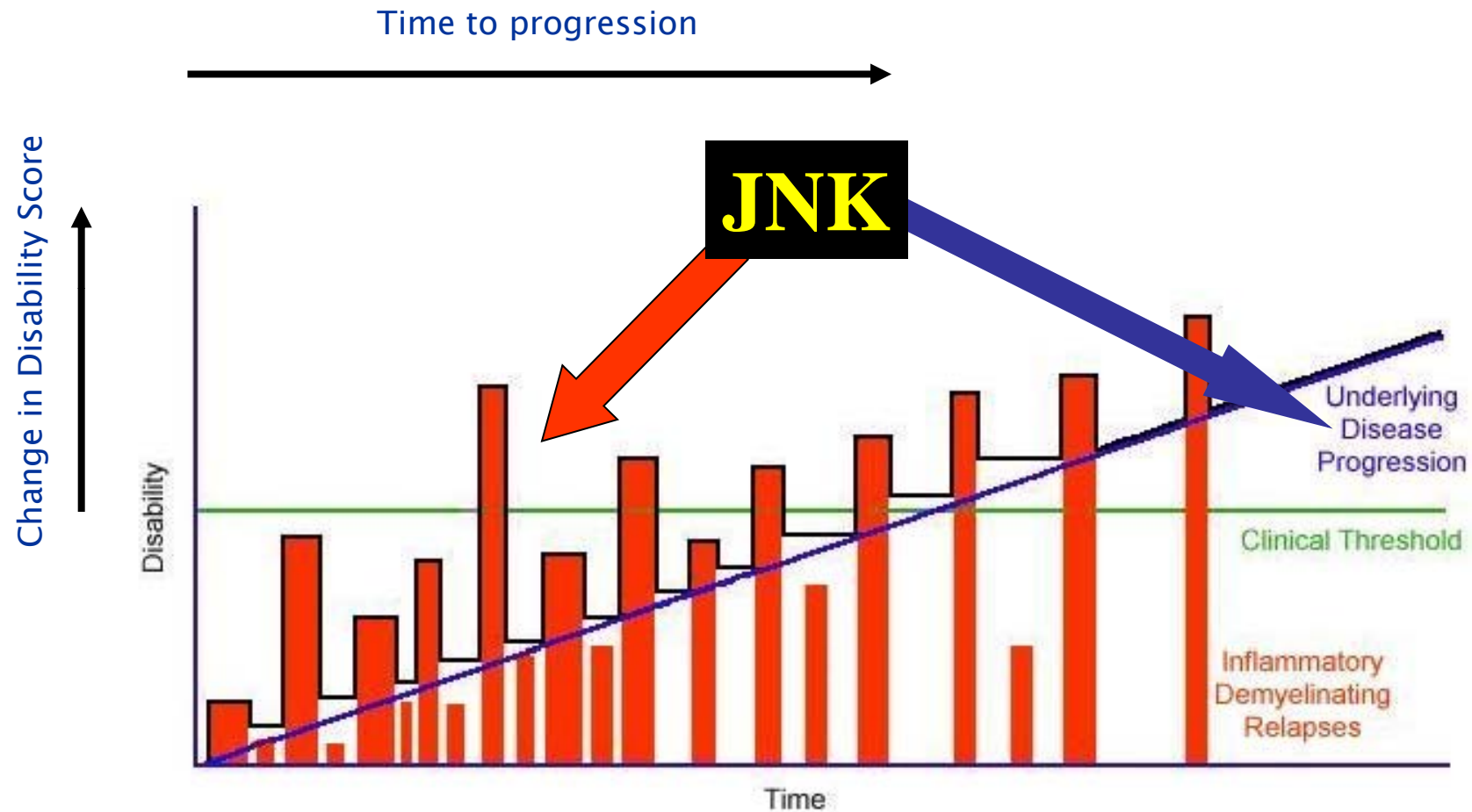
# Role of JNK



*JNK  $\equiv$  c-Jun N-terminal kinase*



# Multiple sclerosis – clinical presentation



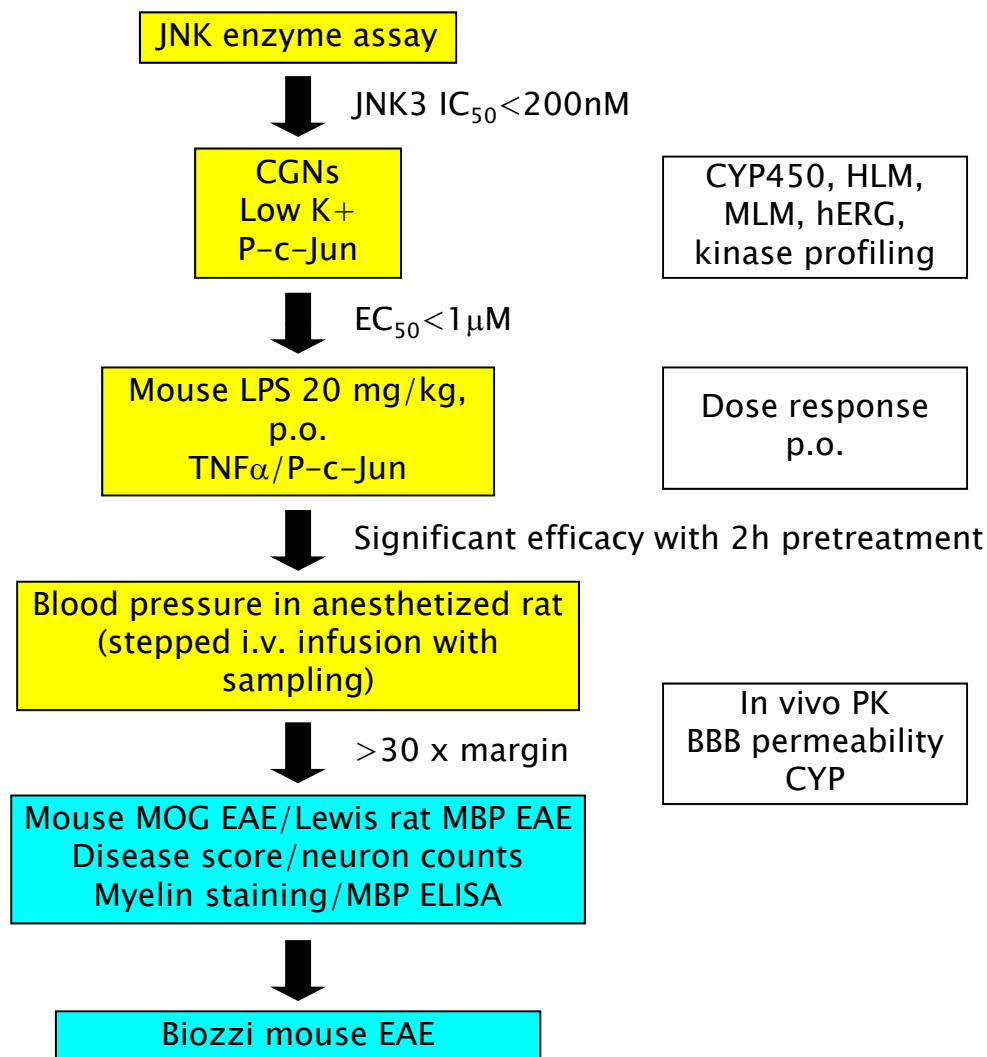
Compston & Coles, 2008

# JNK inhibitors for MS



- Envisaged Clinical Profile
  - neuroprotection (JNK3 inhibition)
  - antiinflammatory/immunomodulatory activity (JNK1 and JNK2 inhibition)
- Selectivity
  - pan – JNK inhibitor
  - Selectivity against all other kinases
- Other requirements
  - CNS penetration
  - Once daily dosing
  - Oral bioavailability
  - Good tolerability

# Screening cascade



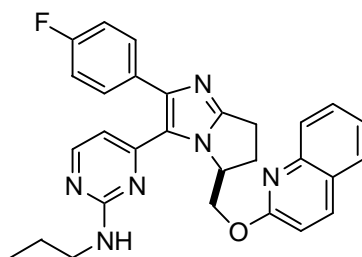
## Explanations:

Biozzi mice =	mice developing chronic relapsing remitting form of EAE
c-Jun =	transcription factor phosphorylated by JNK
CGN Low K <sup>+</sup> =	low-potassium induced death in cerebellar granule neurons
EAE =	experimental autoimmune encephalomyelitis
MBP =	Myelin basic protein
MOG =	Myelin oligodendrocyte glycoprotein
Mouse LPS =	LPS-induced TNFα release in mice
P-c-Jun =	Phospho-c-Jun

# Early JNK research at Eisai



- Neuroprotection
  - Focus on JNK3 inhibition
  - 6,7-dihydro-5H-pyrrolo[1,2-a]imidazole series of compounds (*Bioorg. Med. Chem. Lett.* 2005, 15, 4666)



JNK3 IC<sub>50</sub> [nM] 85

SCG\* ED<sub>50</sub> [μM] 0.3–1.0

B/P (100 mg/kg i.p.)	mouse	0.42 (357 ng/g brain)
	rat	0.57 (709 ng/g brain)

BA (10 mg i.p. rat) 32%

BA (10 mg p.o. rat) 5%

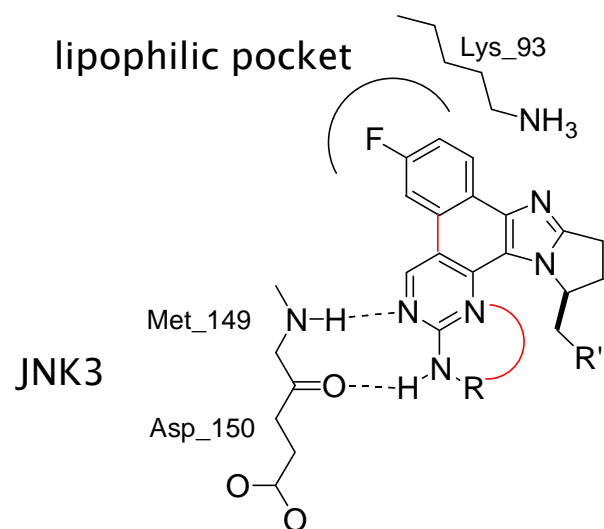
\*SCG – superior cervical ganglion neurons



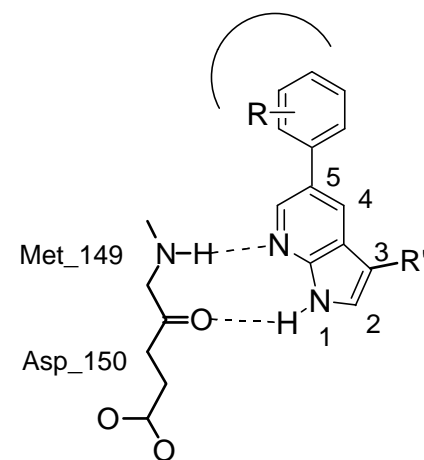
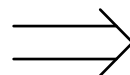
# 7-Azaindoles



Build on past knowledge



6,7-dihydro-5H-pyrrolo[1,2-a]imidazole

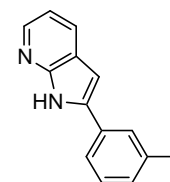
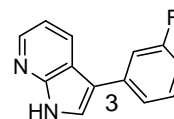
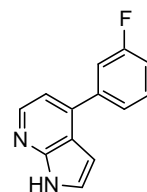
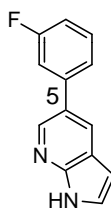
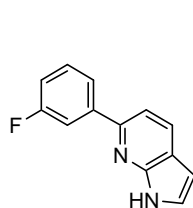


7-azaindole

# Initial SAR



“Active” positions



JNK3 IC<sub>50</sub> [μM]

>10

4.6

>10

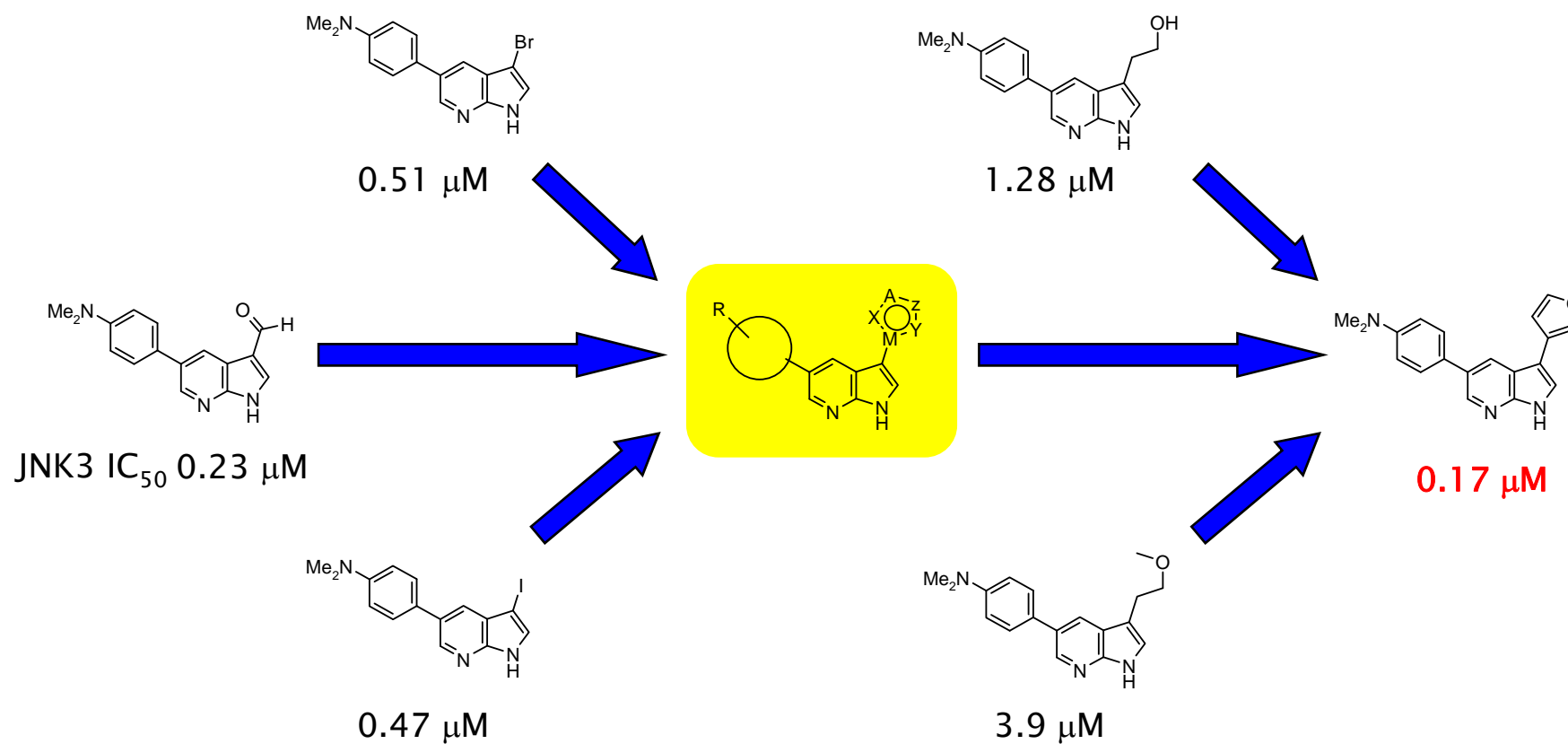
3.4

>10

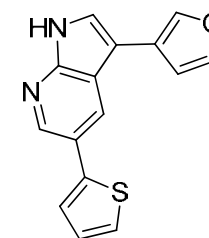
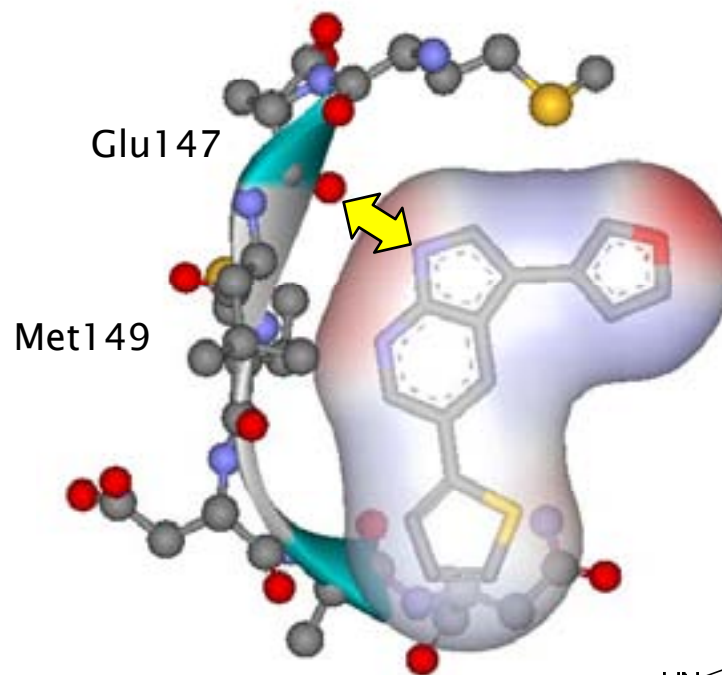
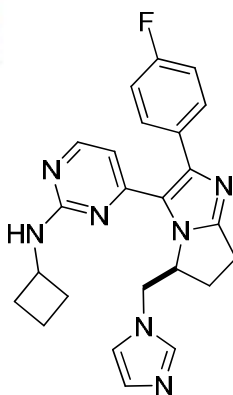
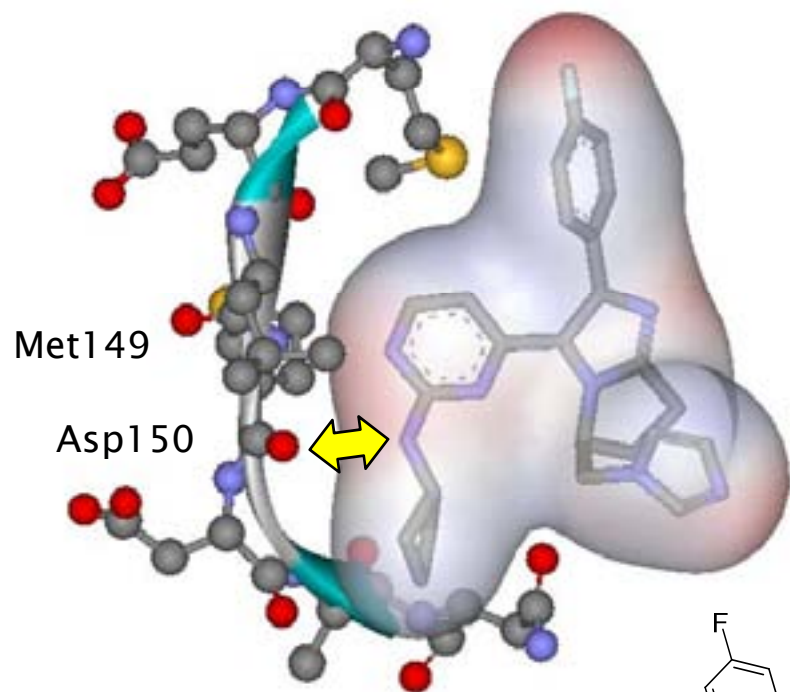
# Initial SAR



## Position (3) – small $\Pi$ -system



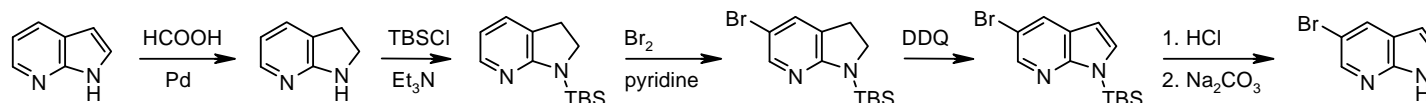
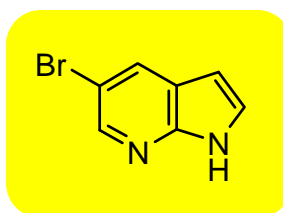
# Serendipity



# Making the chemistry tractable

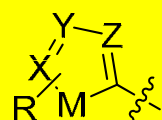
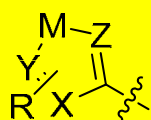
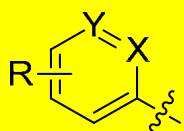


## Synthesis of key intermediate

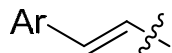
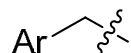
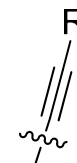
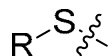
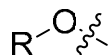
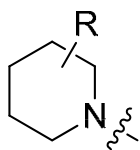
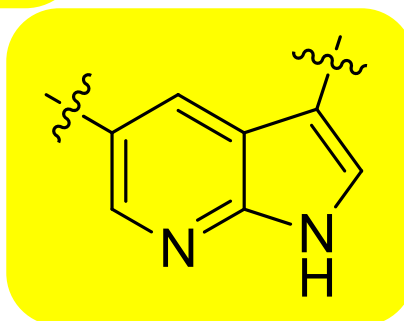
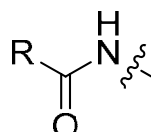
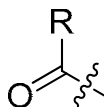
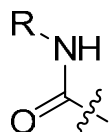
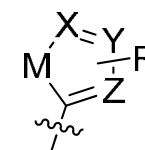
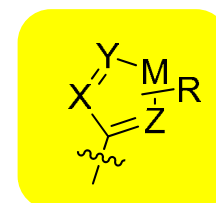
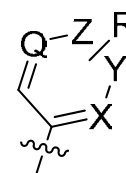


Total yield over 5 steps 205.5 g (82%)

# General SAR



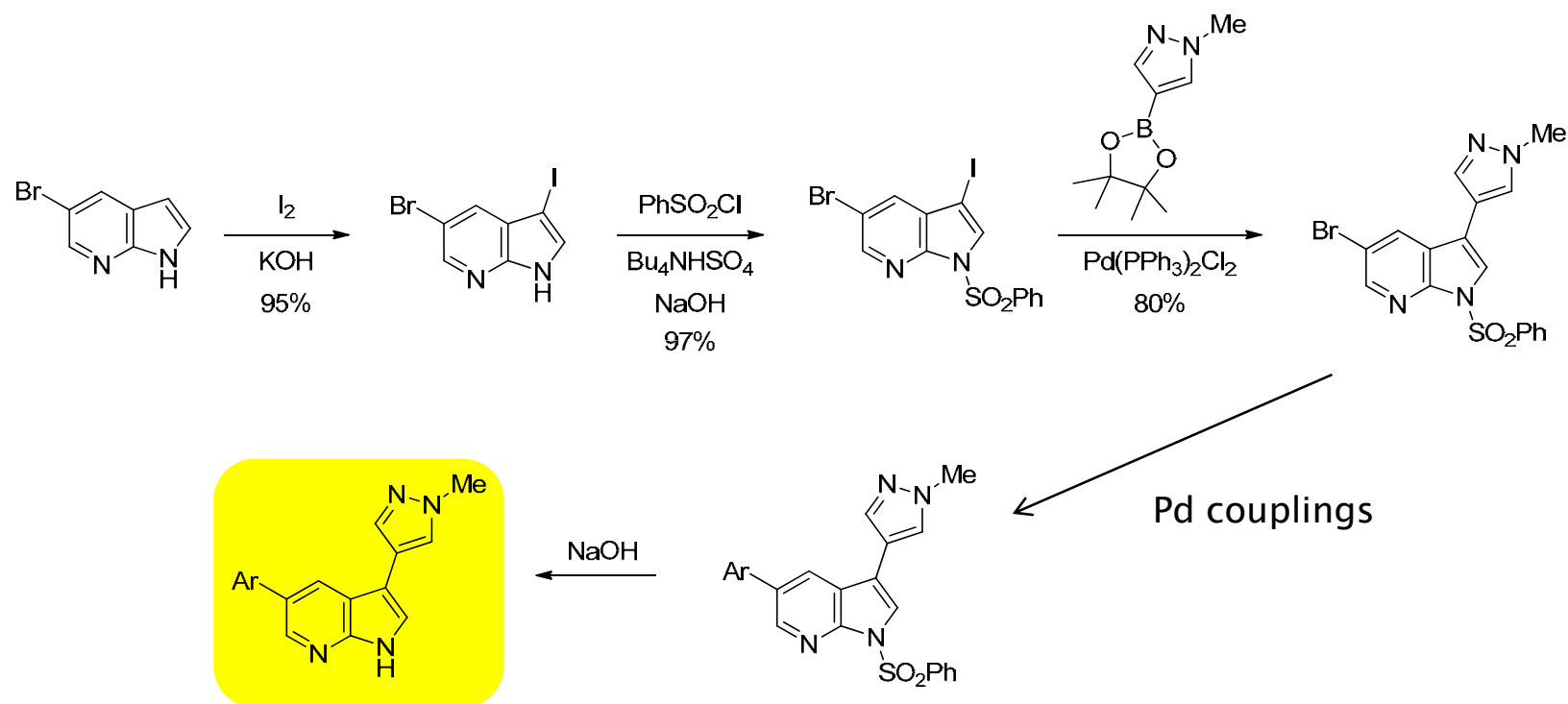
X, Y, Z, Q=CH, N    M=O, S, N-



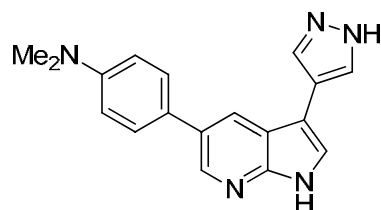
# Making the chemistry tractable



## Approach to C(5) derivatisation



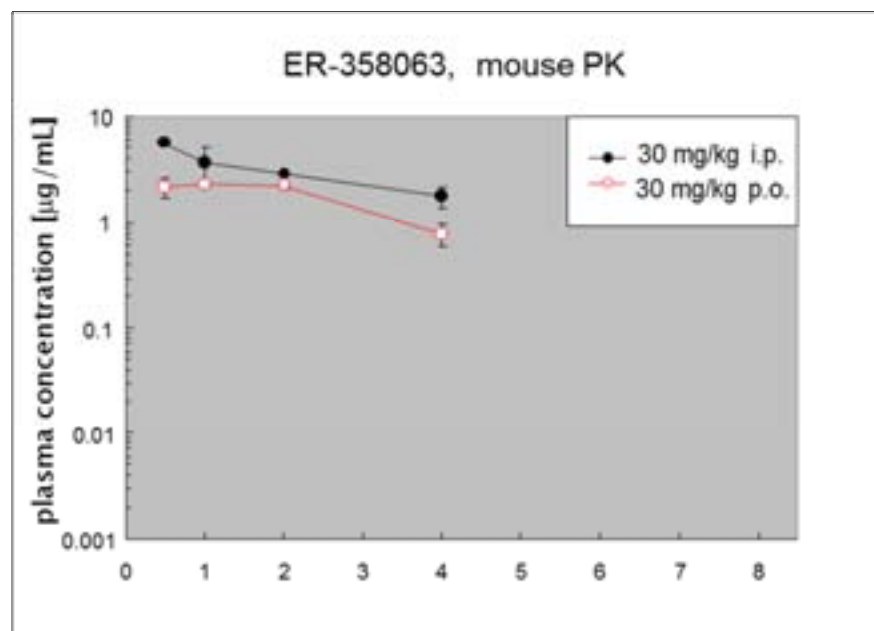
# An example: ER-358063



ER-358063

JNK1, 2, 3 IC<sub>50</sub> 101, 59, 32 nM

3.3 µg/mL PBS (pH 7.2) (around 10 µM)



B/P = 1.4 (after i.p. administration)

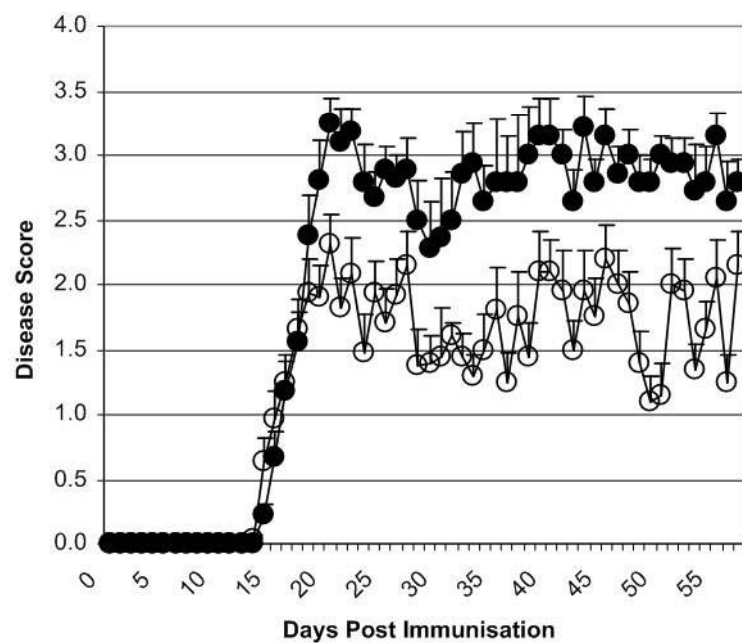
T<sub>1/2</sub> = 2.8 h (i.p.) 1.3 h (p.o.)



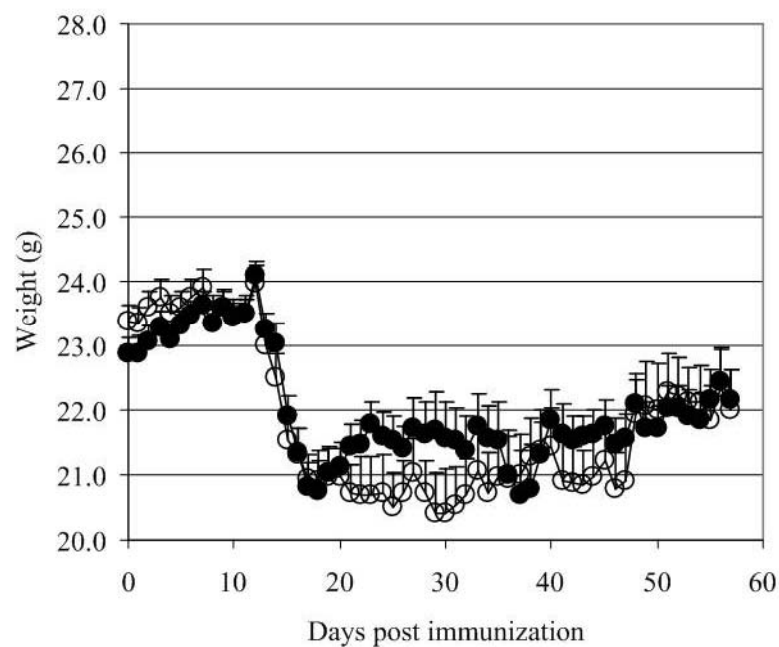
# ER-358063 in the EAE model



EAE model in mice 20 mg/kg p.o. once daily; ○ – compound, ● – vehicle



Disease score



Animal weight

# ER-358063: selectivity at 1 $\mu$ M



	% of Control (10 $\mu$ M ATP) ER-358063 @ 1 $\mu$ M		% of Control (10 $\mu$ M ATP) ER-358063 @ 1 $\mu$ M
Abl(h)	16	MKK4(m)	53
AMPK(r)	22	MKK6(h)	58
Aurora-A(h)	12	MKK7 $\beta$ (h)	49
CaMKII(r)	62	MST2(h)	6
CDK1/cyclinB(h)	2	p70S6K(h)	34
CDK2/cyclinA(h)	5	PDGFR $\alpha$ (h)	77
CDK3/cyclinE(h)	17	PDGFR $\beta$ (h)	30
CDK5/p35(h)	2	PDK1(h)	7
CDK6/cyclinD3(h)	11	PKA(h)	91
CDK7/cyclinH/MAT1(h)	13	PKC $\alpha$ (h)	85
CHK1(h)	17	PKC $\beta$ II(h)	87
CK1 $\delta$ (h)	20	PKC $\gamma$ (h)	76
c-RAF(h)	88	PKC $\delta$ (h)	92
cSRC(h)	46	PKC $\epsilon$ (h)	103
EGFR(h)	114	PKD2(h)	11
EphB2(h)	69	Ret(h)	11
FGFR3(h)	21	ROCK-II(h)	4
Fms(h)	14	Rsk1(h)	24
Fyn(h)	19	SAPK2b(h)	57
GSK3 $\alpha$ (h)	6	SAPK3(h)	77
GSK3 $\beta$ (h)	23	SAPK4(h)	89
IGF-1R(h)	71	TrkA(h)	1
IKK $\alpha$ (h)	5	TrkB(h)	2
MAPK1(h)	41	ZAP-70(h)	98
MAPKAP-K2(h)	90	PI3Kg(h)	72
MEK1(h)	88		

	>70% Inhibition
	50-70% Inhibition
	<50% Inhibition

Problem: low selectivity

# How to improve selectivity?



- In order to improve selectivity one needs to have an objective measure
- Such measure should help identify direction and guide further SAR exploration
- The measure should work with the data which can be obtained quickly and at low cost
- No such measure available at that time

# Chemistry and economy

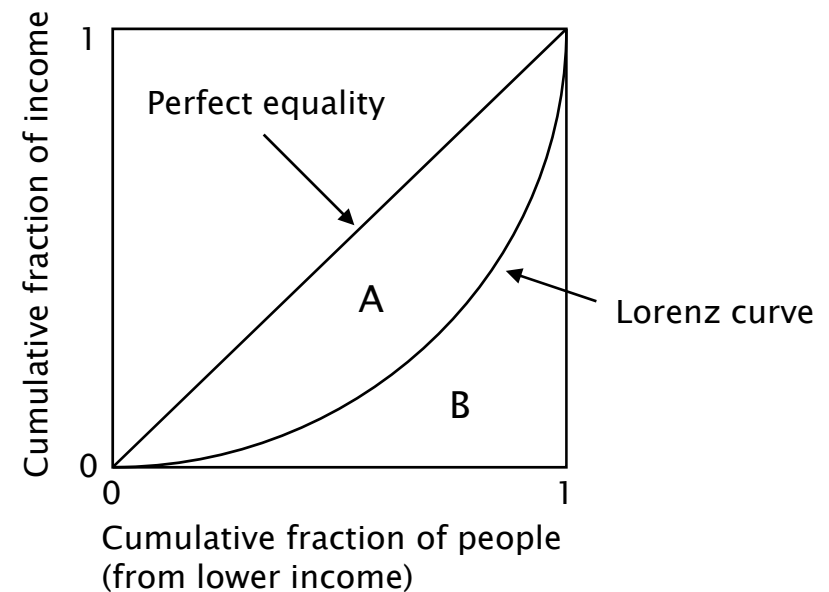


- Selectivity  $\cong$  inequality
- Economy
  - income inequality is measured by Gini coefficient  $G$

$$G = \frac{A}{A+B} = 1 - 2 \times B$$

Perfect equality:  $G = 0$

Extreme inequality:  $G = 1$



# Gini coefficient for selectivity

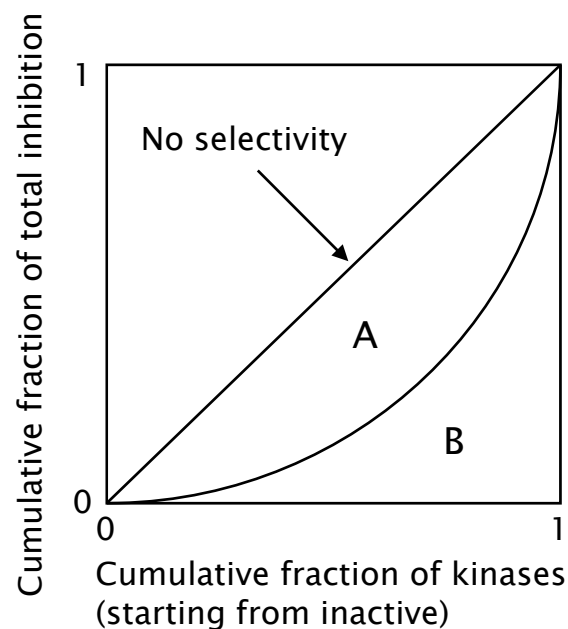


- Let us take:
  - Percentage of kinase inhibition instead of income
  - Kinases instead of people

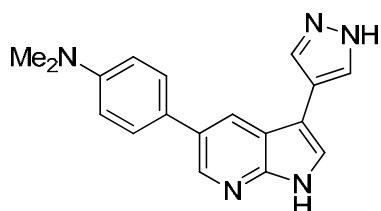
$$G = \frac{A}{A+B} = 1 - 2 \times B$$

No selectivity:  $G = 0$

Perfect selectivity:  $G = 1$

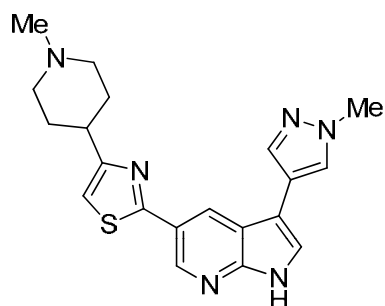


# Examples

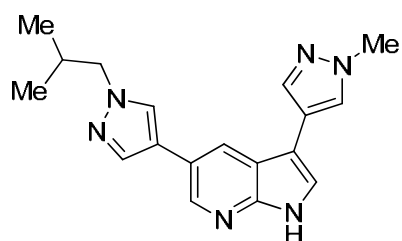


G

0.34

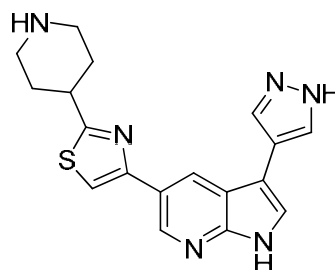


0.38



G

0.38



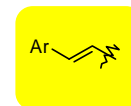
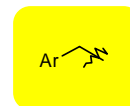
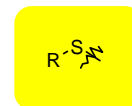
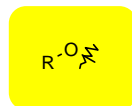
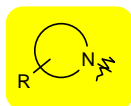
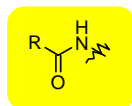
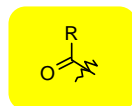
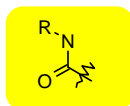
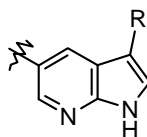
0.41

inhibitor	concn ( $\mu$ M)	G
		10 $\mu$ M ATP
Staurosporine	1	0.093
K252c	10	0.236
Indirubin	10	0.291
Curcumin	50	0.417
Ro31-8220	1	0.432
H89	10	0.442
AG1478	10	0.5
SB202190	10	0.553
AG1024	10	0.568
Genistein	10	0.582
Calphostin C	10	0.606
SU6656	1	0.607
SB203580	10	0.621
Y27632	10	0.628
ZM336372	10	0.635
Lavendustin A	1	0.726
Roscovitine	10	0.744
Wortmannin	1	0.775
Hispidin	10	0.79
PD184352	10	0.802

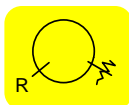
# Where to find selective compounds?



- Already explored at C(5)



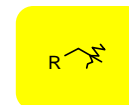
But missing: C(5)-saturated cycles and C(5)-aliphatic chains



C(5)-carbocycle



C(5)-saturated heterocycle



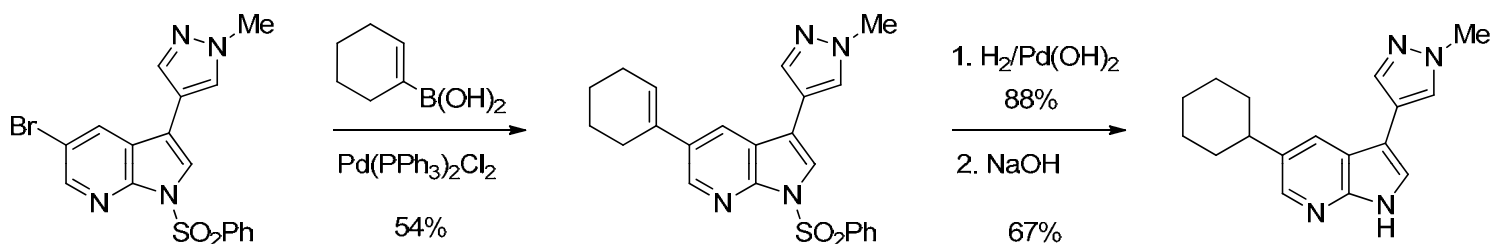
C(5)-carbon chain

Low potency in vitro

# Where to find selective compounds?



- Prototype cyclohexyl derivative

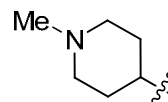
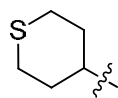
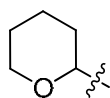
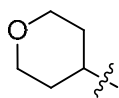


ER-417245

JNK1, 2, 3  $\text{IC}_{50}$  74, 135, 40 nM

*G* 0.57

- Other saturated rings



JNK3  $\text{IC}_{50}$  [nM]

60

640

148

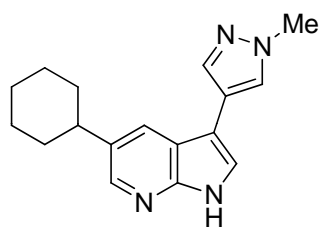
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# Cyclohexyl derivatives



- Prototype cyclohexyl derivative



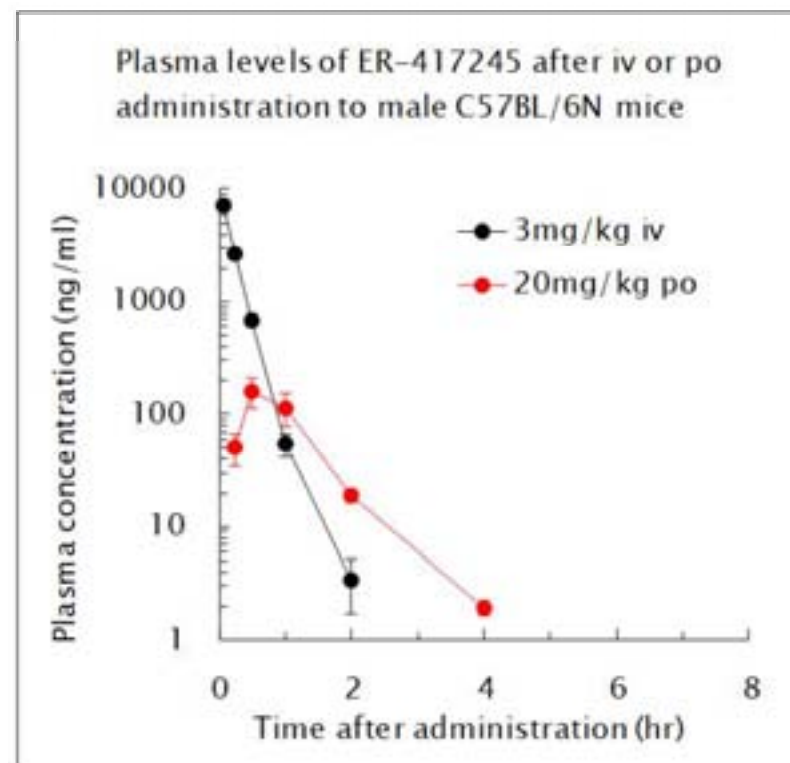
ER-417245

mLM  $T_{1/2}$  [min]                      4.37

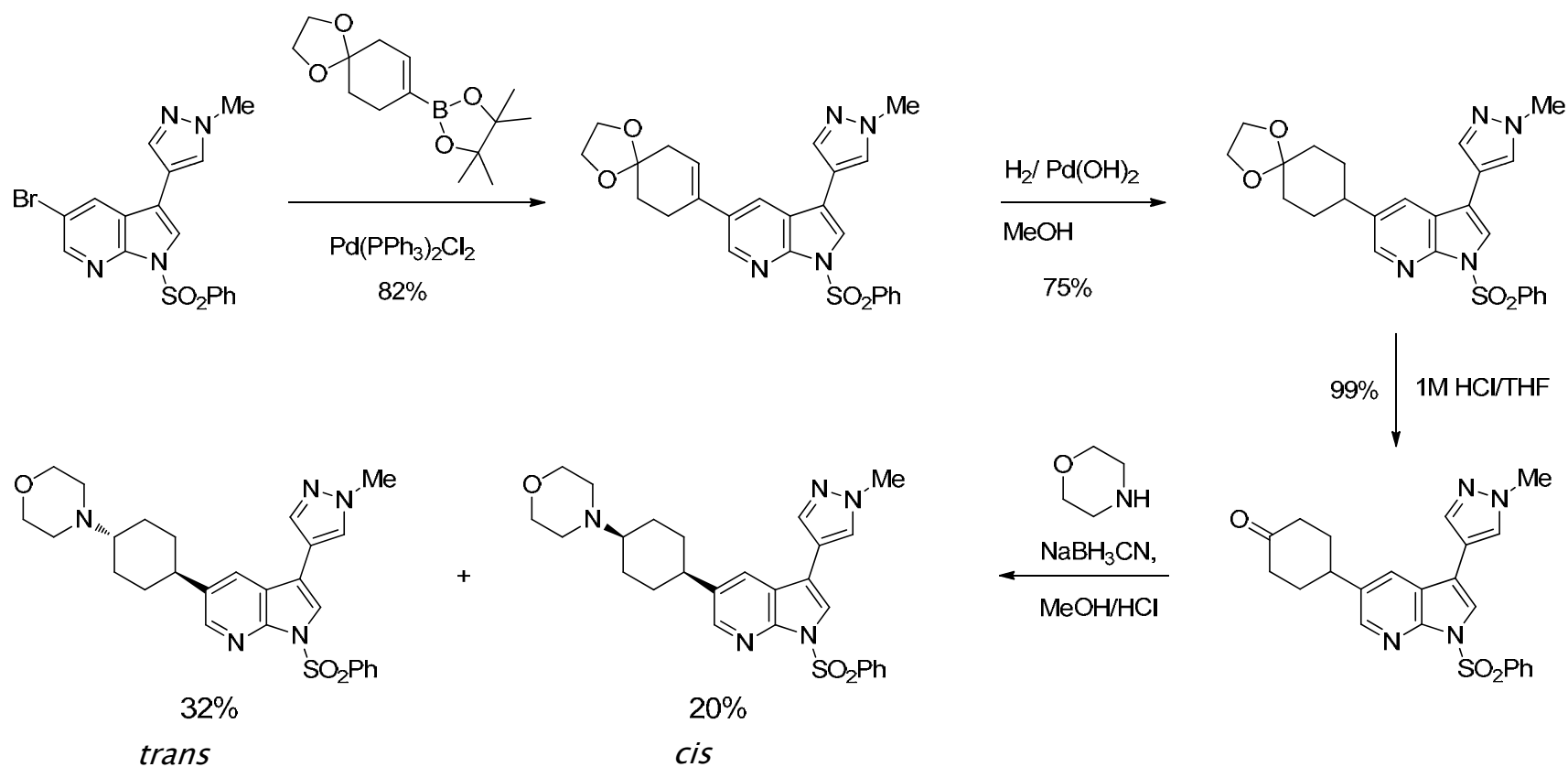
Peripheral *in vivo* model

TNF- $\alpha$ /P-c-Jun                      45%/20%

(LPS-induced TNF- $\alpha$  production in BCG-primed C57Bl/6 mice)



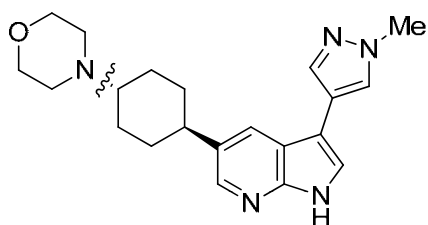
## • Approach to an initial SAR study



# Cyclohexyl derivatives

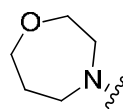


## • *Trans* derivatives



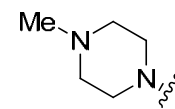
ER-409903

JNK3 IC<sub>50</sub> [nM] 80



ER-417258

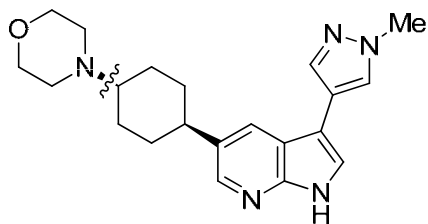
129



ER-409923

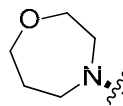
90

## • *Cis* derivatives



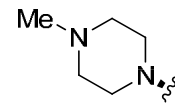
ER-409904

JNK3 IC<sub>50</sub> [nM] 60



ER-417257

184



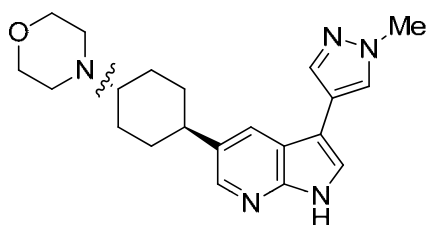
ER-409924

94

# Cyclohexyl derivatives



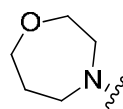
## • *Trans* derivatives



ER-409903

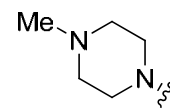
*G*

0.72



ER-417258

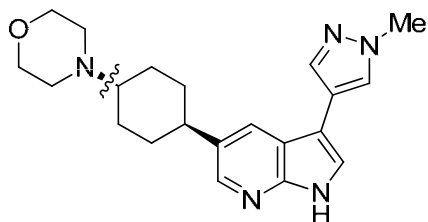
0.77



ER-409923

0.68

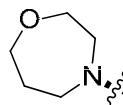
## • *Cis* derivatives



ER-409904

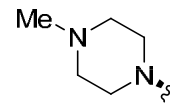
*G*

0.70



ER-417257

0.77



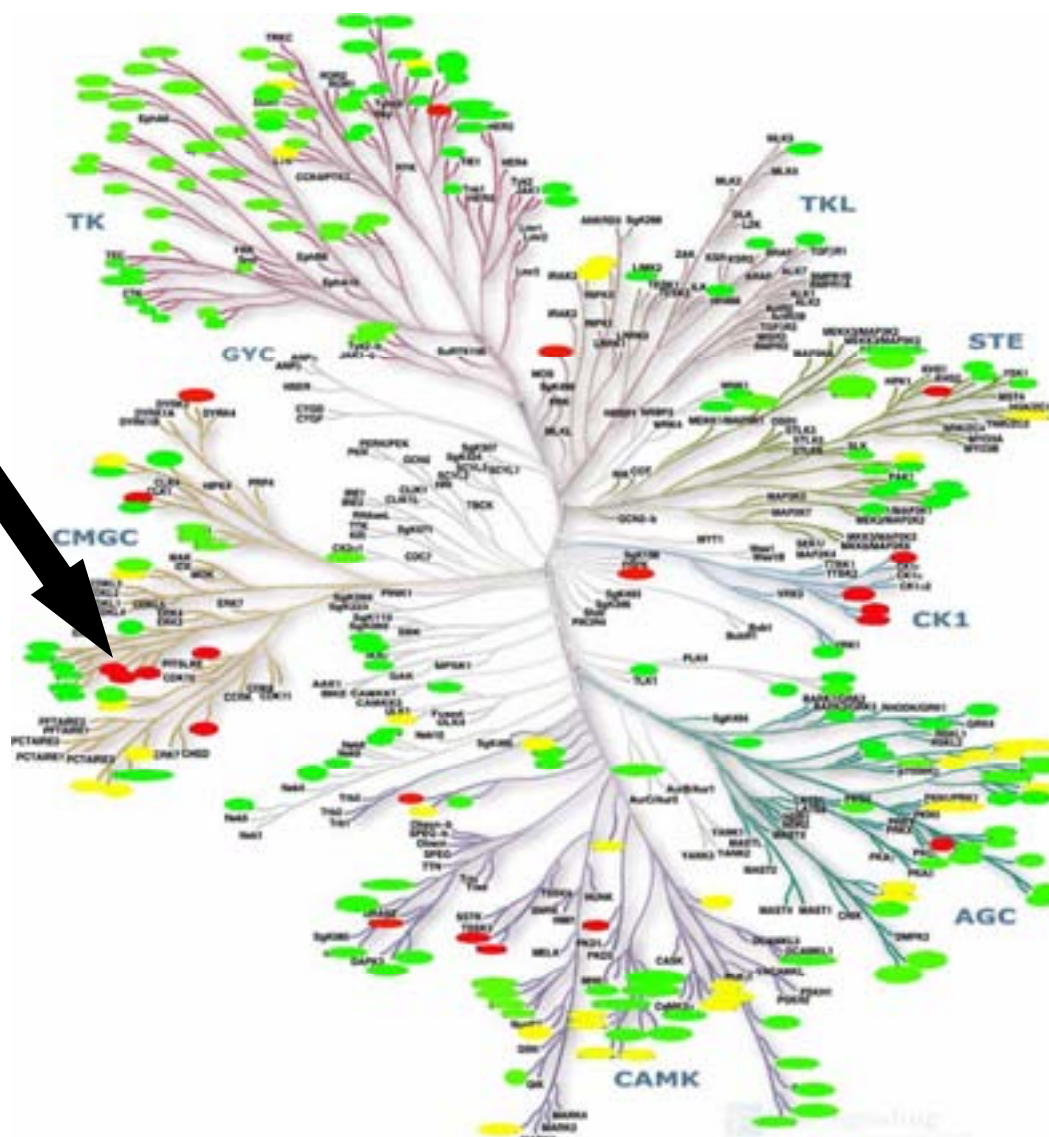
ER-409924

0.67

# Selectivity of ER-417258



JNKs



% Activity

■ <30%

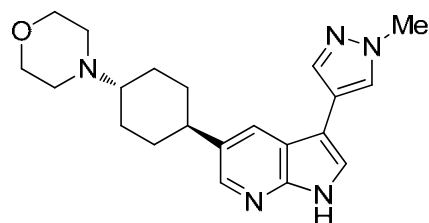
■ 30-70%

■ >70%

# Origin of selectivity



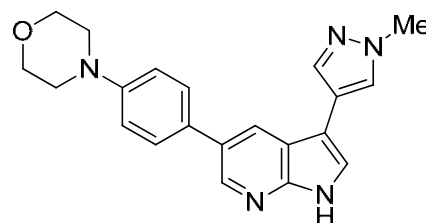
- Cyclohexyl derivatives are more selective than aromatic derivatives
- To explain this we carried out X-ray analysis of JNK3 with two representative compounds



ER-409903

*G*

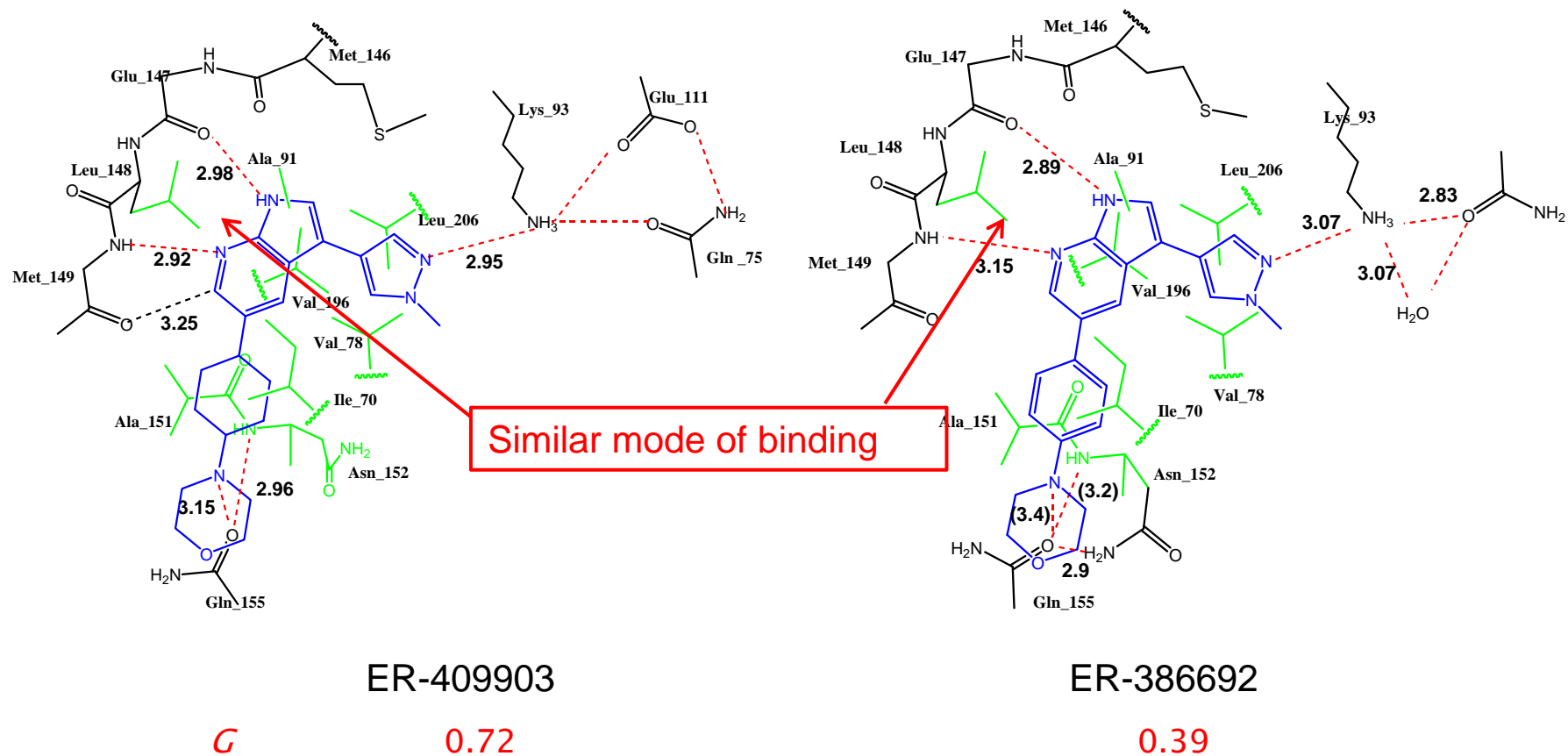
0.72



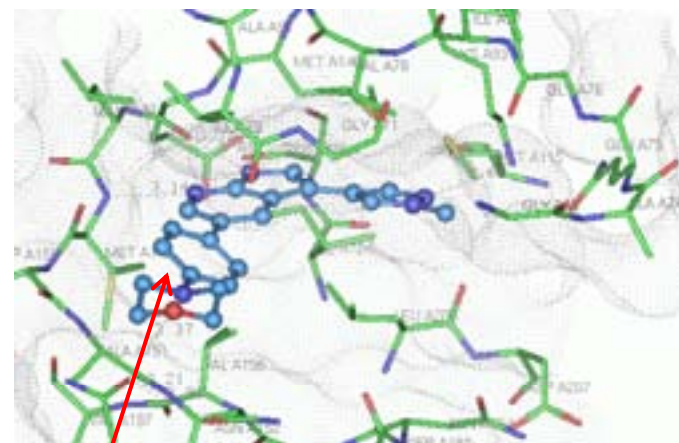
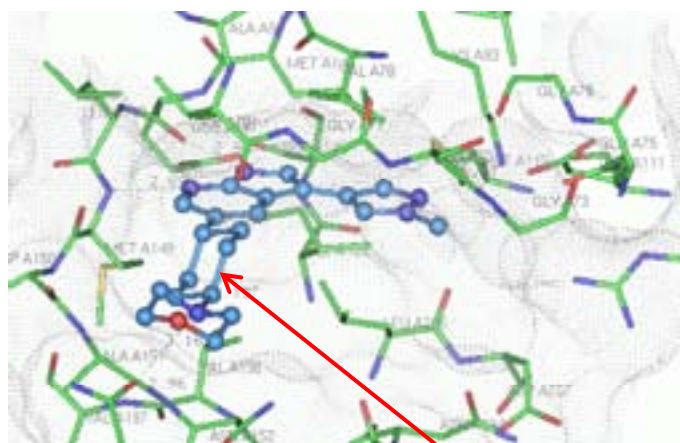
ER-386692

0.39

# Origin of selectivity



# Origin of selectivity



Different conformations

ER-409903

*G*

0.72

ER-386692

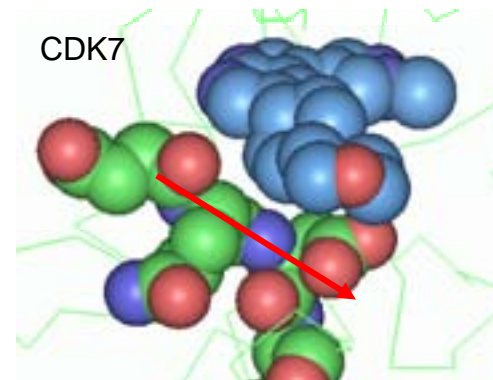
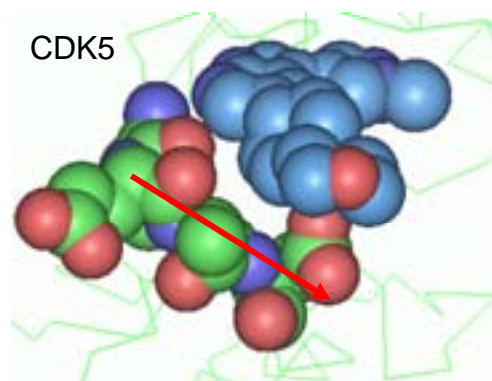
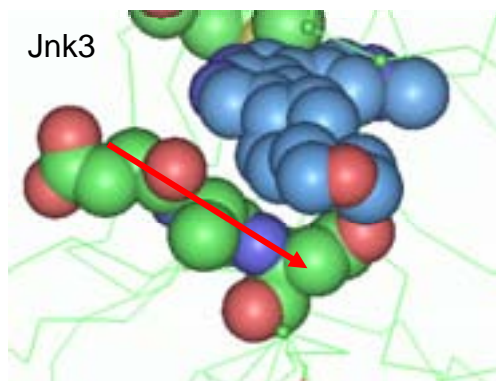
0.39



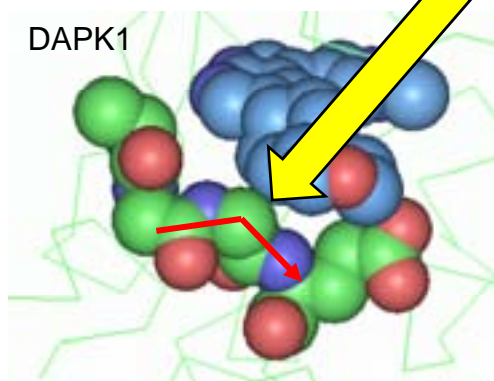
# Loop next to the hinge region



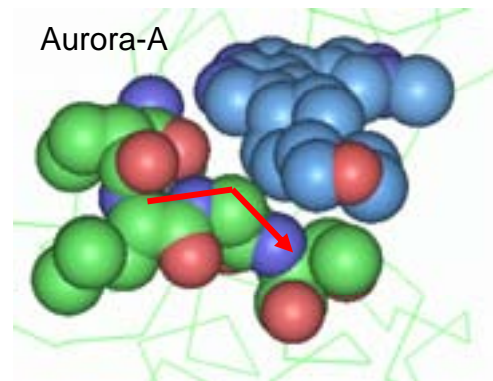
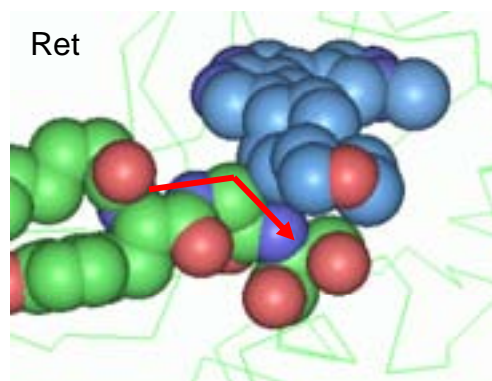
Kinases inhibited



Kinases not inhibited



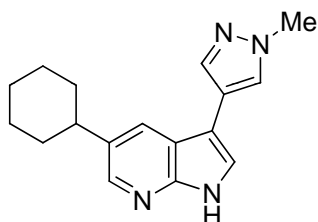
bent conformation at Gly residue. Not enough room to accommodate the cyclohexyl ring



# Cyclohexyl derivatives



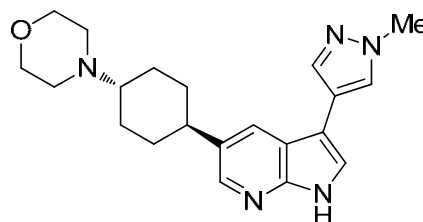
- Metabolic stability



ER-417245

mLM  $T_{1/2}$  [min]

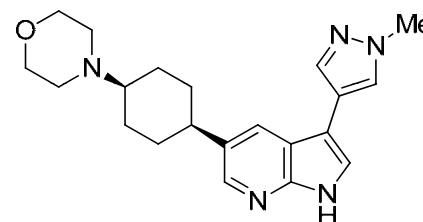
4.37



*trans*

ER-409903

151



*cis*

ER-409904

13.4

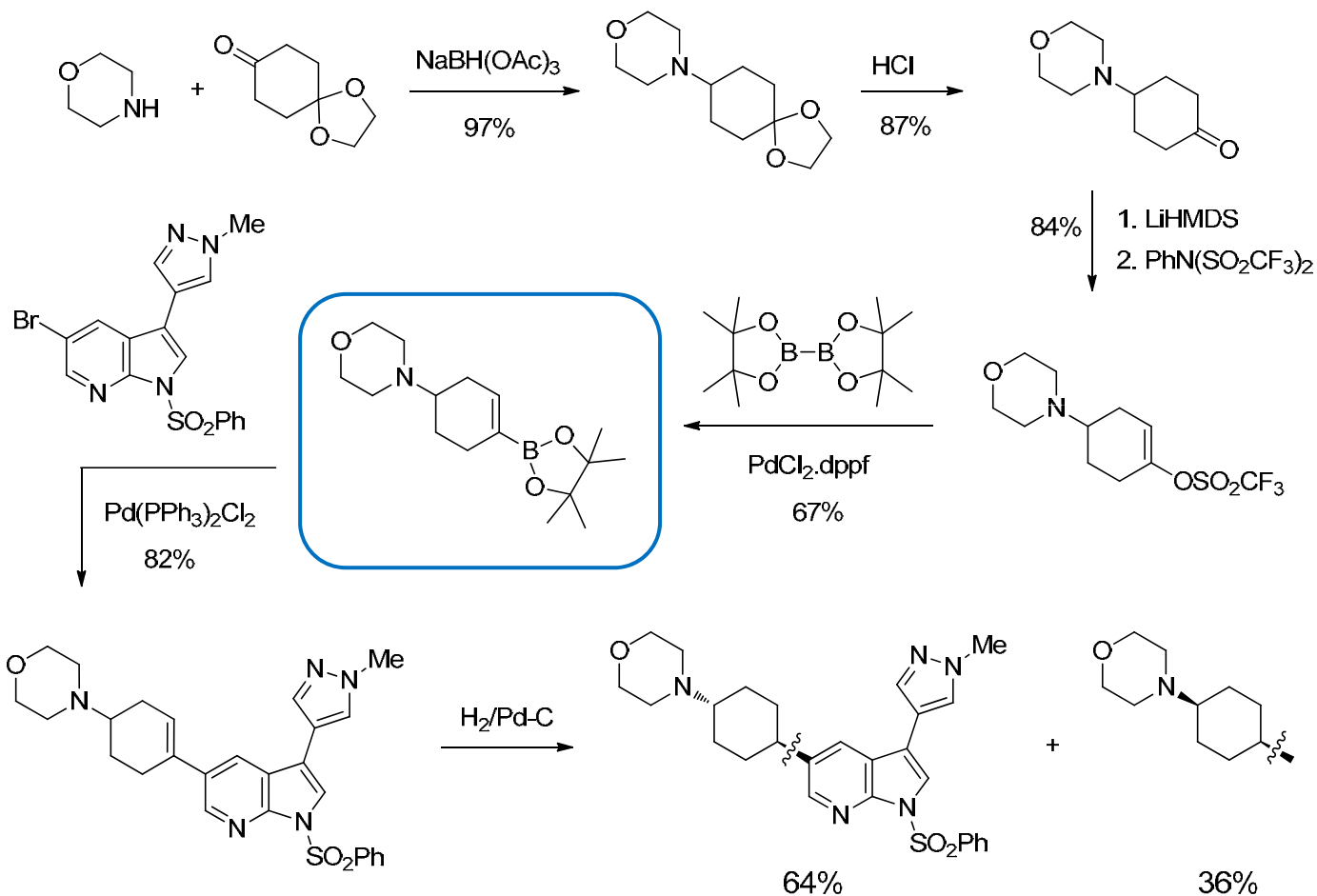
*Trans* isomers more metabolically stable

Need an efficient method of preparation

# Synthesis of trans derivatives



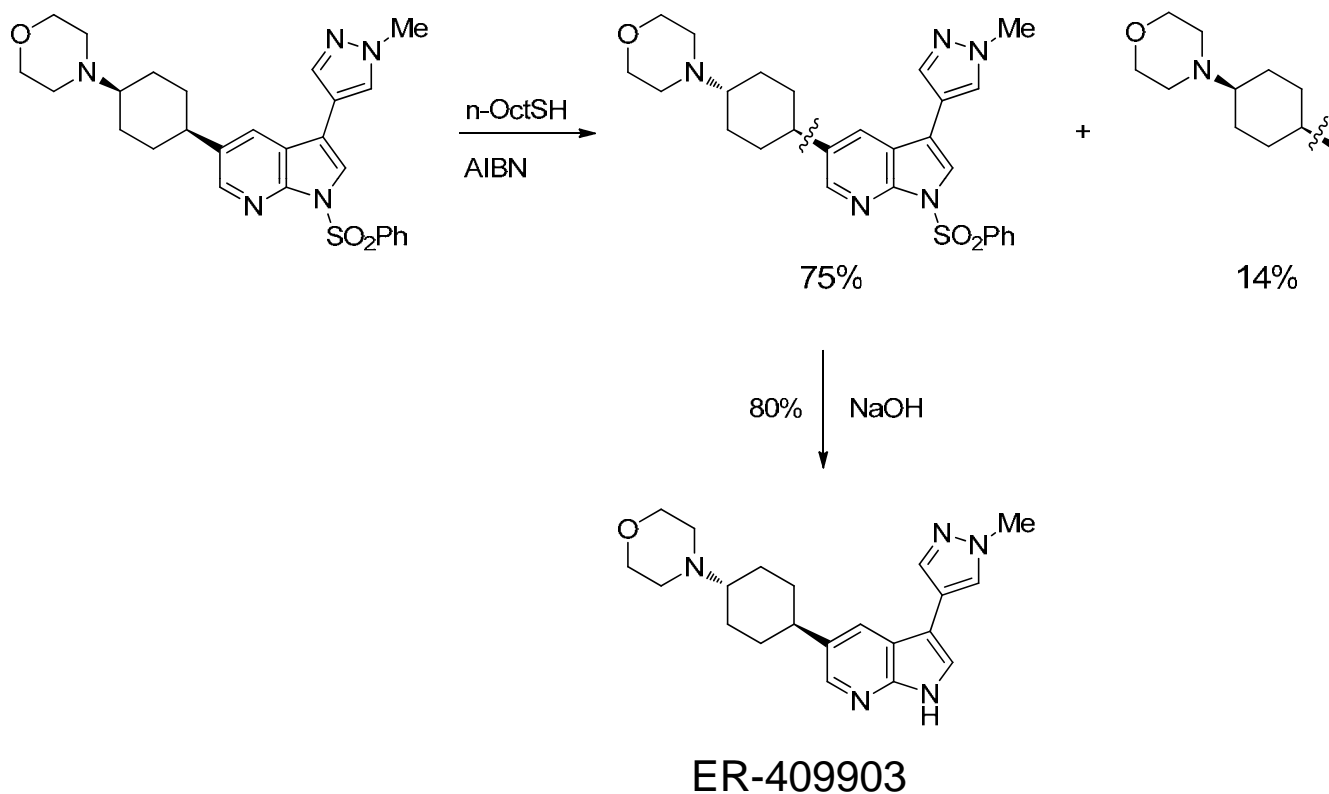
- Key material: the relevant boronic ester



# Synthesis of trans derivatives



## • Isomerisation



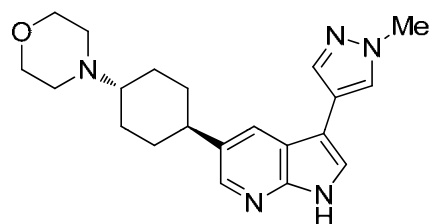
Isomerisation method adopted from Bertrand *et al.* (*J. Org. Chem.* 2006, 71, 7288)

# Activity in vivo - comparison



EAE model in mice 20 mg/kg p.o. once daily; ○ – compound, ● – vehicle

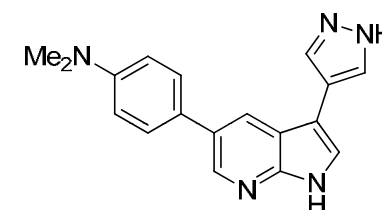
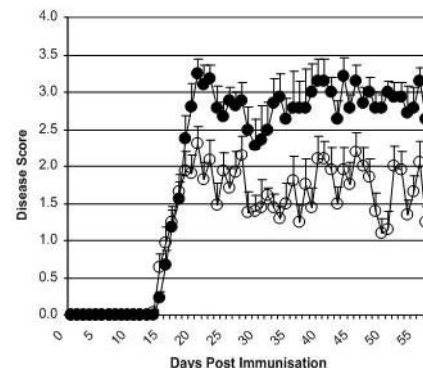
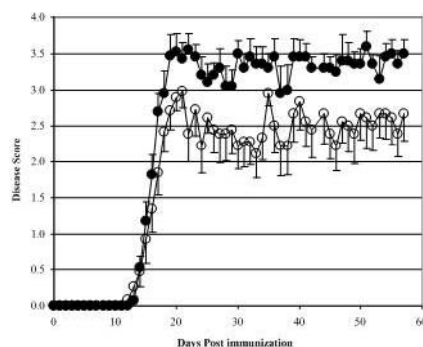
Similar pharmacological effect



ER-409903

JNK3 IC<sub>50</sub> 80 nM

*G* 0.72

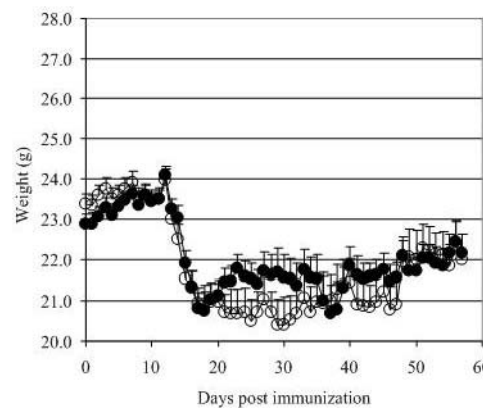
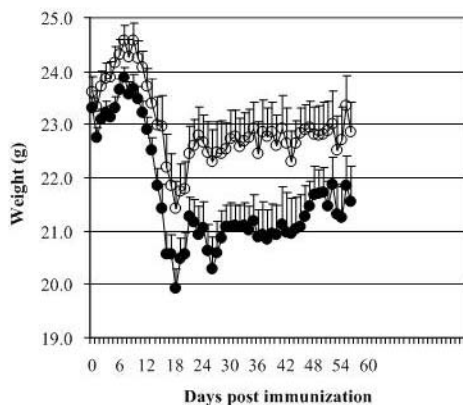


ER-358063

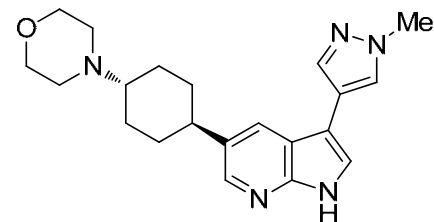
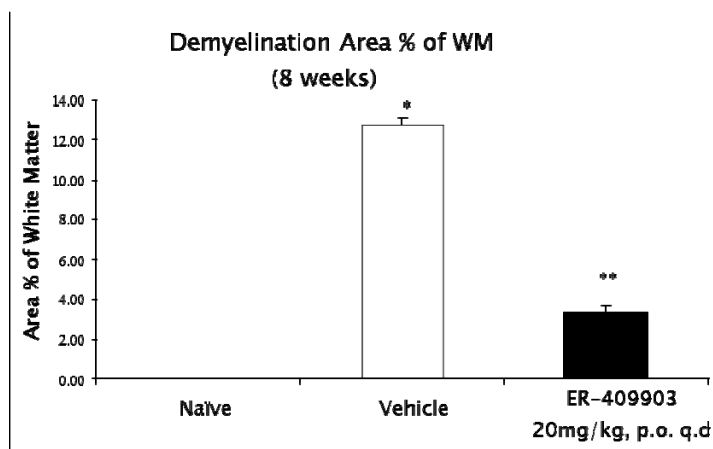
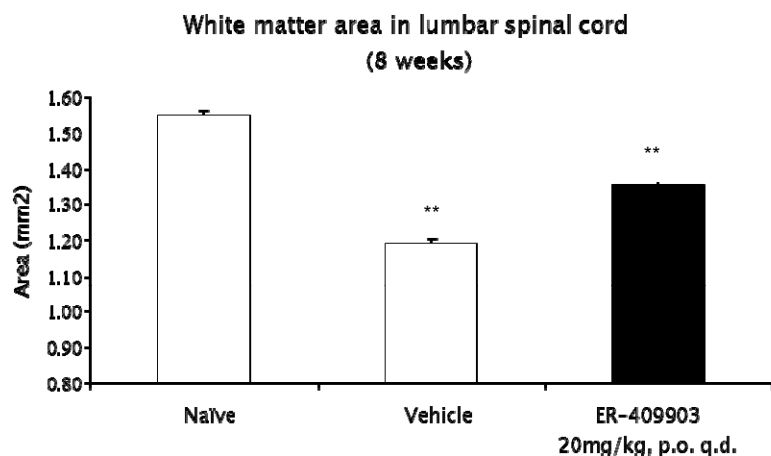
JNK3 IC<sub>50</sub> 52 nM

*G* 0.34

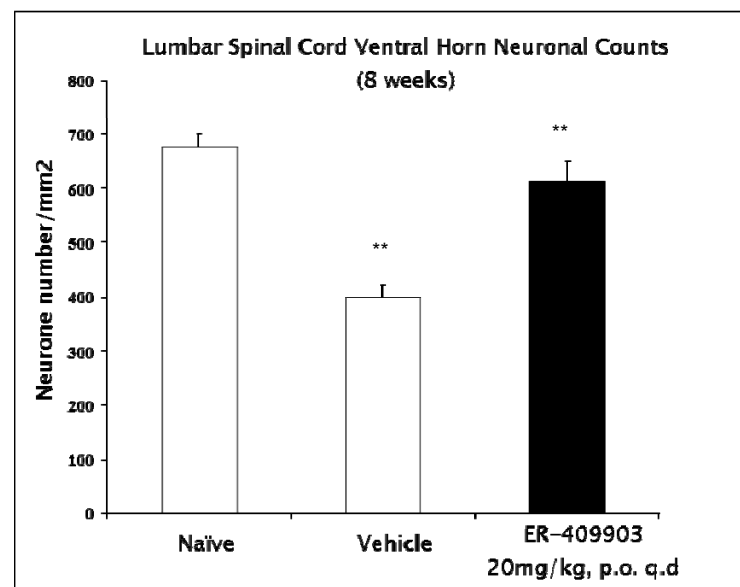
but significant difference in the effect on weight



# Activity in vivo - histopathology



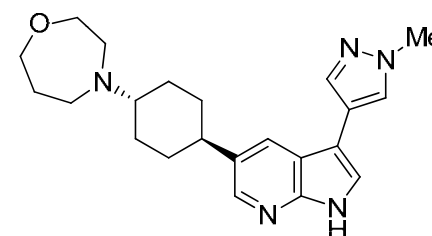
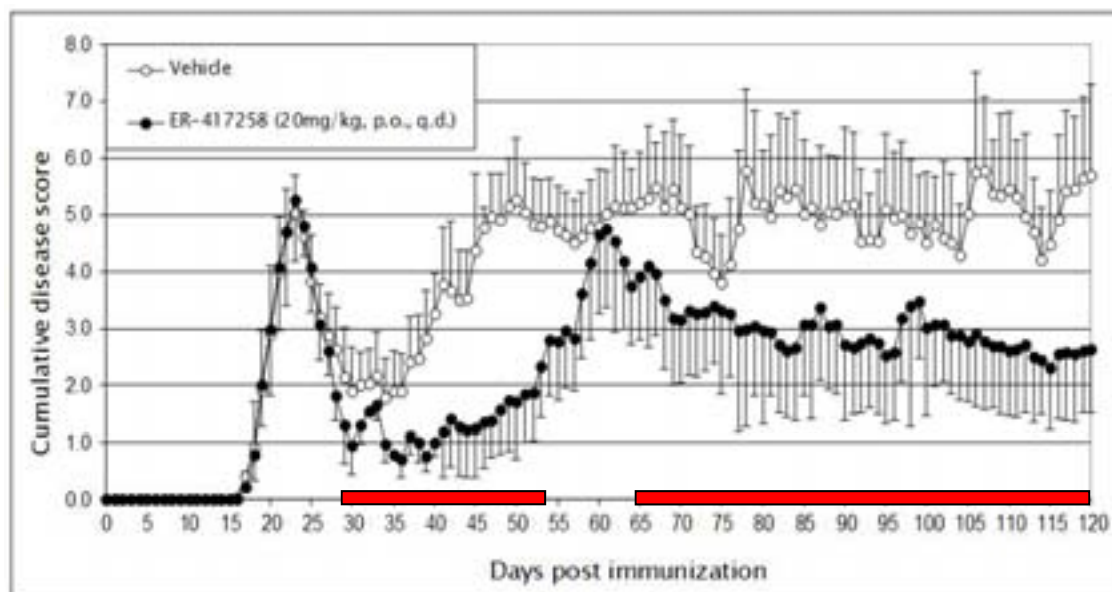
ER-409903




# EAE in Biozzi mouse



- Biozzi mice develop a chronic relapsing remitting form of EAE analogous to human disease
- ER-417258 was dosed between day 28 and 53. Dosing was then stopped between day 54 and 63. Dosing was restarted on day 64 until end of experiment



ER-417258

 Period of drug administration

- We were able to demonstrate the efficacy of ER-417258 even after the dosing was suspended for a short period

# Additional data



## Plasma protein binding

Compound	Fraction unbound [%]			
	human	rat	mouse	dog
ER-409903	36.7	29.5	27.3	42.4
ER-409923	50.5	39.2	32.2	61.0
ER-417258	50.7	35.4	34.3	55.7

## Solubility

ER-409903 62 µg/mL (about 1.7 mM) @ pH 6.8

## CYP inhibition

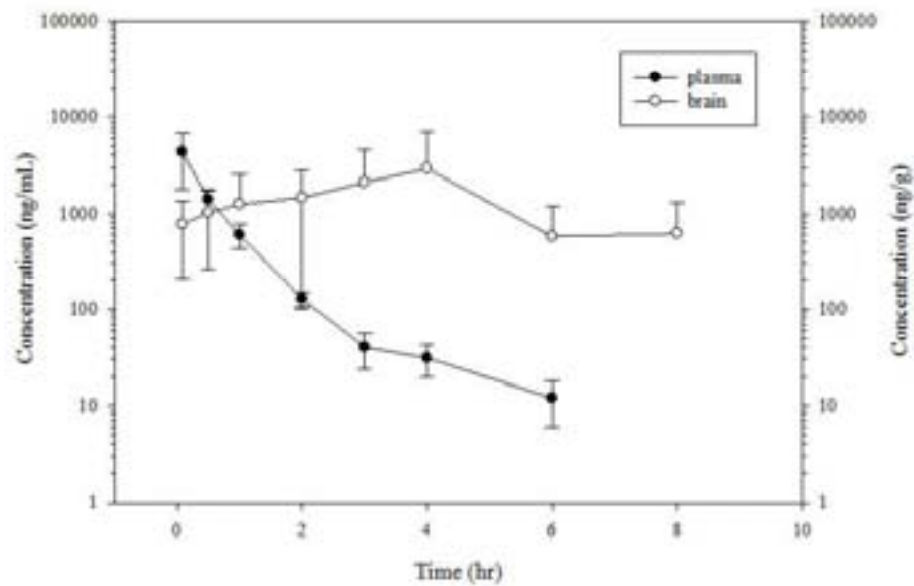
Compound	CYP inhibition IC <sub>50</sub> [µM]				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
ER-409903	>25	>25	>25	18.9	>25
ER-409923	>25	>25	>25	>25	>25
ER-417258	>25	>25	>25	11.3	>25



# Additional data



Brain PK parameters for ER-417258 after 10 mg/kg i.v. in mice



BPI = 4.4

$$\text{BPI} = \text{brain penetration index} = \text{AUC}_{0-t}(\text{brain}) / \text{AUC}_{0-t}(\text{plasma})$$

- The new series of JNK inhibitors is characterised by:
  - Good solubility
  - Moderate protein binding
  - Good selectivity against the rest of the kinome
  - Clean CYP450 inhibition profile
  - No significant liabilities in the 270 receptor binding screen
  - *In vivo* activity with once daily dosing
  - CNS penetration
  - Activity in a number of *in vivo* models of MS as well as models of peripheral indications, e.g. collagen-induced arthritis in mice and adjuvant-induced arthritis in rats
- The positive *in vivo* characteristics of ER-409903 and ER-417258 need to be balanced with preclinical safety observations when determining future investment strategy.

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