



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

14 September, 2011

Piotr Graczyk

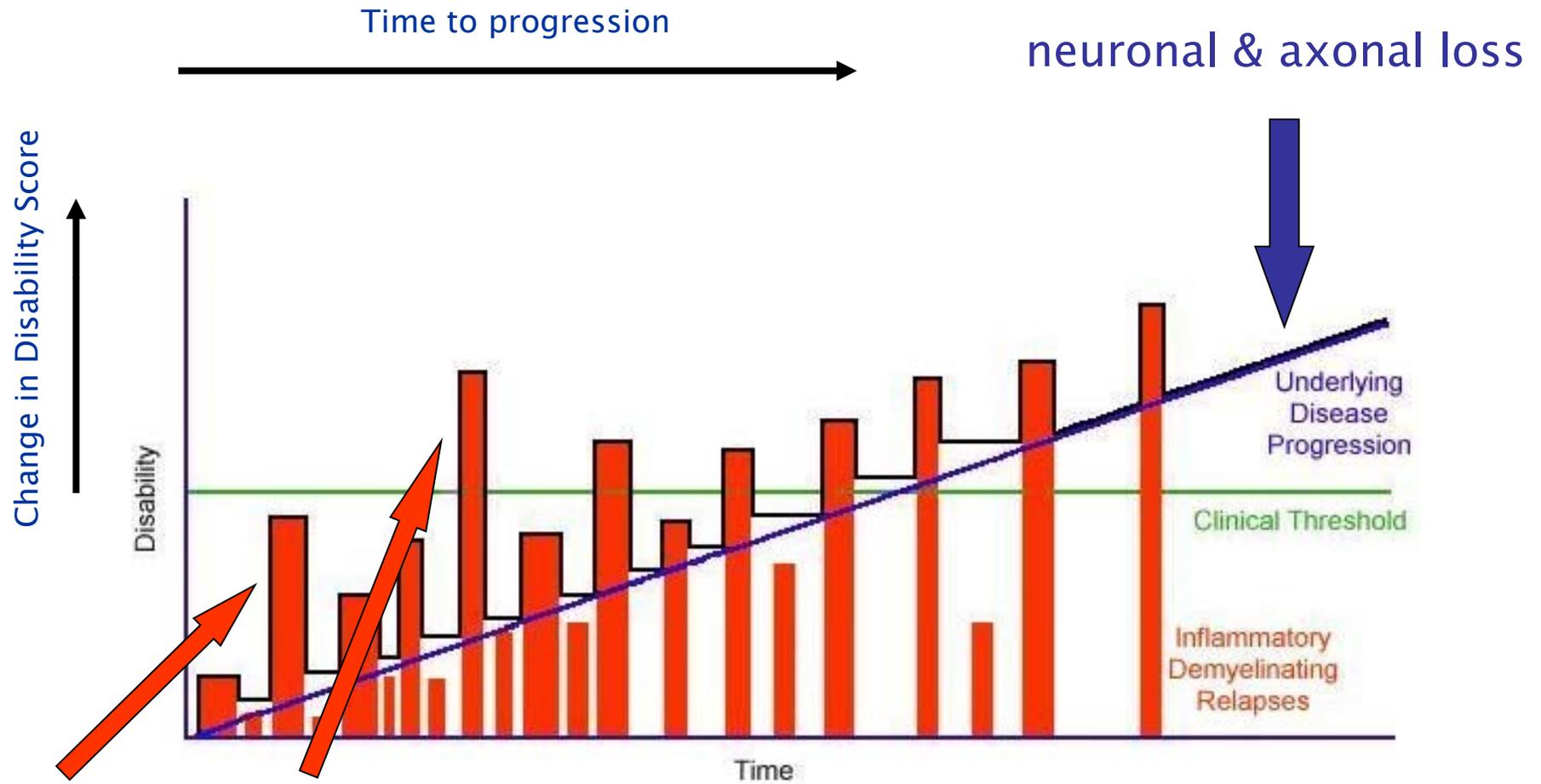
Eisai Ltd. Hatfield, UK

hvc

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



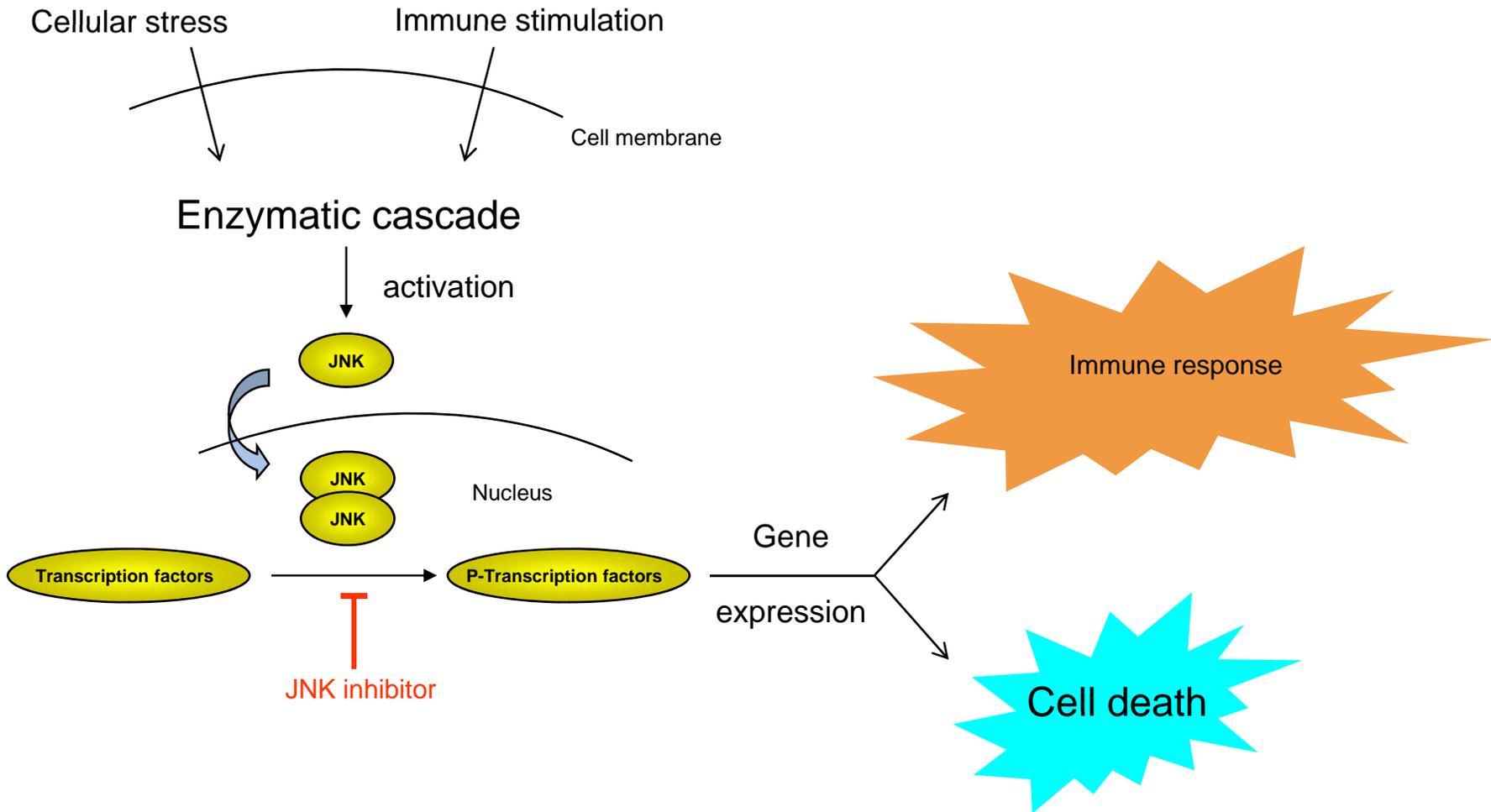
Inflammation
IFN β , Tysabri, Copaxone, FTY720

Compston & Coles, 2008

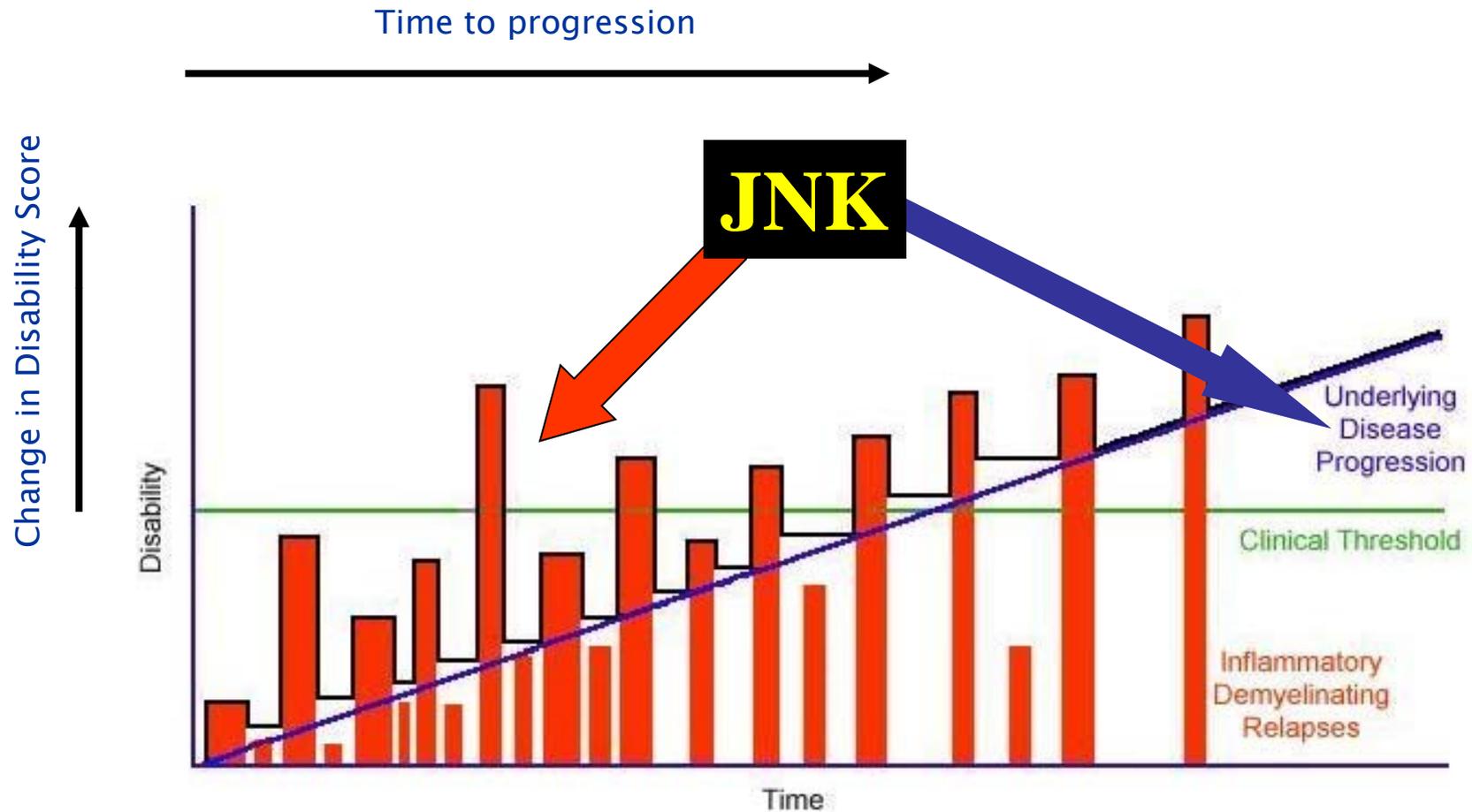
Role of JNK



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



Multiple sclerosis – clinical presentation



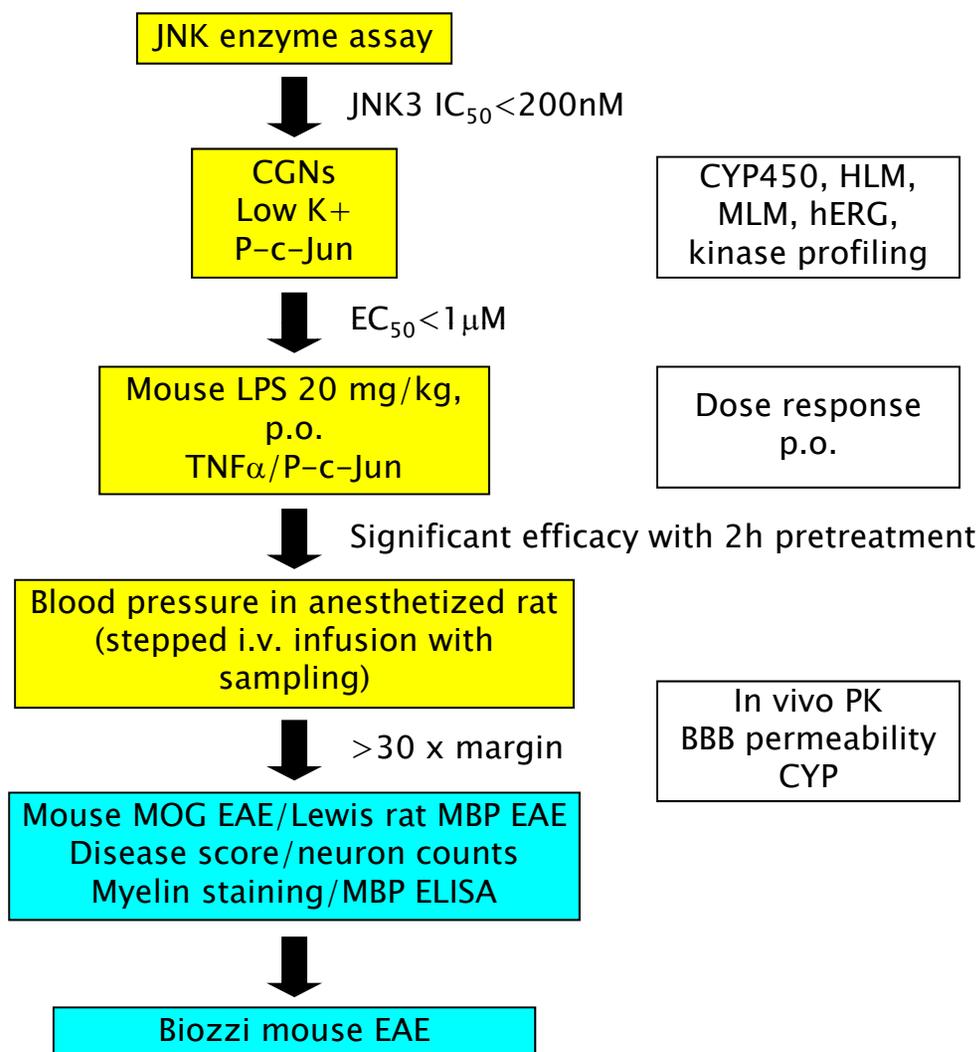
Compston & Coles, 2008

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



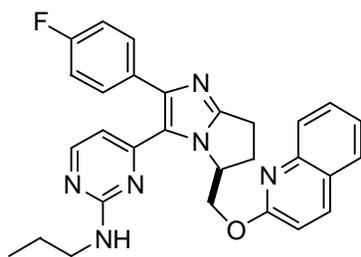
Explanations:

| | |
|--------------------------|---|
| Biozzi mice = | mice developing chronic relapsing remitting form of EAE |
| c-Jun = | transcription factor phosphorylated by JNK |
| CGN Low K ⁺ = | low-potassium induced death in cerebellar granule neurons |
| EAE = | experimental autoimmune encephalomyelitis |
| MBP = | Myelin basic protein |
| MOG = | Myelin oligodendrocyte glycoprotein |
| Mouse LPS = | LPS-induced TNF α 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)



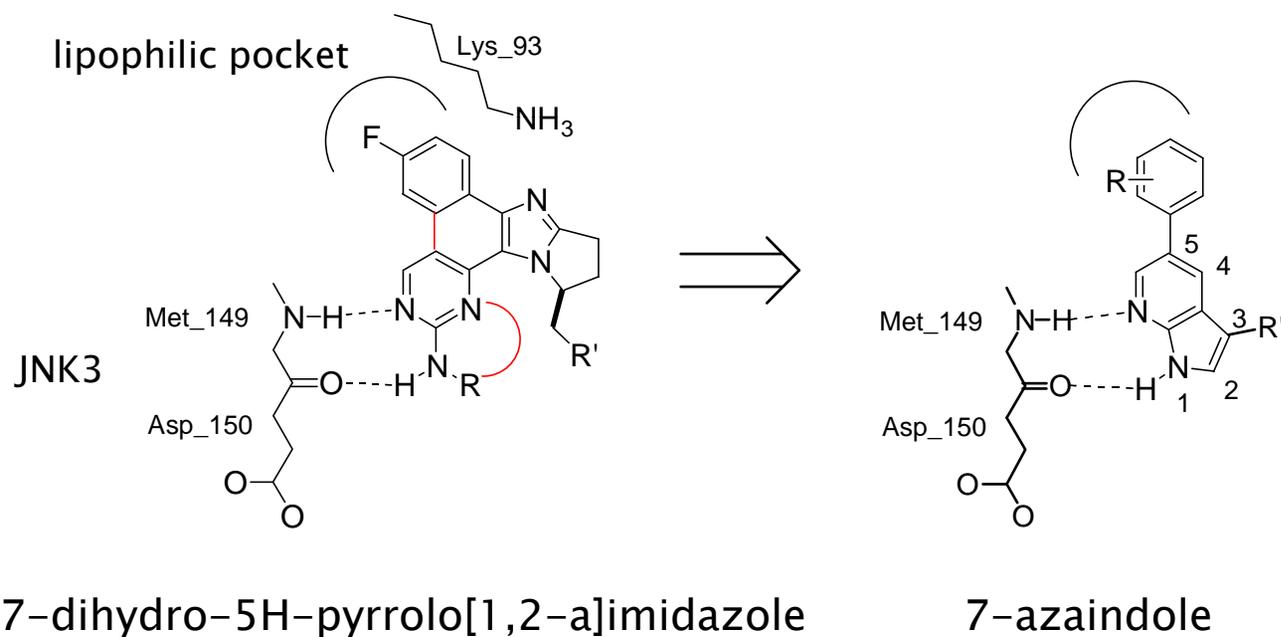
| | | |
|----------------------|----------------------------|-----------------------|
| | JNK3 IC ₅₀ [nM] | 85 |
| | SCG* ED ₅₀ [μM] | 0.3–1.0 |
| B/P (100 mg/kg i.p.) | mouse | 0.42 (357 ng/g brain) |
| | rat | 0.57 (709 ng/g brain) |
| | BA (10 mg i.p. rat) | 32% |
| | BA (10 mg p.o. rat) | 5% |

*SCG – superior cervical ganglion neurons

7-Azaindoles



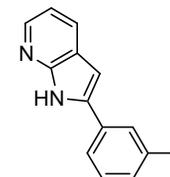
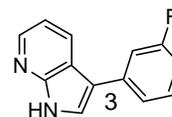
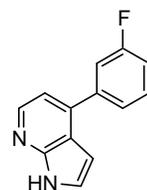
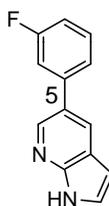
Build on past knowledge



Initial SAR



“Active” positions



JNK3 IC₅₀ [μM]

>10

4.6

>10

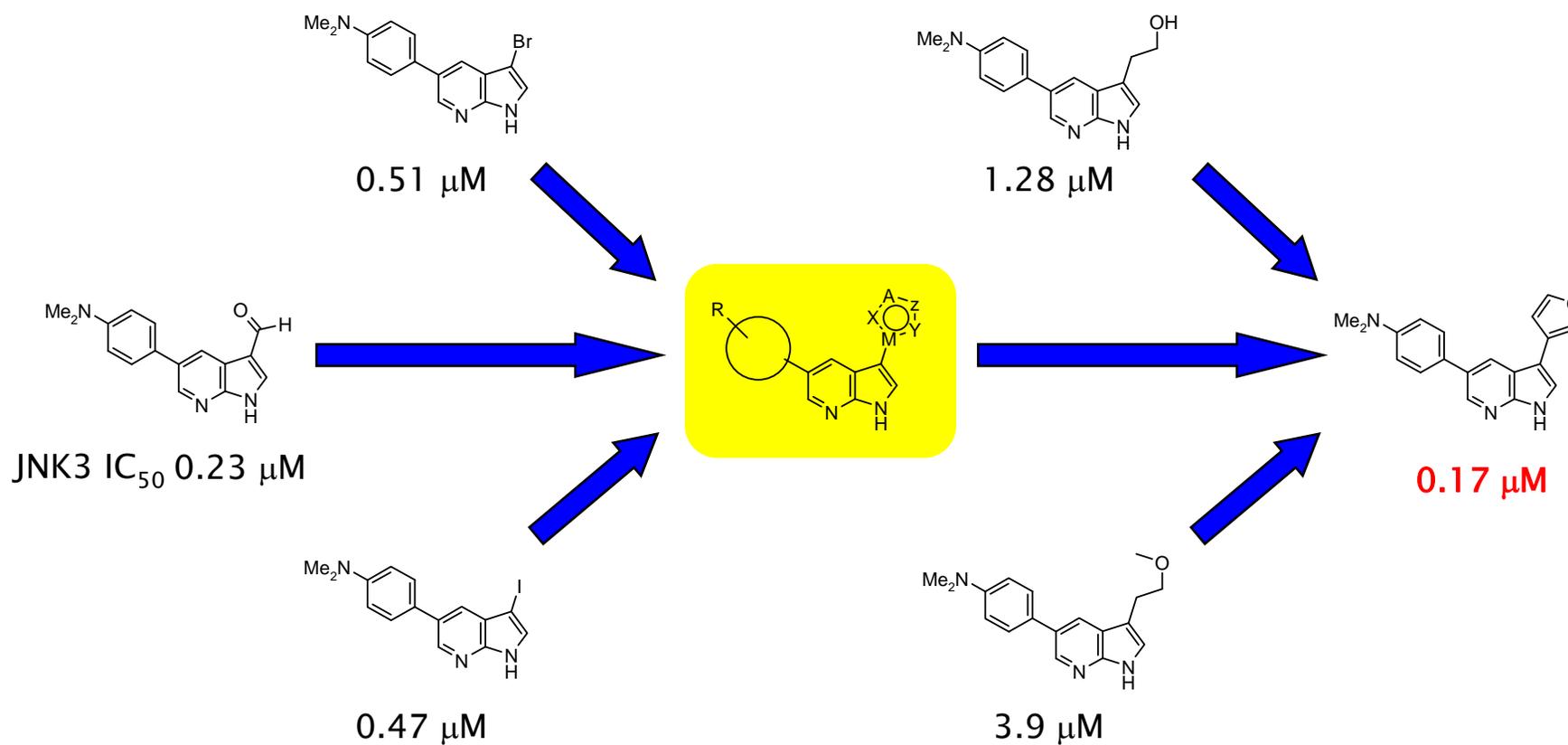
3.4

>10

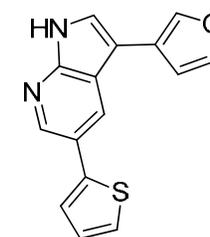
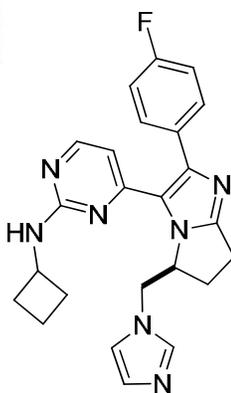
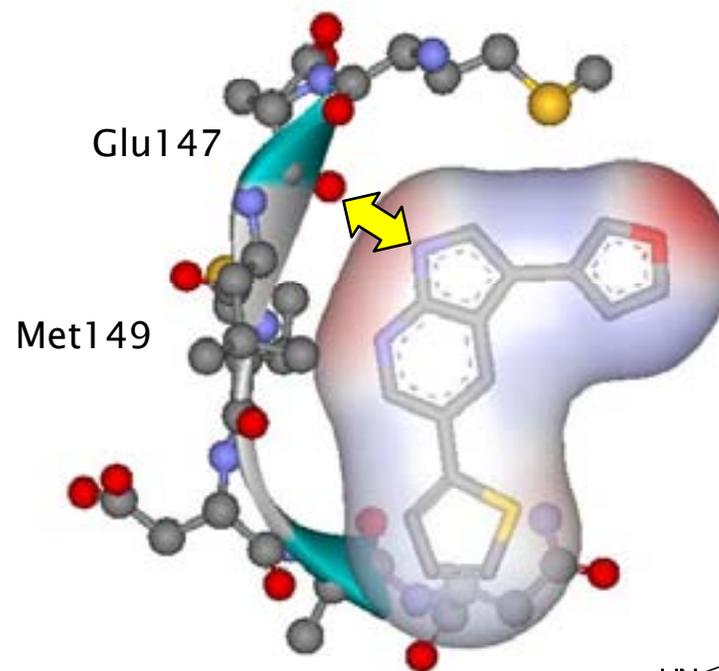
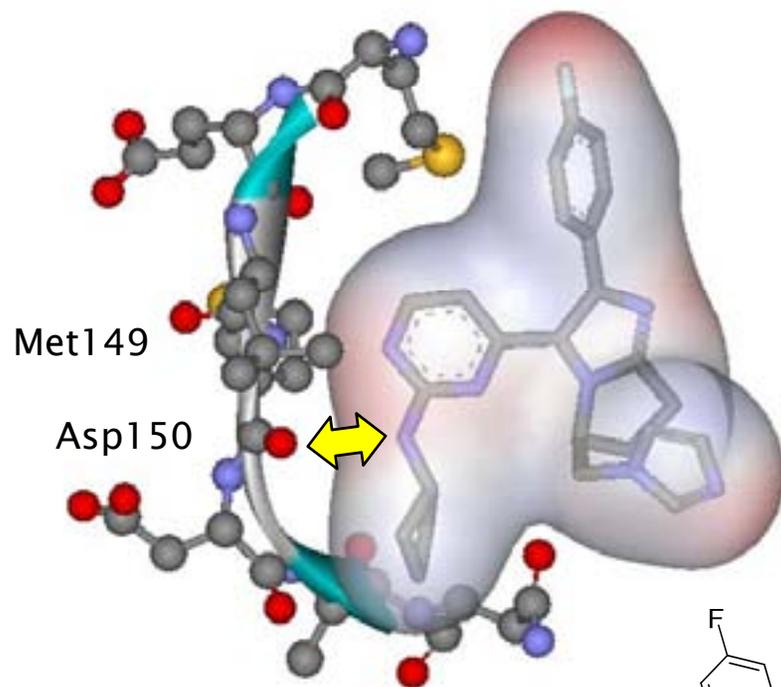
Initial SAR



Position (3) – small Π -system



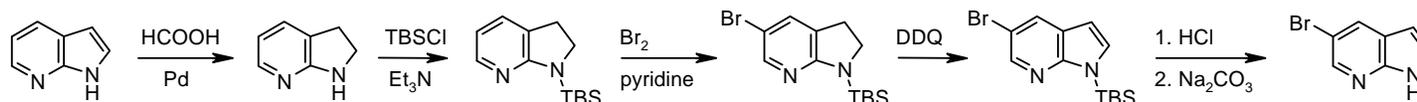
Serendipity



Making the chemistry tractable



Synthesis of key intermediate

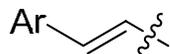
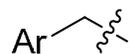
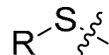
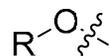
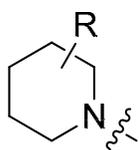
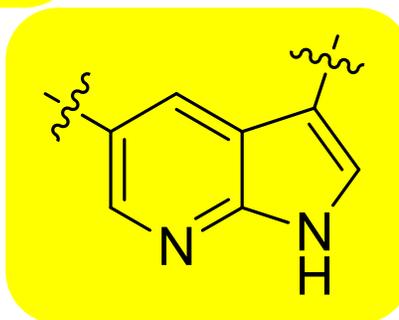
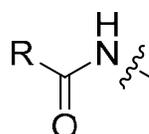
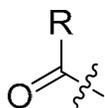
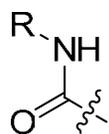
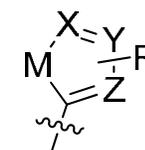
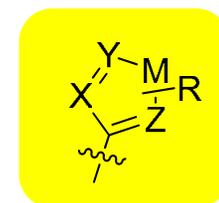


Total yield over 5 steps 205.5 g (82%)

General SAR



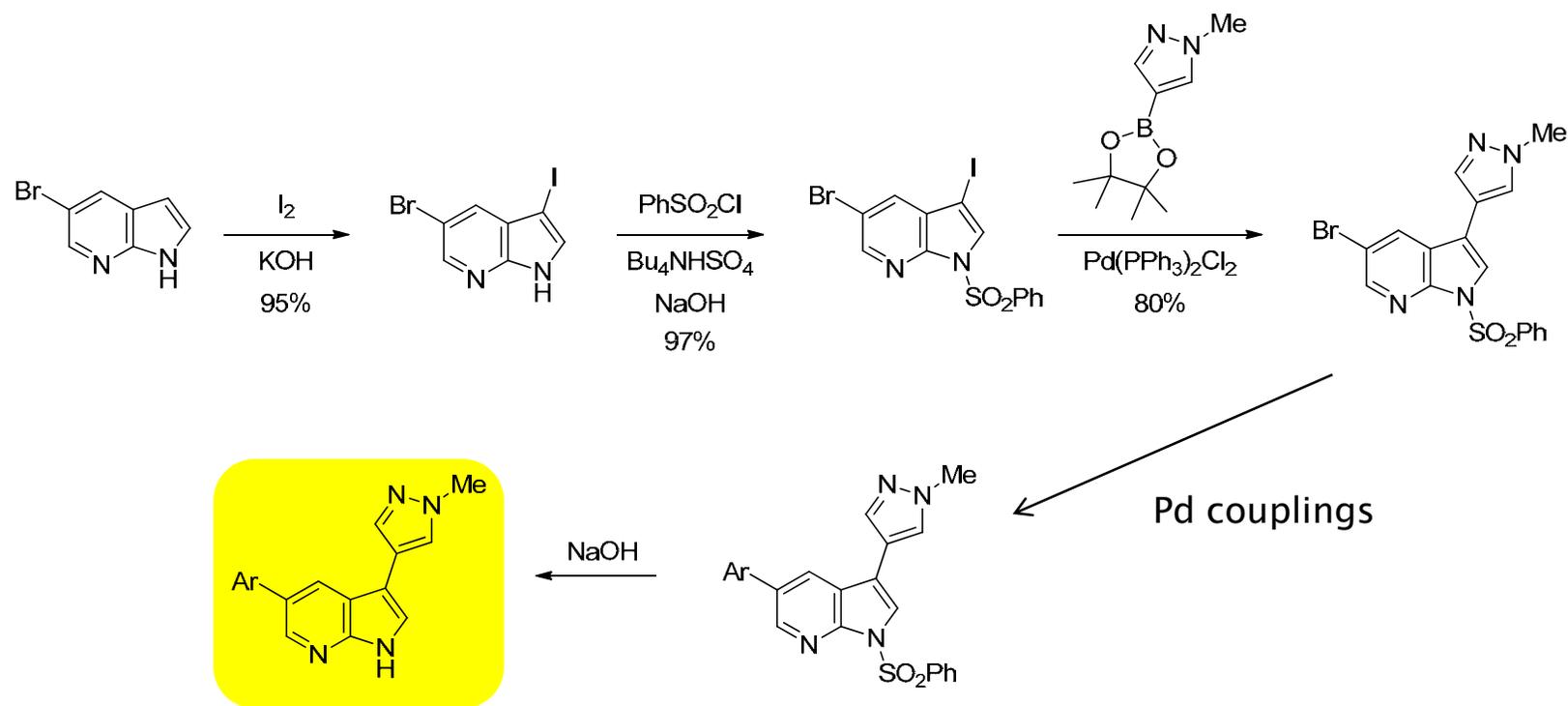
X, Y, Z, Q=CH, N M=O, S, N-



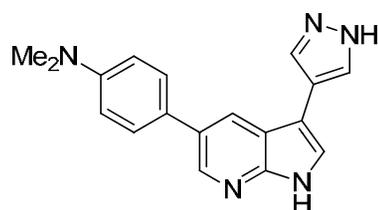
Making the chemistry tractable



Approach to C(5) derivatisation



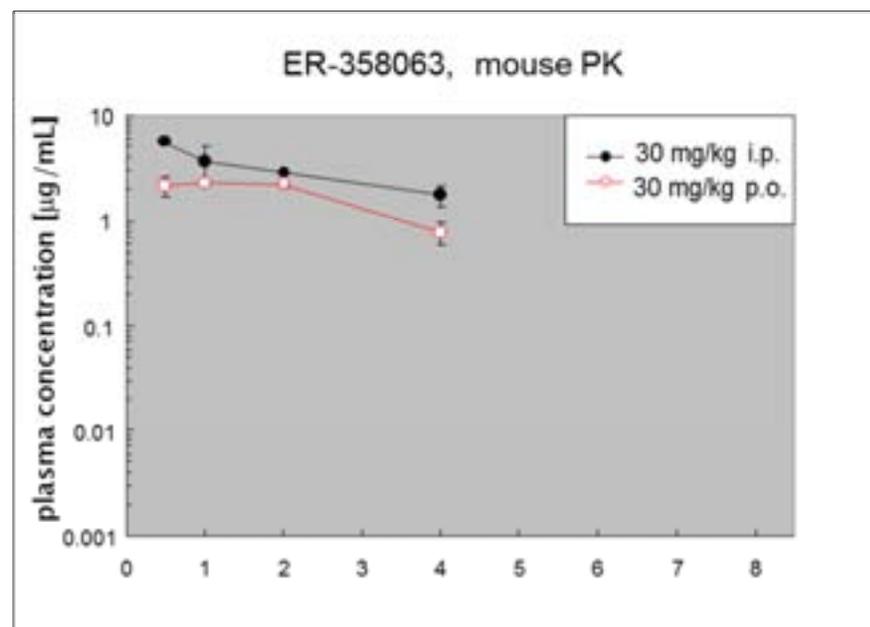
An example: ER-358063



ER-358063

JNK1, 2, 3 IC₅₀ 101, 59, 32 nM

3.3 µg/mL PBS (pH 7.2) (around 10 µM)



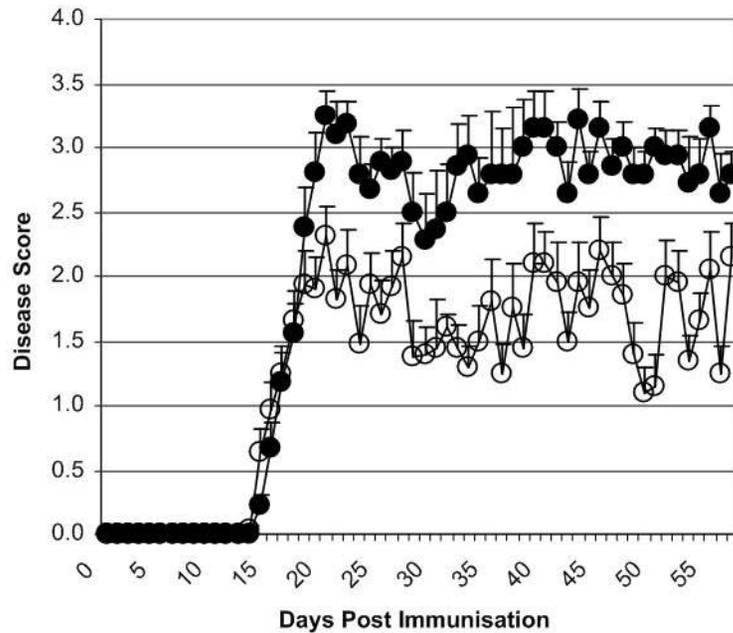
B/P = 1.4 (after i.p. administration)

T_{1/2} = 2.8 h (i.p.) 1.3 h (p.o.)

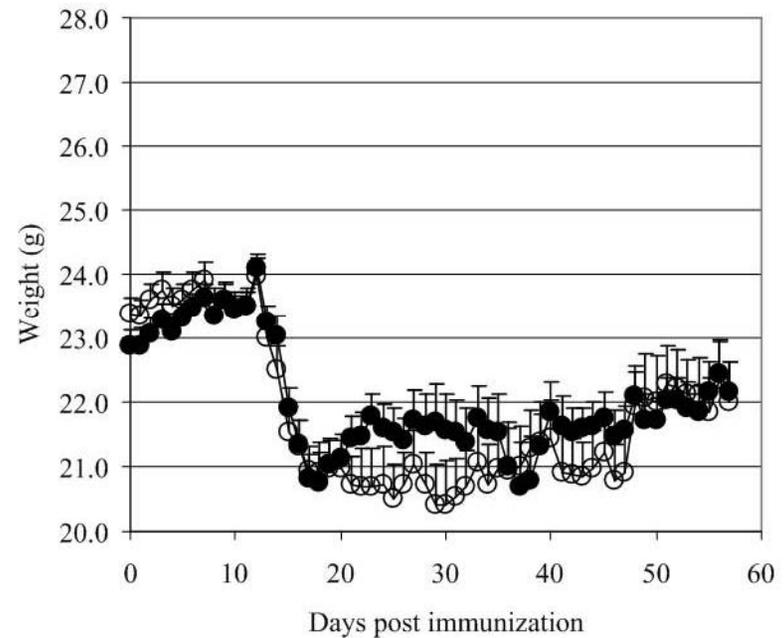
ER-358063 in the EAE model



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



Disease score



Animal weight

ER-358063: selectivity at 1 μ M



| | % of Control (10 μ M ATP) ER-358063 @ 1 μ M | | % of Control (10 μ M ATP) ER-358063 @ 1 μ M |
|----------------------|--|--------------------|--|
| Abl(h) | 16 | MKK4(m) | 53 |
| AMPK(r) | 22 | MKK6(h) | 58 |
| Aurora-A(h) | 12 | MKK7 β (h) | 49 |
| CaMKII(r) | 62 | MST2(h) | 6 |
| CDK1/cyclinB(h) | 2 | p70S6K(h) | 34 |
| CDK2/cyclinA(h) | 5 | PDGFR α (h) | 77 |
| CDK3/cyclinE(h) | 17 | PDGFR β (h) | 30 |
| CDK5/p35(h) | 2 | PDK1(h) | 7 |
| CDK6/cyclinD3(h) | 11 | PKA(h) | 91 |
| CDK7/cyclinH/MAT1(h) | 13 | PKC α (h) | 85 |
| CHK1(h) | 17 | PKC β II(h) | 87 |
| CK1 δ (h) | 20 | PKC γ (h) | 76 |
| c-RAF(h) | 88 | PKC δ (h) | 92 |
| cSRC(h) | 46 | PKC ϵ (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 α (h) | 6 | SAPK3(h) | 77 |
| GSK3 β (h) | 23 | SAPK4(h) | 89 |
| IGF-1R(h) | 71 | TrkA(h) | 1 |
| IKK α (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

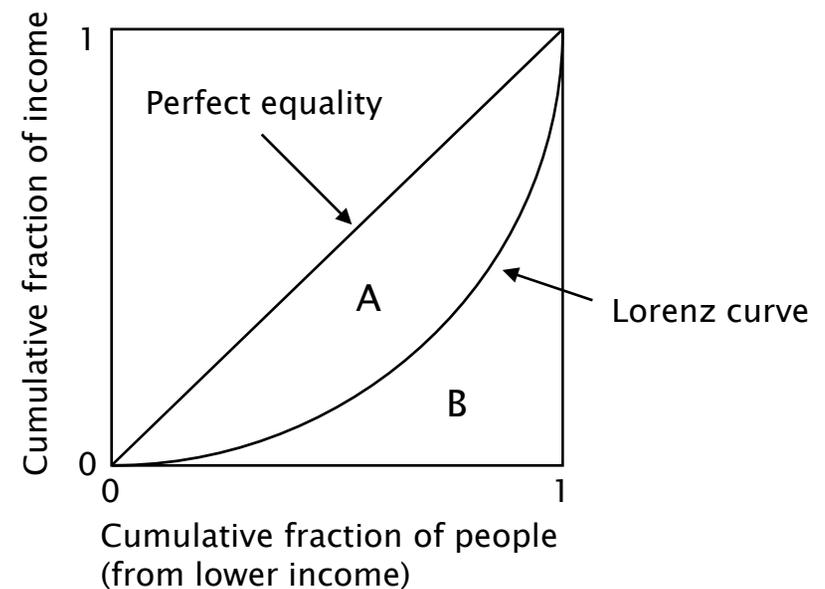


- Selectivity \cong inequality
- Economy
 - income inequality is measured by Gini coefficient G

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

Perfect equality: $G = 0$

Extreme inequality: $G = 1$



Gini coefficient for selectivity

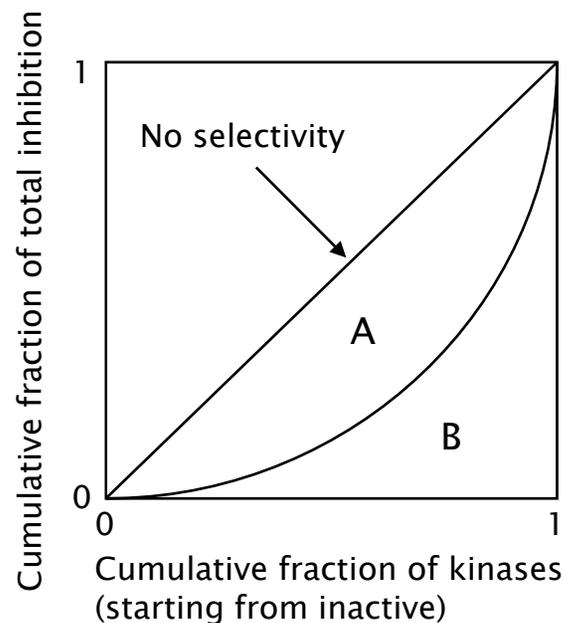


- Let us take:
 - Percentage of kinase inhibition instead of income
 - Kinases instead of people

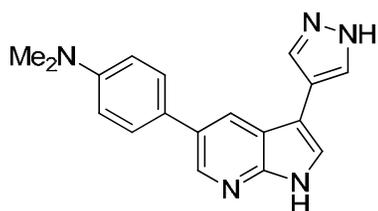
$$G = \frac{A}{A+B} = 1 - 2 \times B$$

No selectivity: $G = 0$

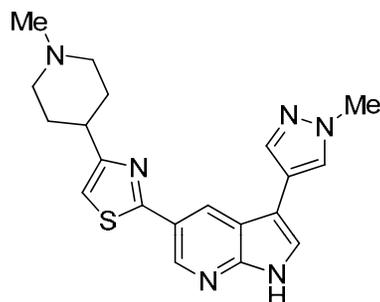
Perfect selectivity: $G = 1$



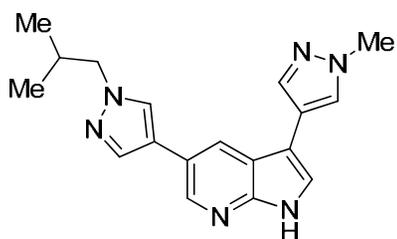
Examples



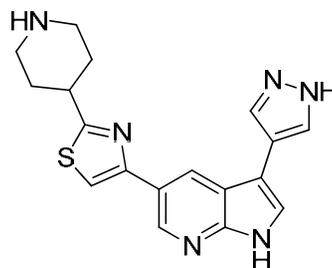
G 0.34



0.38



G 0.38



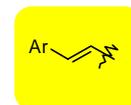
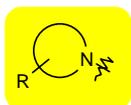
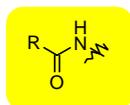
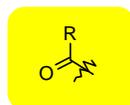
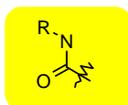
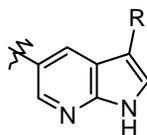
0.41

| inhibitor | concn (μM) | G |
|---------------|-------------------------|----------------------|
| | | 10 μM 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 |

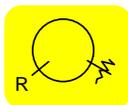
Where to find selective compounds?



- Already explored at C(5)



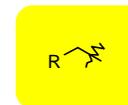
But missing: C(5)-saturated cycles and C(5)-aliphatic chains



C(5)-carbocycle



C(5)-saturated heterocycle



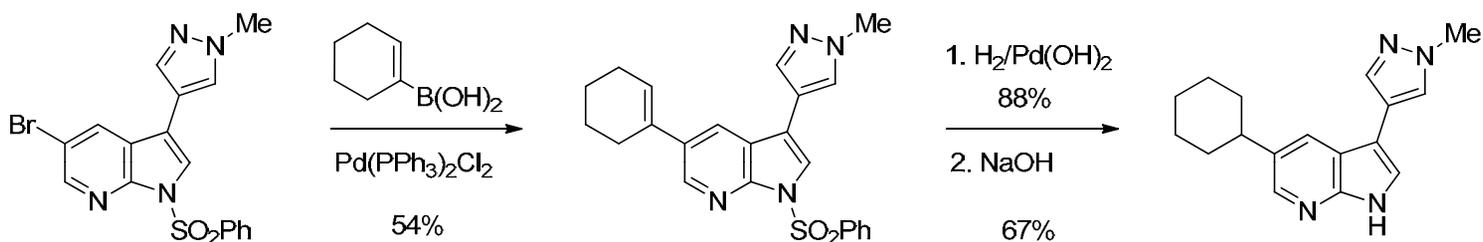
C(5)-carbon chain

Low potency in vitro

Where to find selective compounds?



• Prototype cyclohexyl derivative

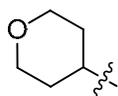


ER-417245

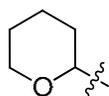
JNK1, 2, 3 IC₅₀ 74, 135, 40 nM

G 0.57

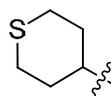
• Other saturated rings



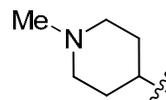
JNK3 IC₅₀ [nM] 60



640



148

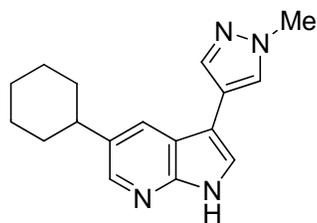


>2000

Cyclohexyl derivatives



- Prototype cyclohexyl derivative



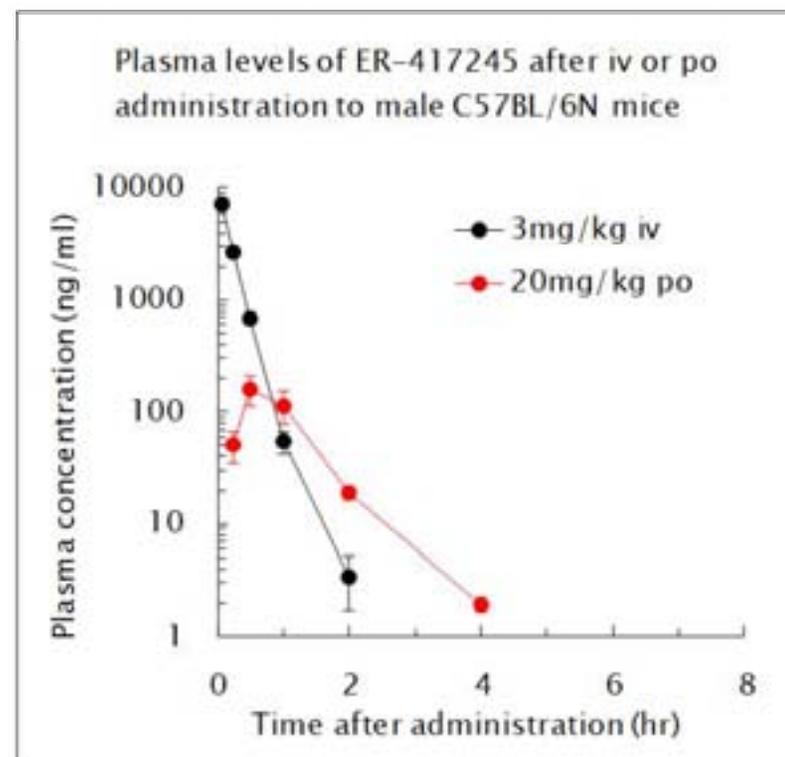
ER-417245

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

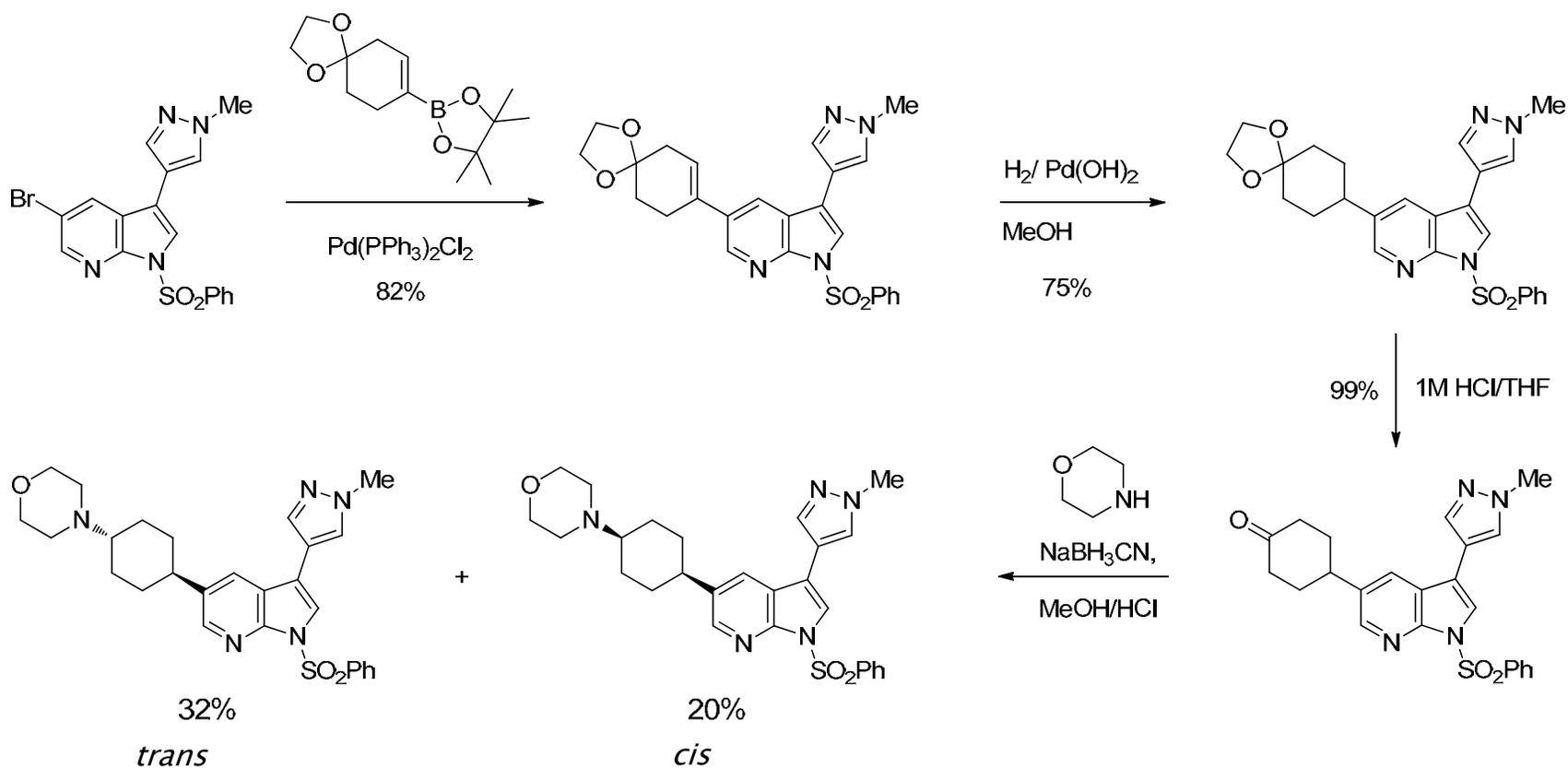
Peripheral *in vivo* model

TNF- α /P-c-Jun 45%/20%

(LPS-induced TNF- α production in BCG-primed C57Bl/6 mice)



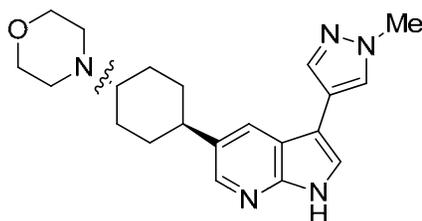
• Approach to an initial SAR study



Cyclohexyl derivatives

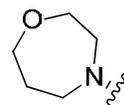


• *Trans* derivatives



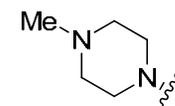
ER-409903

JNK3 IC₅₀ [nM] 80



ER-417258

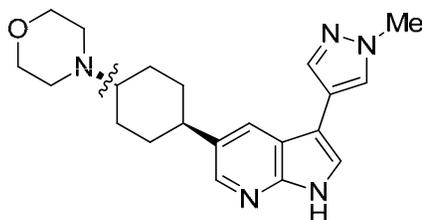
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ER-409923

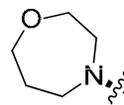
90

• *Cis* derivatives



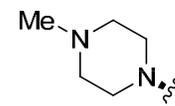
ER-409904

JNK3 IC₅₀ [nM] 60



ER-417257

184



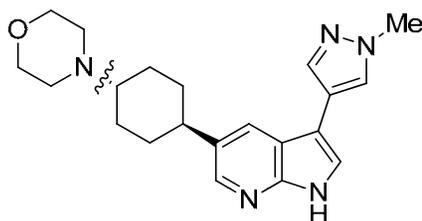
ER-409924

94

Cyclohexyl derivatives



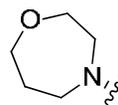
• *Trans* derivatives



ER-409903

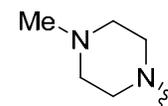
G

0.72



ER-417258

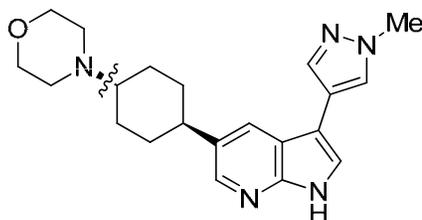
0.77



ER-409923

0.68

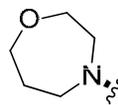
• *Cis* derivatives



ER-409904

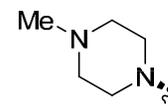
G

0.70



ER-417257

0.77



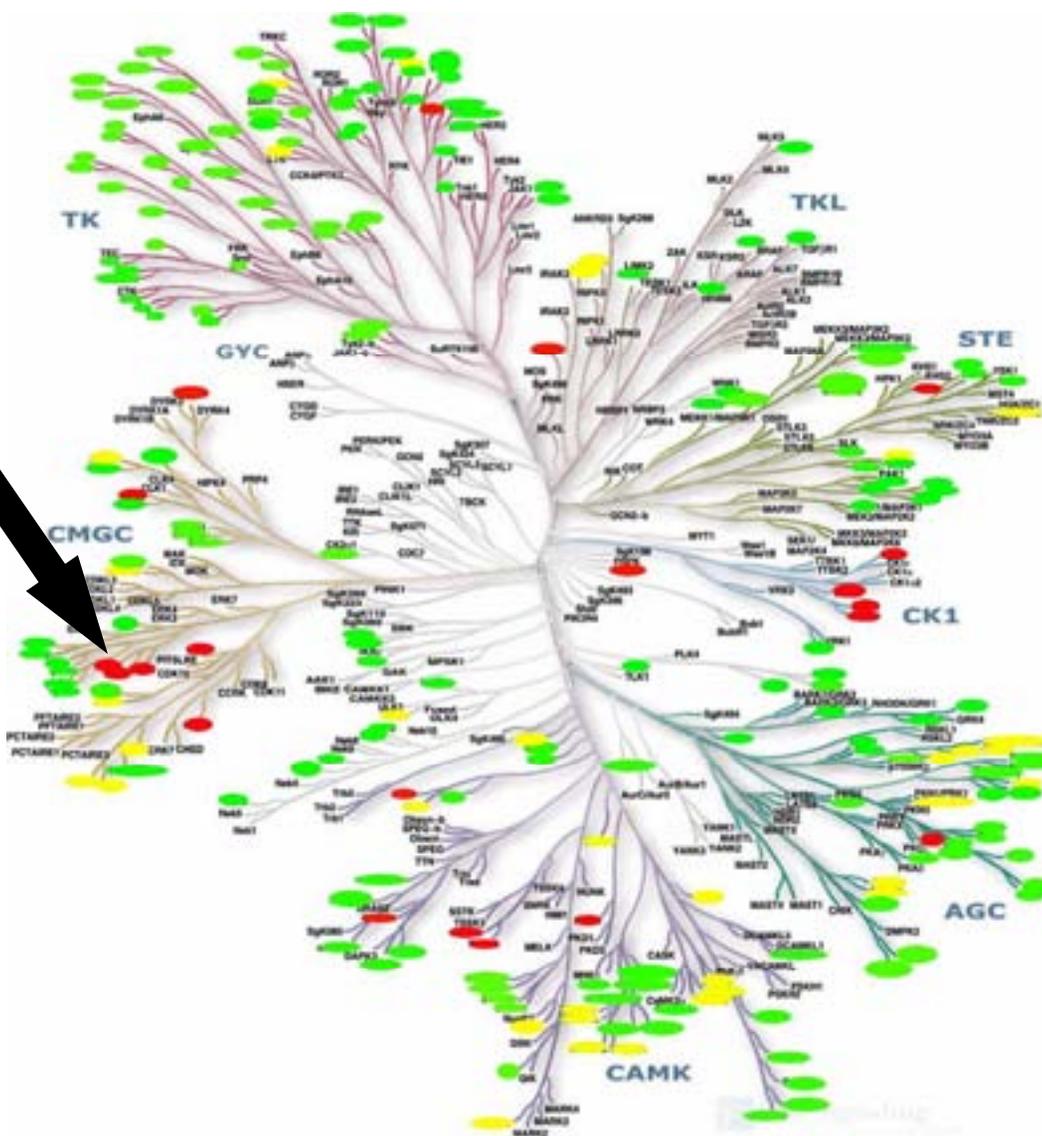
ER-409924

0.67

Selectivity of ER-417258



JNKs



% Activity

 <30%

 30-70%

 >70%

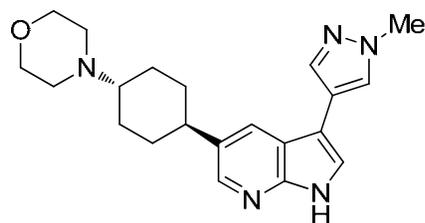
270 kinases examined

hvc
human health care

Origin of selectivity



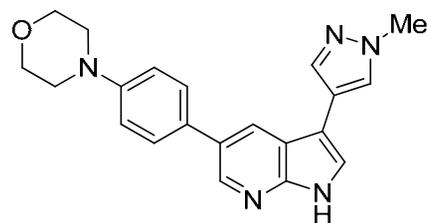
- Cyclohexyl derivatives are more selective than aromatic derivatives
- To explain this we carried out X-ray analysis of JNK3 with two representative compounds



ER-409903

G

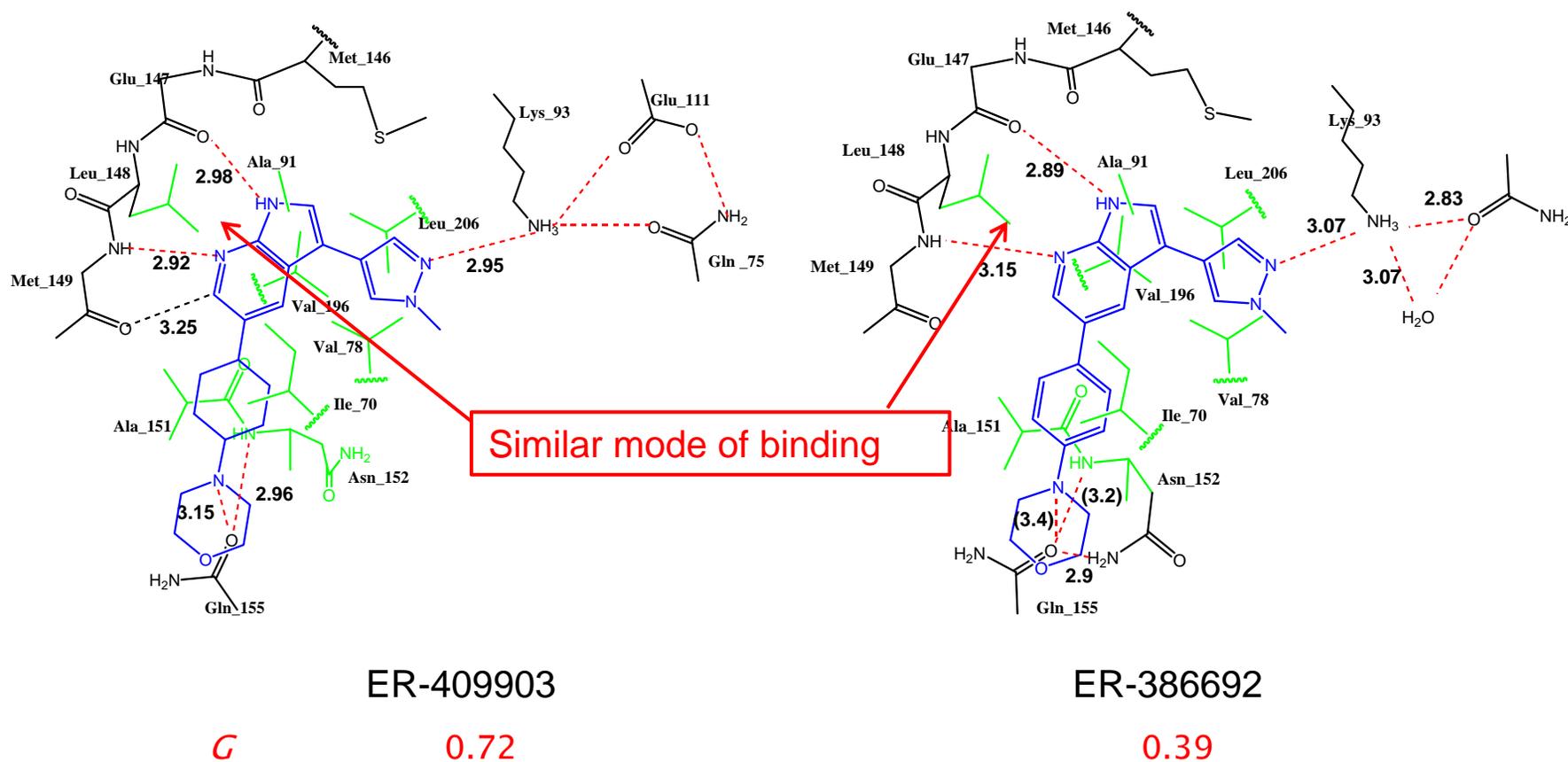
0.72



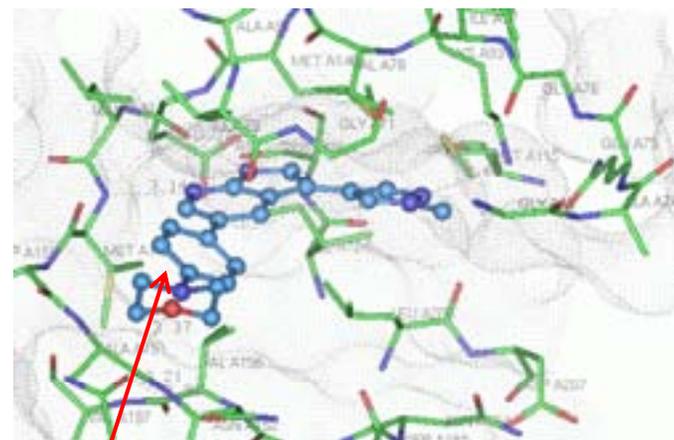
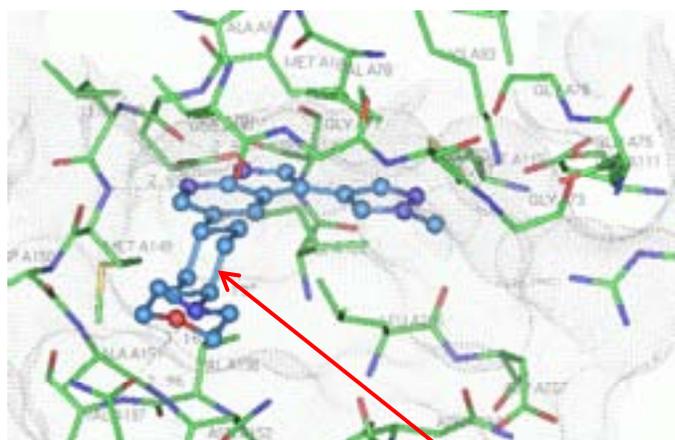
ER-386692

0.39

Origin of selectivity



Origin of selectivity



Different conformations

ER-409903

G

0.72

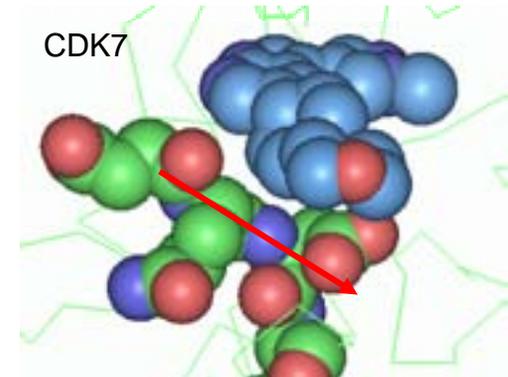
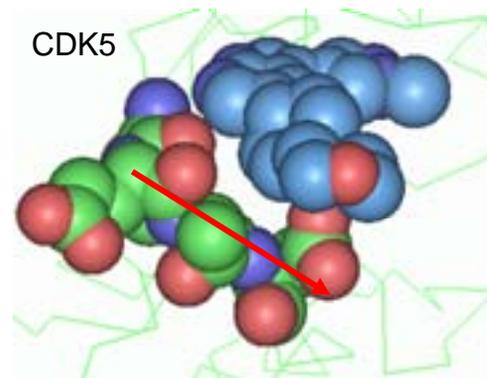
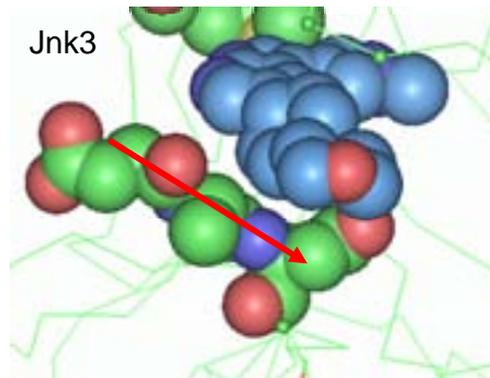
ER-386692

0.39

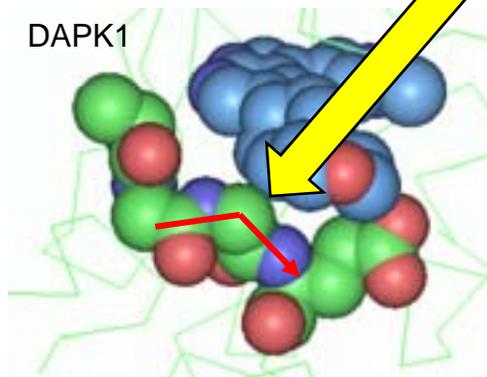
Loop next to the hinge region



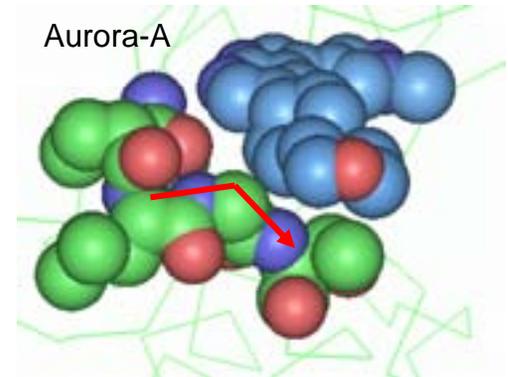
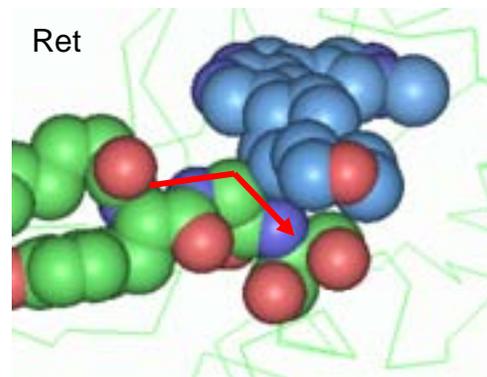
Kinases inhibited



Kinases not inhibited



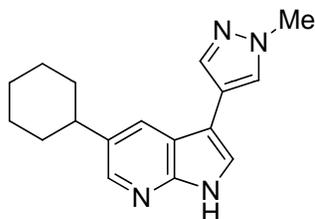
bent conformation at Gly residue. Not enough room to accommodate the cyclohexyl ring



Cyclohexyl derivatives



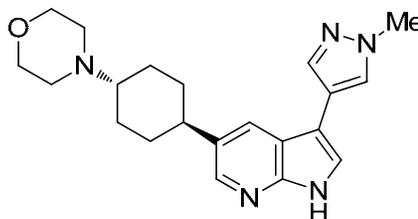
- Metabolic stability



ER-417245

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

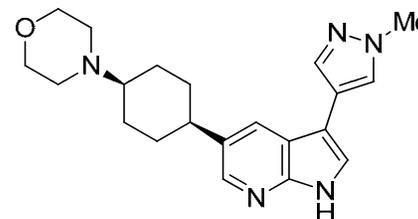
4.37



trans

ER-409903

151



cis

ER-409904

13.4

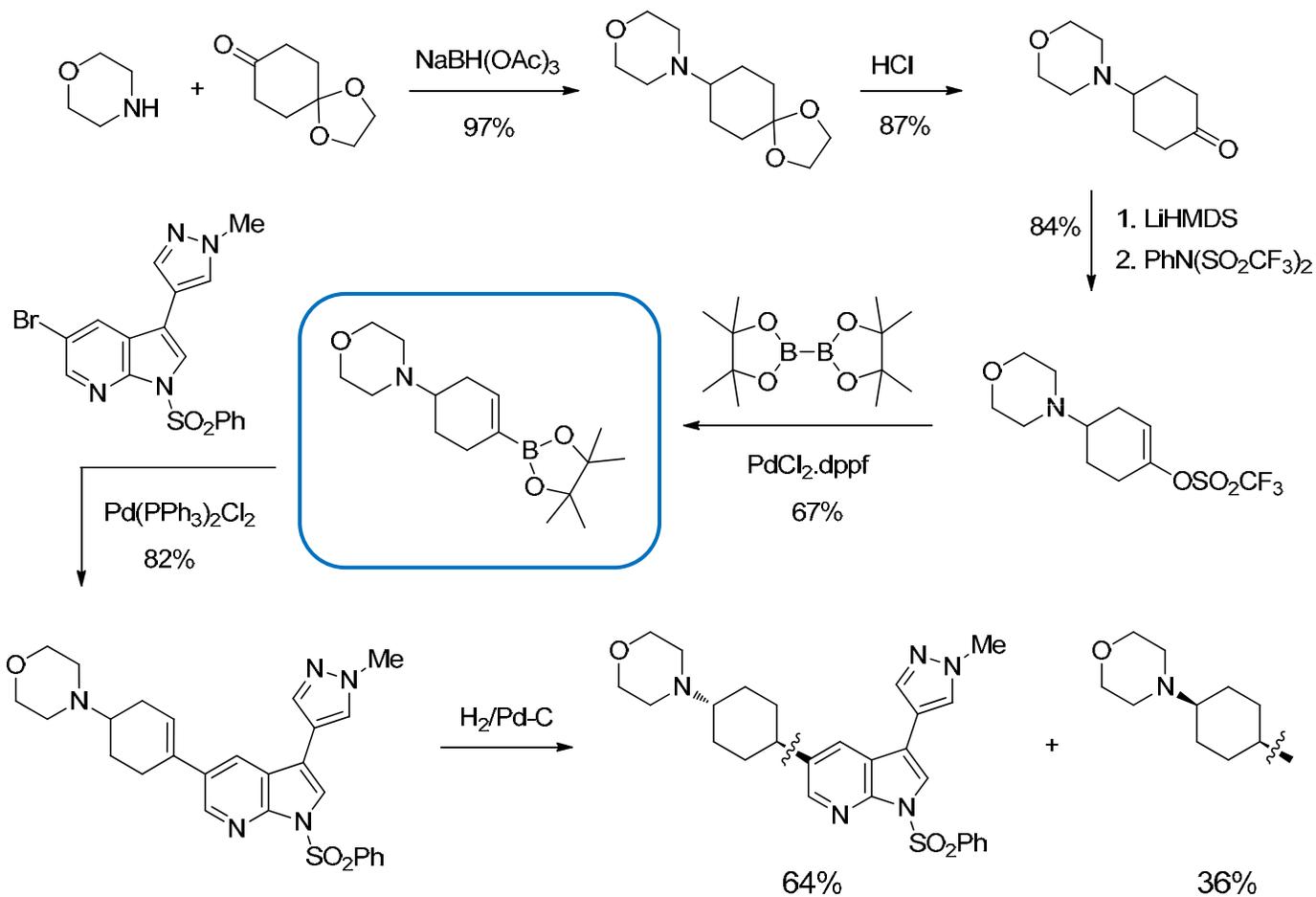
Trans isomers more metabolically stable

Need an efficient method of preparation

Synthesis of trans derivatives



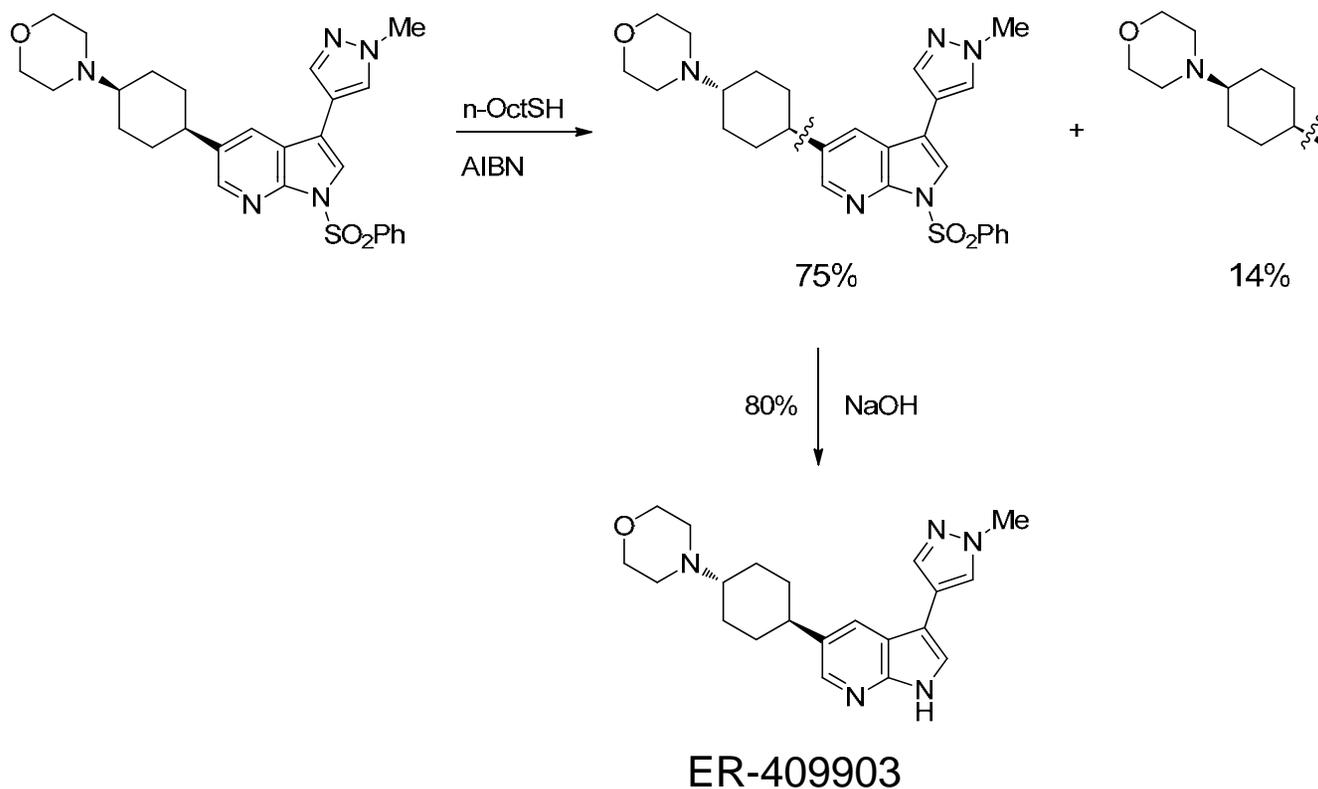
- Key material: the relevant boronic ester



Synthesis of trans derivatives



• Isomerisation



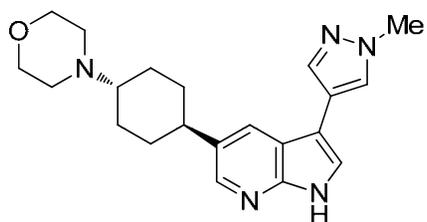
Isomerisation method adopted from Bertrand *et al.* (*J. Org. Chem.* 2006, 71, 7288)

Activity in vivo - comparison



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

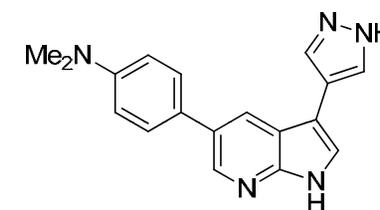
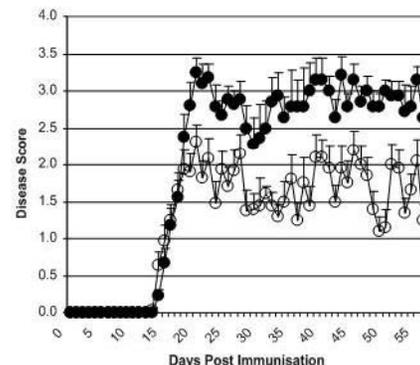
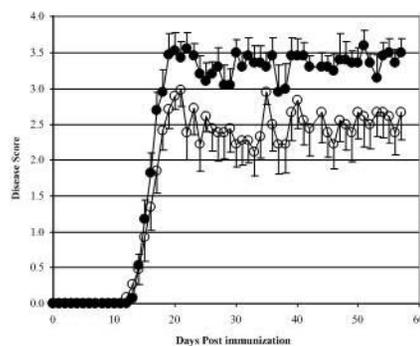
Similar pharmacological effect



ER-409903

JNK3 IC₅₀ 80 nM

G 0.72

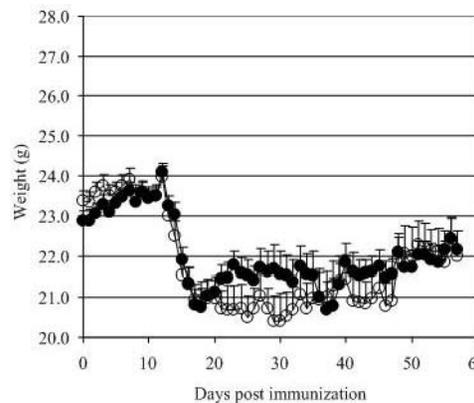
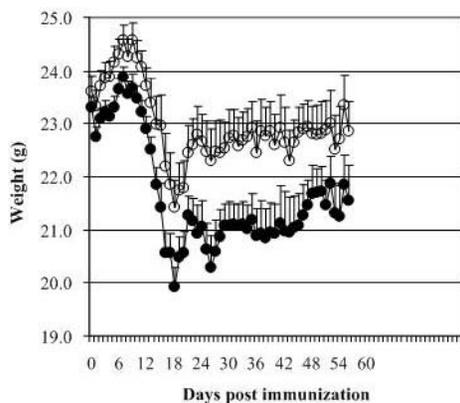


ER-358063

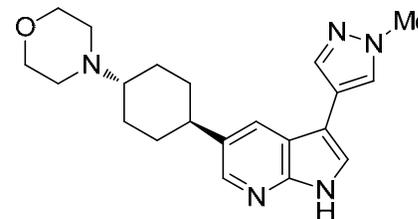
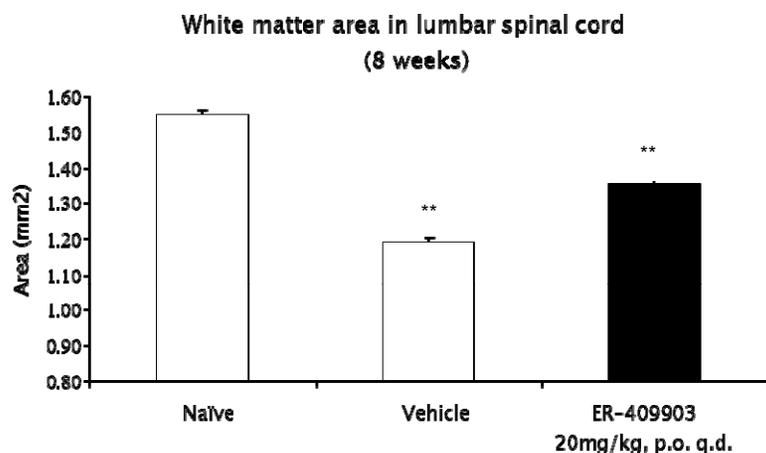
JNK3 IC₅₀ 52 nM

G 0.34

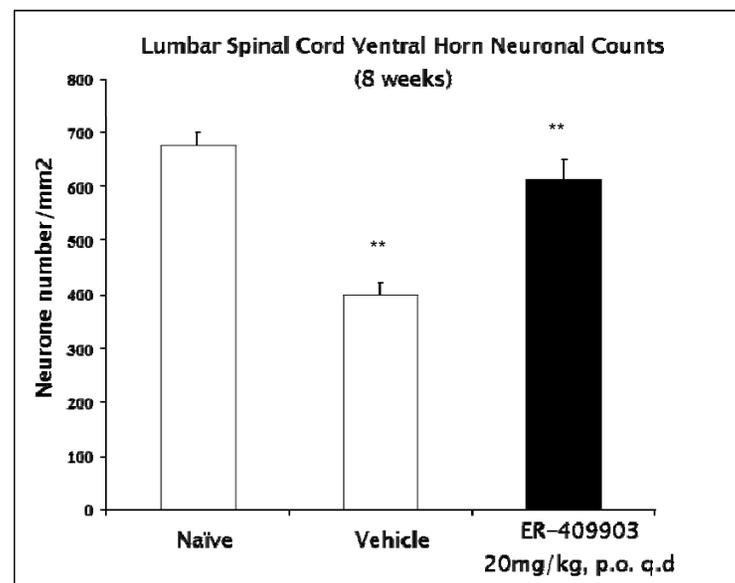
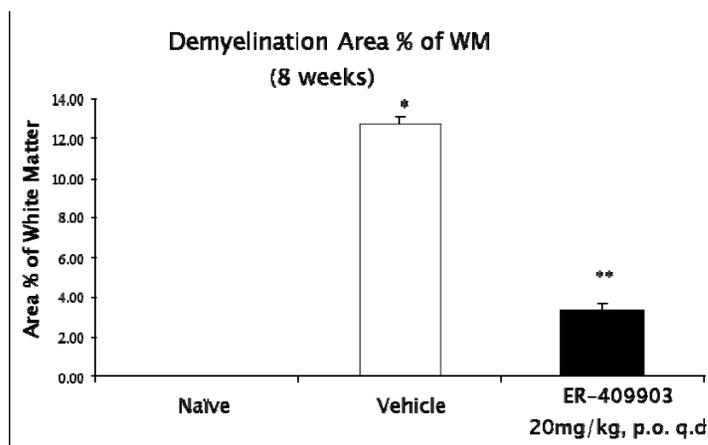
but significant difference in the effect on weight



Activity in vivo - histopathology



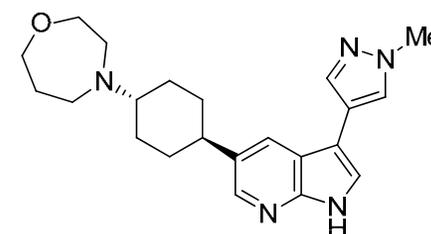
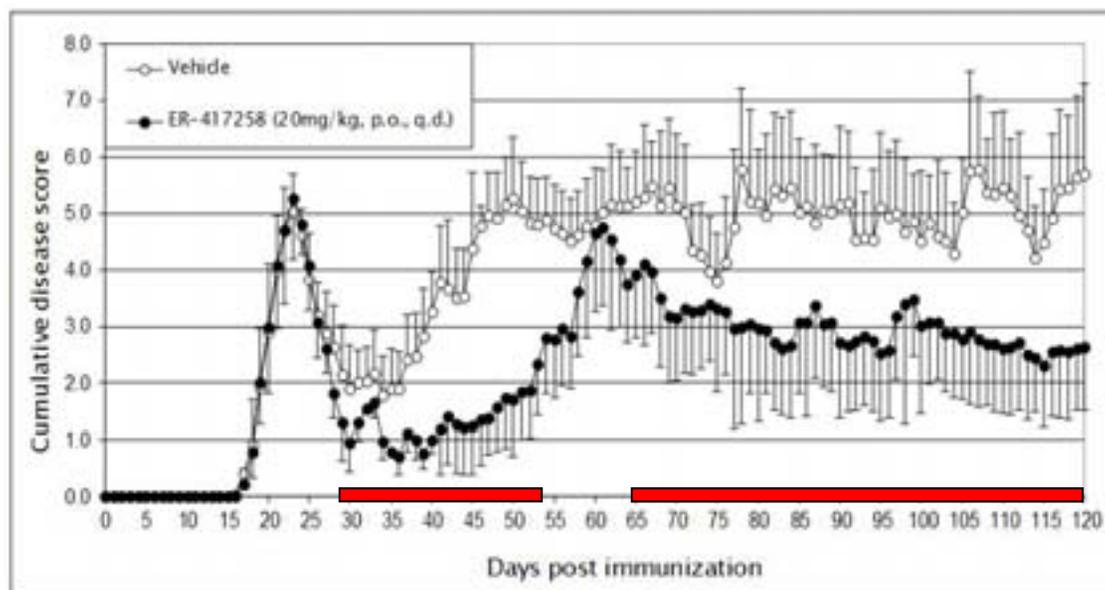
ER-409903



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

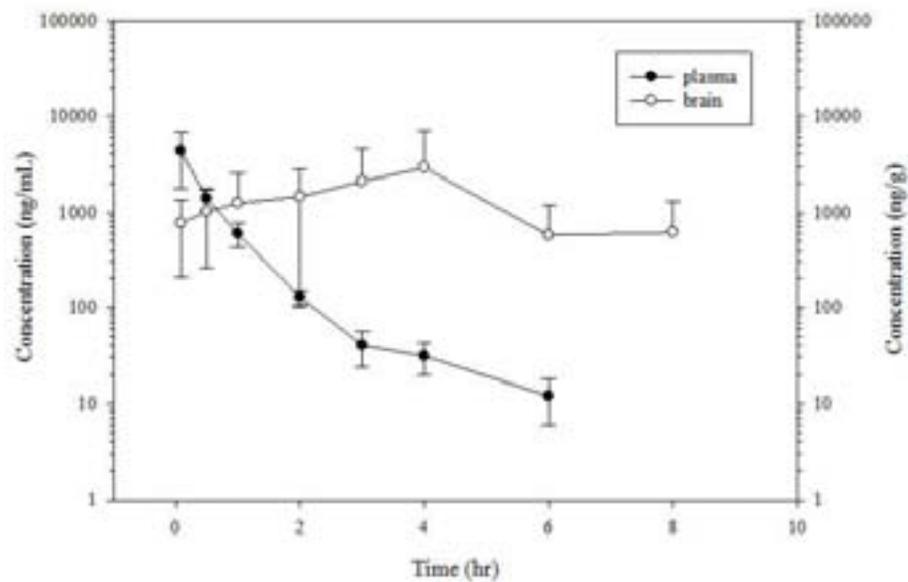
CYP inhibition

| Compound | CYP inhibition IC ₅₀ [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

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

Summary



- 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.

Acknowledgement



Molecular Biology

Stephen Neame

Pharmacology

Laura Barden

Martin Gill

Jonathan Papworth

Anthony Groom

Janette Robertson

David Reynolds

Toshal Patel*

Pharmacology (Japan)

Toshihiko Yamauchi

Naoki Tokuhara

Makoto Ogo

Kenzo Muramoto

*project leaders

Cell Biology

Bina Shah

Louise Morgan

Biochemistry

Raymond Chung

Jim Staddon

ADME (Japan)

Susumu Takakuwa

Osamu Takenaka

ADME (US)

Nancy Wong

Vipul Kumar

Edgar Schuck

Medicinal Chemistry

Afzal Khan

Gurpreet Bhatia

Vanessa Palmer

Darren Medland

Hirotohi Numata

Paschalis Dimopoulos

Christopher Farthing

Piotr Graczyk*

Chemistry (US)

Keith Wilcoxon

Tom Horstman

SBDD (Japan)

Atsushi Inoue

Masahiro Sakurai

Scientific and managerial support

Luis Castro