A Small Molecule Bidentate-Binding Dual Inhibitor Probe of the LRRK2 and JNK Kinases

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ACS Chem. Biol., 2013, ASAP, June 10th Web

(Scripps Research Institute, Florida)

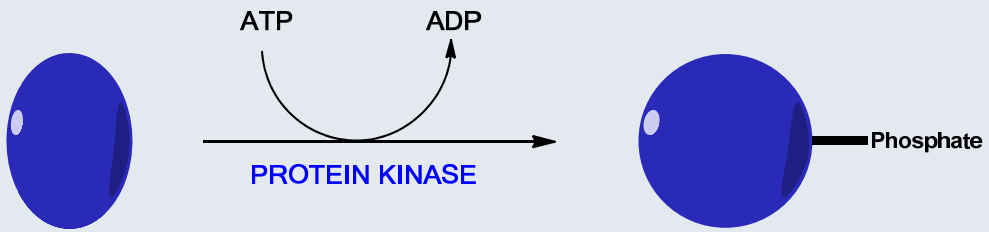
A. Manos-Turvey,

Wipf Group Current Literature

July 13th, 2013

Protein Kinases

- Protein kinases phosphorylate ~30% proteins, and are key to signal transduction in cells, through activation of relevant proteins.



- They are considered an attractive target in diseases where phosphorylation of a protein triggers continued growth or initiates apoptosis

G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, Science, **2002**, *298*, 1912-1934
P. Cohen, European Journal of Biochemistry, **2001**, *268*, 5001-5010

JNK Kinases

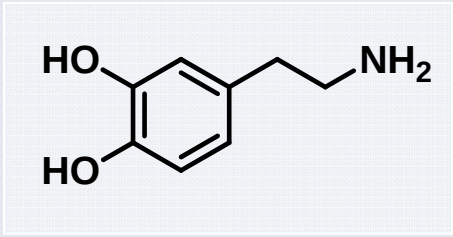
- JNK = c-jun-*N*-terminal kinase
  - a member of the MAP (mitogen-activated protein) kinases
  - regulates survival, proliferation, differentiation and apoptosis
- three forms: JNK1, JNK2 and JNK3
  - JNK3 is found predominantly in the brain
- JNK is activated by external stress such as UV-irradiation or reactive oxygen species (ROS)
- contributor to tumour promotion, and onset of neuronal apoptosis in neurodegenerative diseases

H. Okazawa, S. Estus, American Journal of Alzheimer's Disease and Other Dementias, **2002**, *17*, 79-88

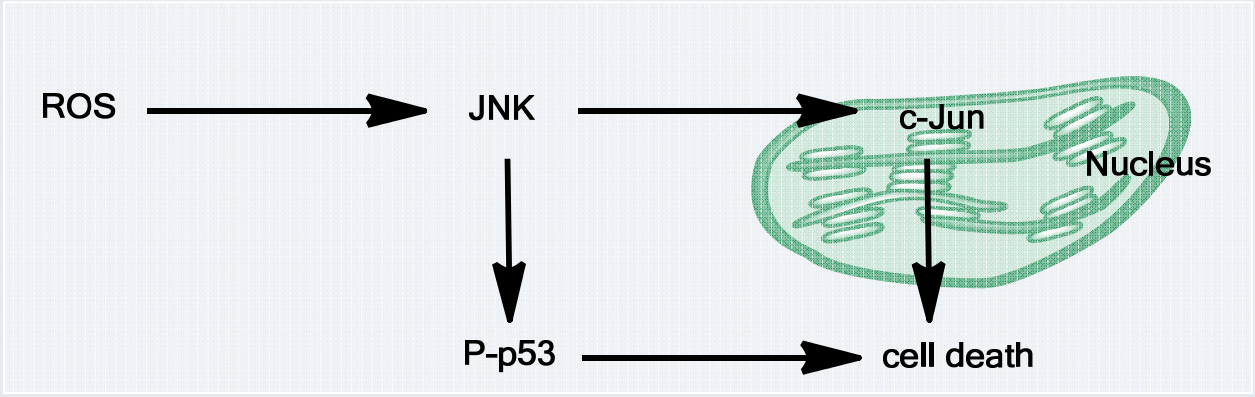
J. Peng, J. Andersen, IUBMB Life, **2003**, *55*, 267-271

Parkinson's Disease and JNK

- characterised by loss of dopaminergic neurons
- dopamine can induce oxidative stress leading to ROS which activates JNK



- under normal conditions wild type α-synuclein has been shown to protect against ROS
- in Parkinson's disease α-synuclein aggregates form Lewy bodies and the JNK pathway is no longer inhibited



H. Okazawa, S. Estus, American Journal of Alzheimer's Disease and Other Dementias, **2002**, *17*, 79-88

J. Peng, J. Andersen, IUBMB Life, **2003**, *55*, 267-271

LRRK2

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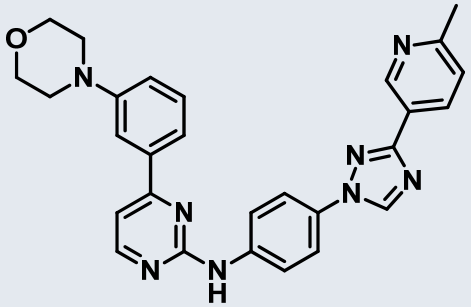
- leucine-rich repeat kinase-2
  - involved in many cellular processes in neurons, controlling multiple signaling pathways
  - High structural homology with a related MAPK family
  - at least 20 mutations in *LRRK2* are linked to autosomal-dominant Parkinson's disease
  - inhibition of kinase activity has been shown to disrupt LRRK2 related toxicities

I. F. Mata, W. J. Wedemeyer, M. J. Farrer, J. P. Taylor, K. A. Gallo, Trends in Neurosciences, **2006**, *29*, 286-293

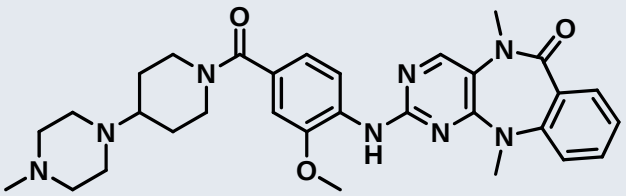
B. D. Lee, J. H. Shin, J. VanKampen, L. Petrucelli, A. B. West, H. S. Ko, Y. I. Lee, *et al.*, Nature Medicine, **2010**, *16*, 998-1000.

Proof of Concept

- proven that selective inhibition of the JNKs can treat neurodegenerative diseases
  - SR-3306 = orally bioavailable and can penetrate the brain (30 mg/kg) in mouse models of Parkinson's disease
- selective inhibition of LRRK2 is possible
  - LRRK2-IN-1 = a reversible competitive inhibitor of LRRK2
  - half-life of 4.5 hours when intravenously injected
  - inhibitors suffer from inability to penetrate the brain



SR-3306
JNK3 IC_{50} = 159 nM
Cell IC_{50} = 216 nM

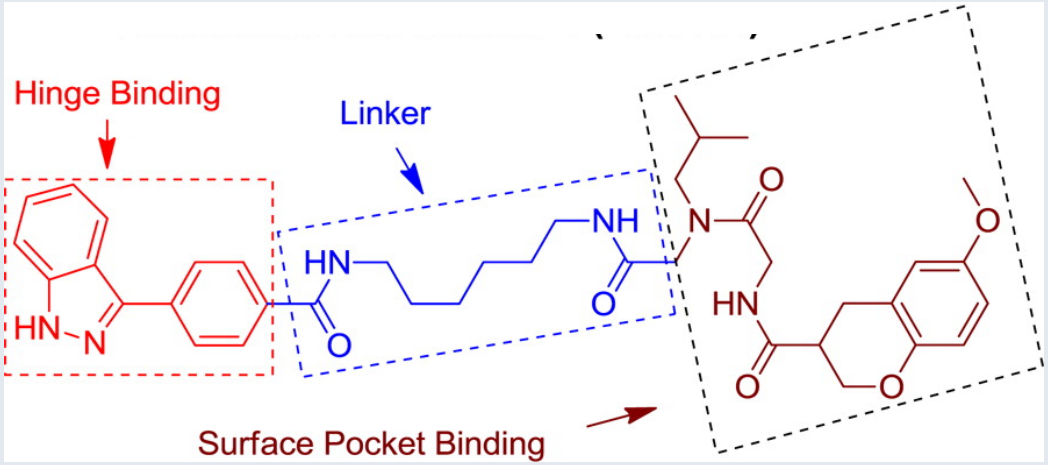


LRRK2-IN-1
wild type LRRK2 IC_{50} = 13 nM
a mutant LRRK2 IC_{50} = 6 nM

J. W. Chambers, A. Pachori, S. Howard, M. Ganno, D. Hansen, T. Kamenecka, *et al.*, ACS Chemical Neuroscience, **2011**, *2*, 198-206
C. E. Crocker, S. Khan, M. D. Cameron, H. A. Robertson, G. S. Robertson, P. LoGrasso, ACS Chemical Neuroscience, **2011**, *2*, 207-212
T. Kramer, F. Lo Monte, S. Göring, G. M. Okala Amombo, B. Schmidt, ACS Chemical Neuroscience, **2012**, *3*, 151-160.

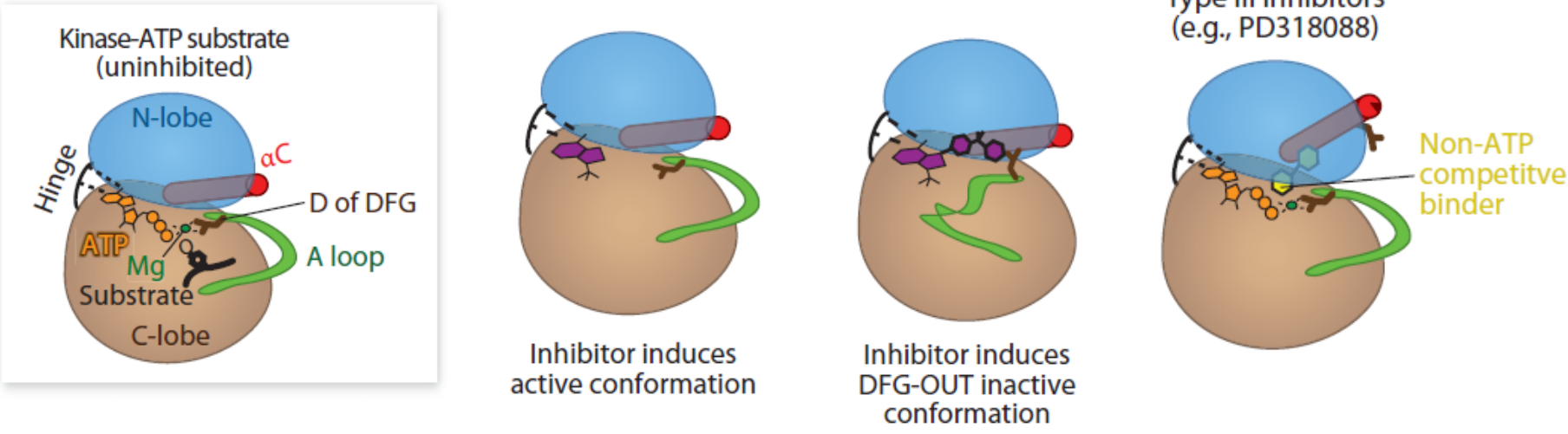
Aim: To Identify Bidentate-Binding Inhibitors

- discover “unique JNK inhibitors from diversified scaffolds”
- create dual inhibitors of JNK3 and LRRK2
  - investigate if inhibition is additive or synergistic
- combine traits of known kinase inhibitor types in a single bidentate-binding inhibitor



Types of Kinase Inhibitors

- Type I: target the active conformation of the ATP binding site
- Type II: bind to an inactive conformation of the ATP binding site
- Type III: are non-ATP competitive, usually bind to an allosteric pocket

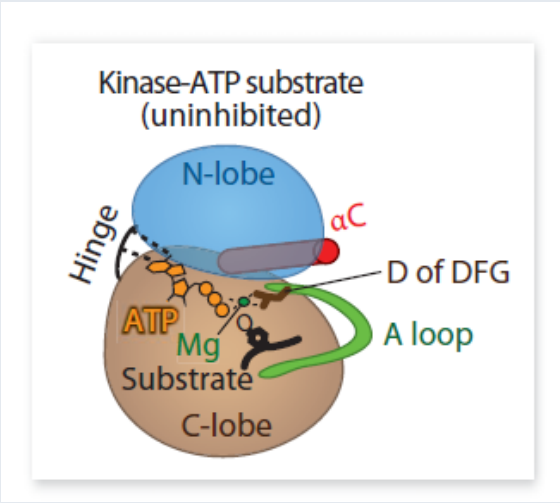


A. C. Dar, K. M. Shokat, Annual Review of Biochemistry, **2011**, *80*, 769-795

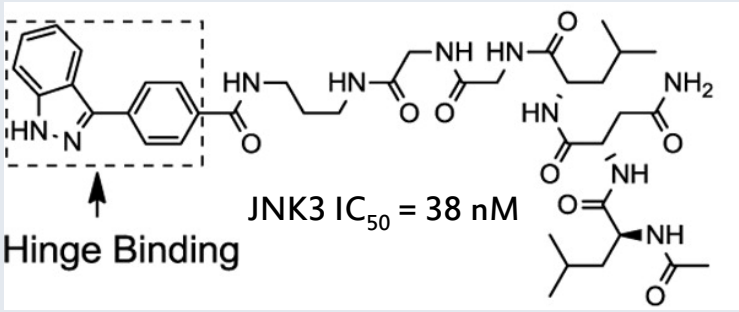
L. Garuti, M. Roberti, G. Bottegoni, Current Medicinal Chemistry, **2010**, *17*, 2804-2821

Optimisation of a JNK inhibitor

- in the JNKs, the substrate-binding pocket is close to the “hinge region” of the ATP binding site
- JIP is a native JNK interacting protein
  - 11-mer peptide derivatives can compete with JIP for the substrate-binding site



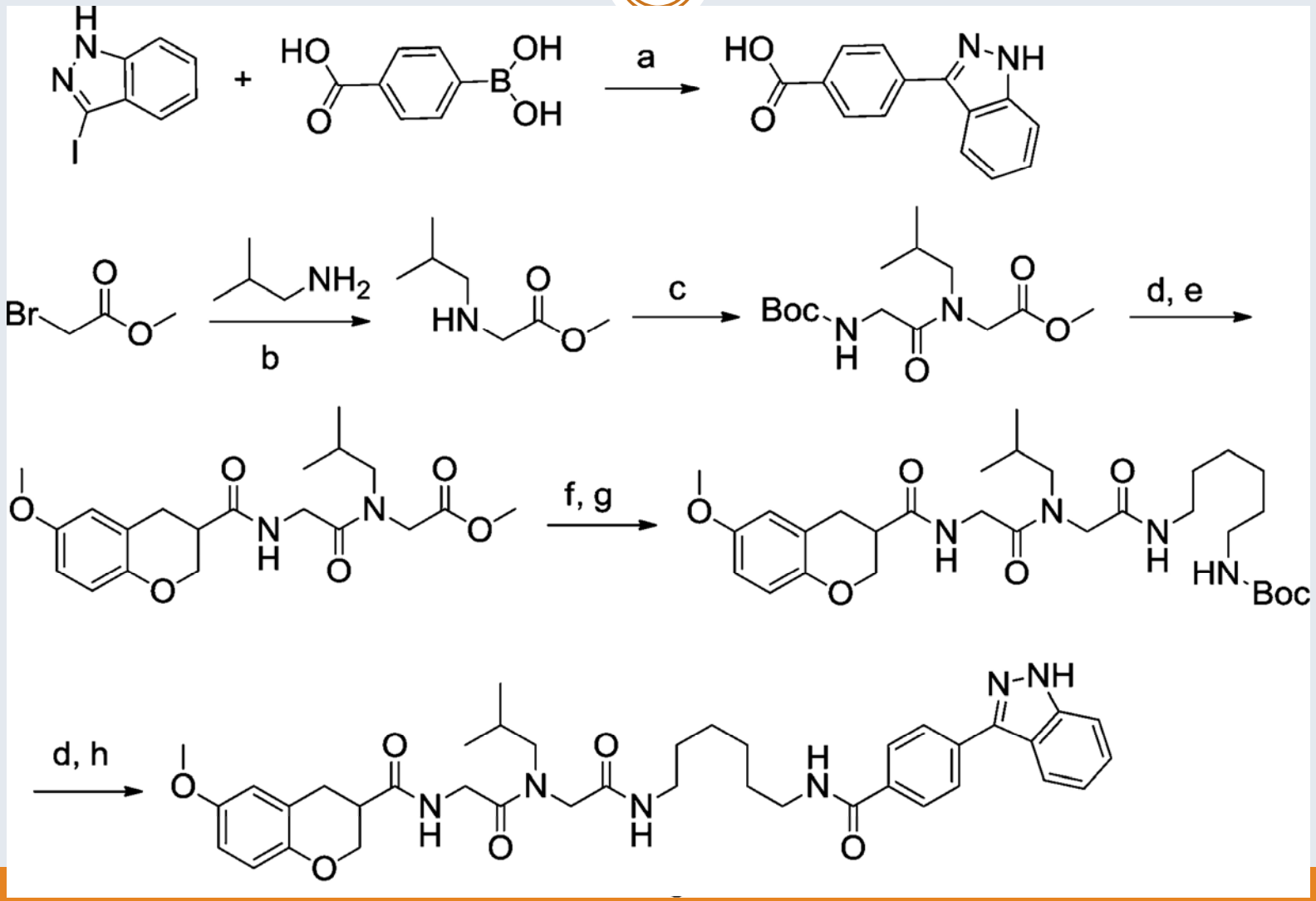
- bi-dentate binders were synthesised based upon a hinge binder coupled with the 11-mer peptides
  - optimised the 11-mer to tripeptide LNL inhibitors



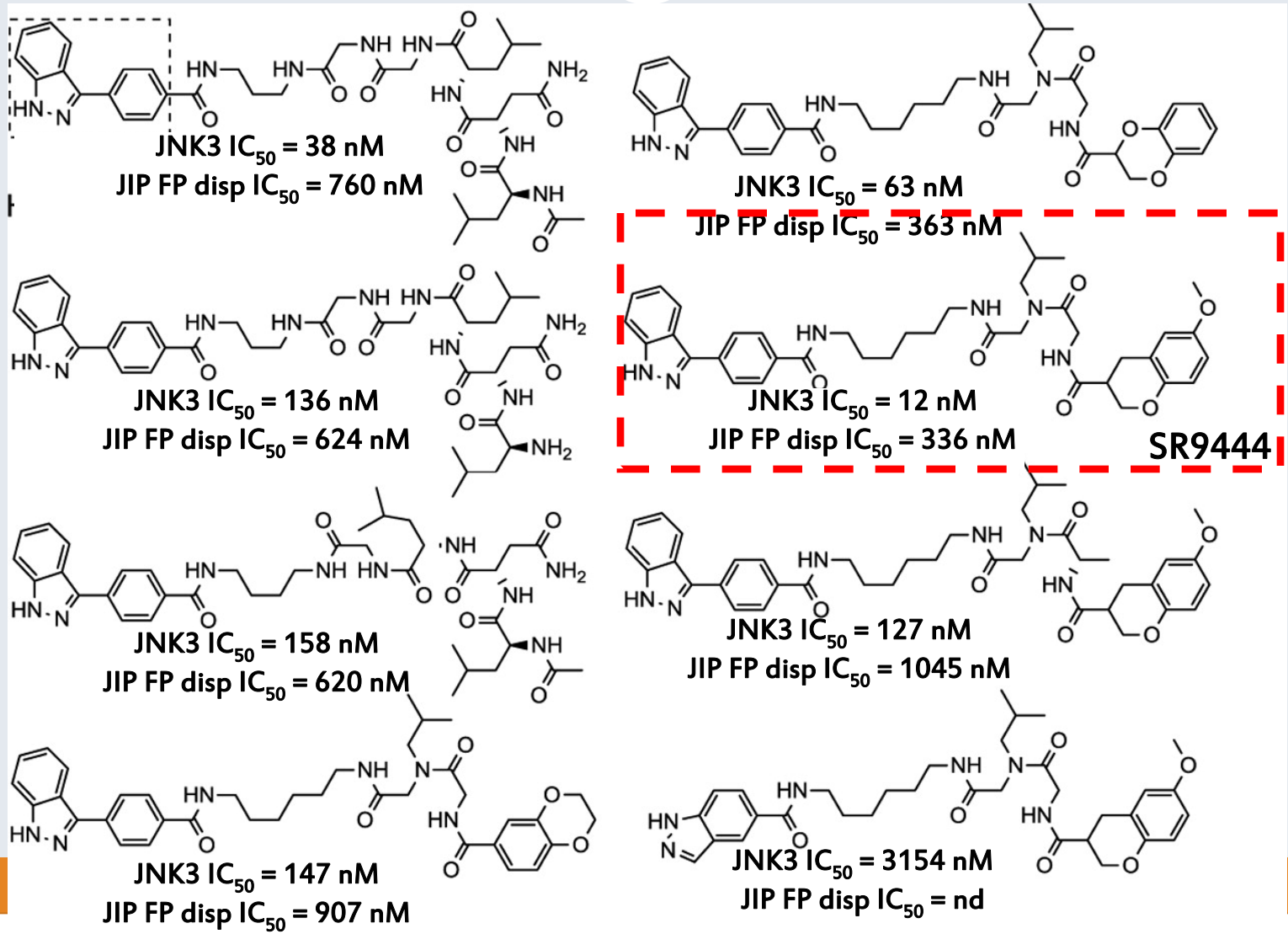
R. K. Barr, T. S. Kendrick, M. A. Bogoyevitch, Journal of Biological Chemistry, **2002**, *277*, 10987-10997

J. L. Stebbins, S. K. De, P. Pavlickova, V. Chen, T. Machleidt, *et al.*, Journal of Medicinal Chemistry, **2011**, *54*, 6206-6214

Synthesis of New Bidentate-Binders

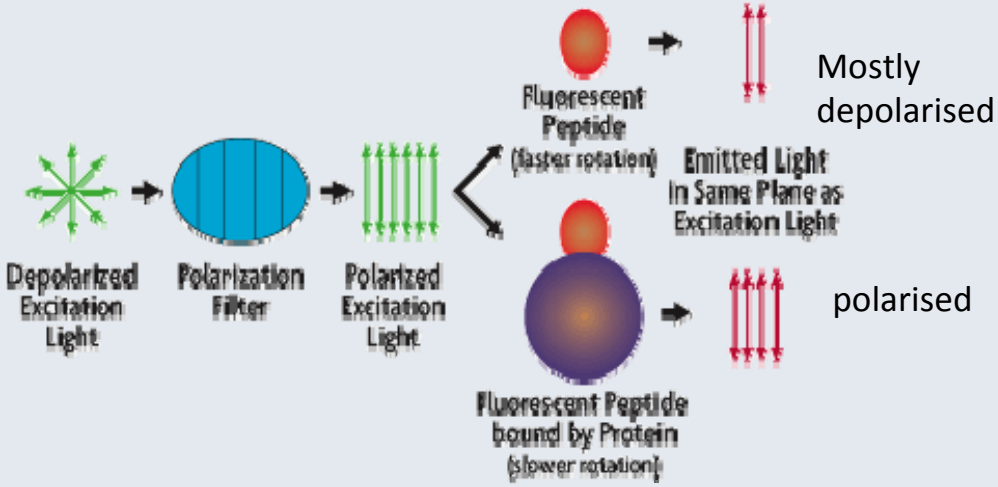


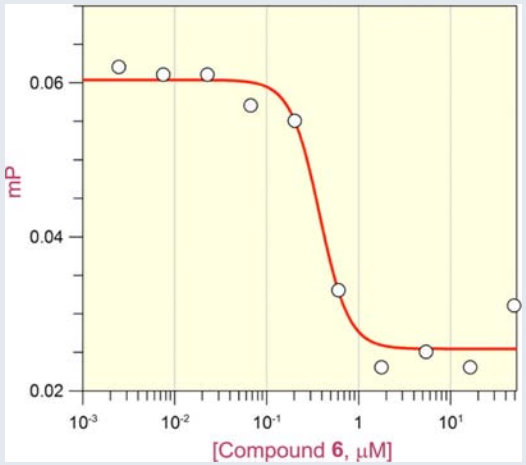
JNK Inhibition Results



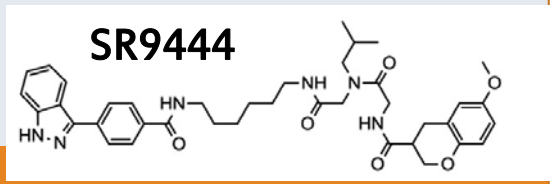
JIP Fluorescence Polarisation Displacement

- an 11-mer segment of native JIP protein bearing a fluorophore is placed in solution with JNK3 39-422 (containing the binding pocket)
- the fluorescence polarisation (FP) in the presence/absence of the inhibitors can then be measured





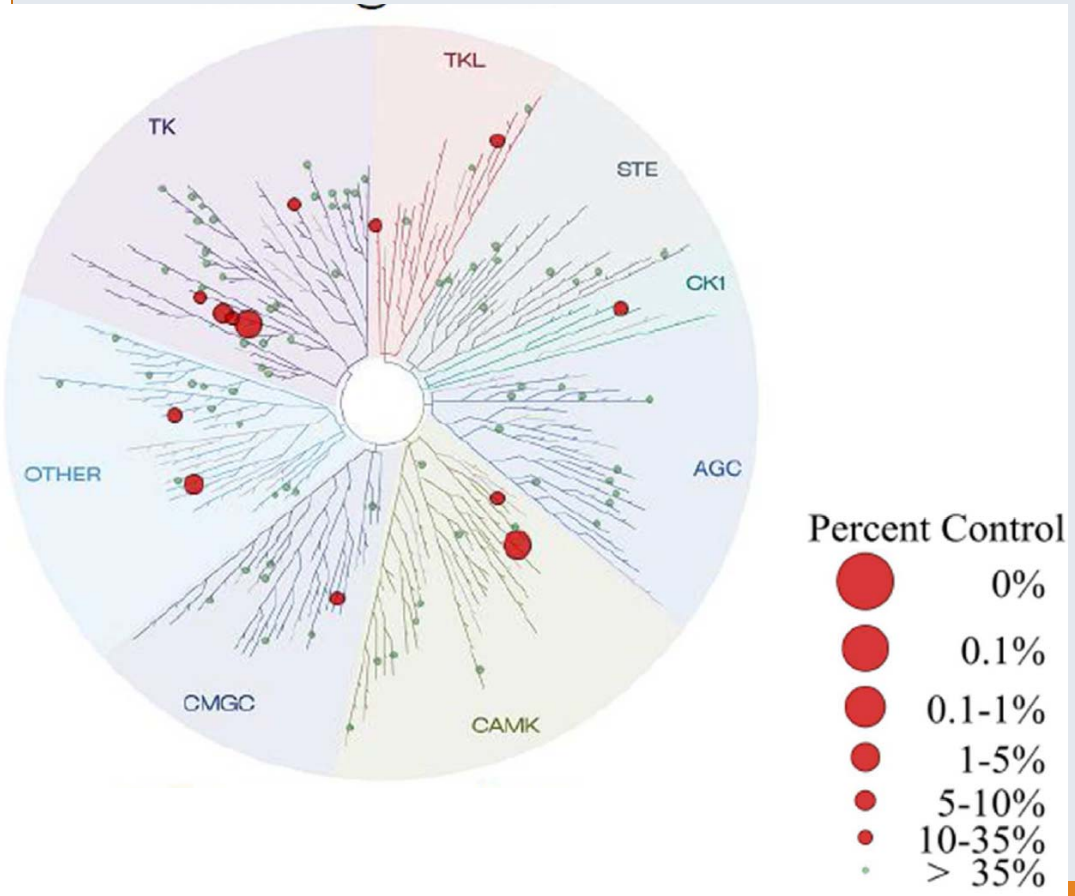
- if JNK3 inhibition is purely through substrate inhibition, the IC_{50}'s should be similar (IC_{50} = 12 nM JNK3 *vs* 336 nM FP)
  - SR9444 is also a competitive ATP inhibitor

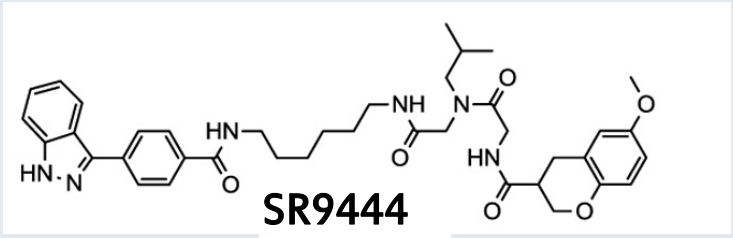


Cool Gif: http://www.activemotif.com/images/products/FP_diagram_animated.gif

Selectivity of SR9444

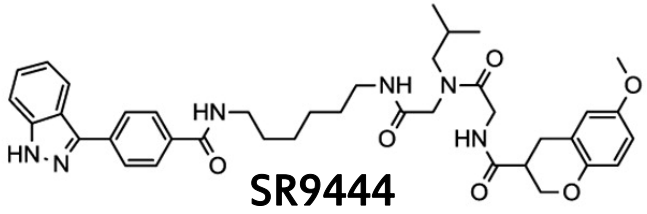
- screened at 10 µM against 117 kinases from different families
  - only six kinases tested had > 90% binding



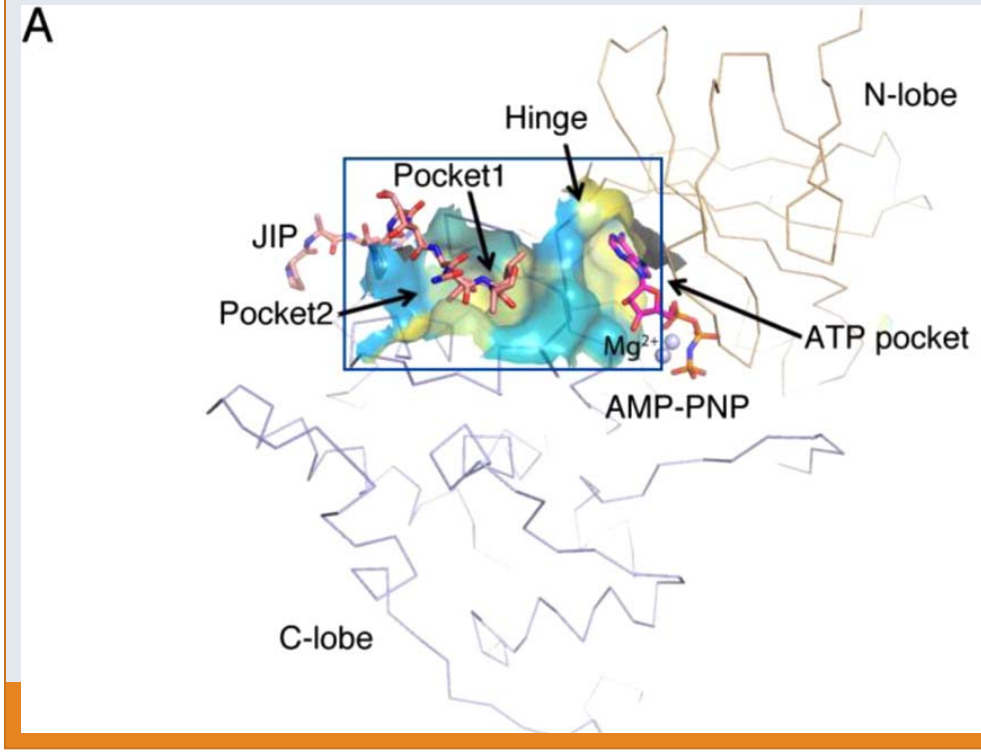


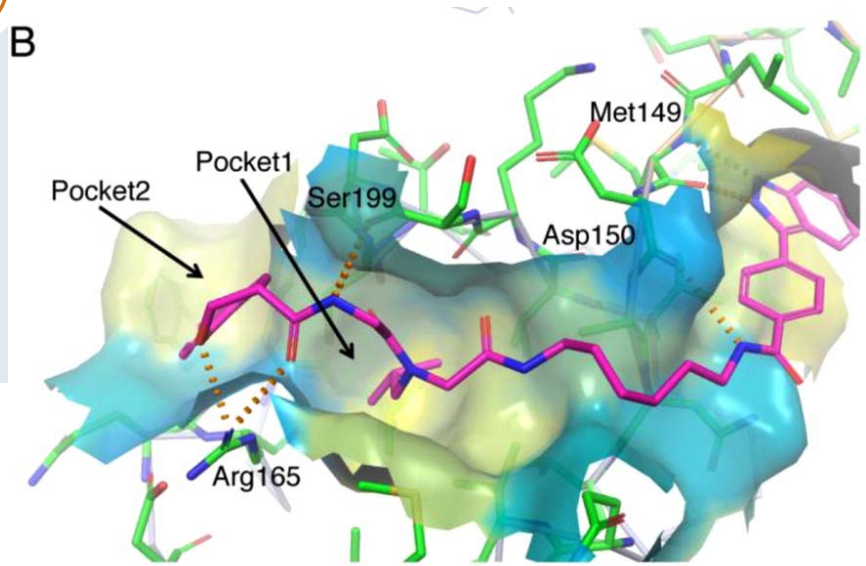
- with values of binding for LRRK2 > 95% and JNK1 and JNK3 ~100%, SR9444 was deemed largely selective
- proved to inhibit both wild-type LRRK2 and PD-specific mutant LRRK2-G2019S (IC_{50} = 100 nM)
  - Similar trends seen for other compounds from the series

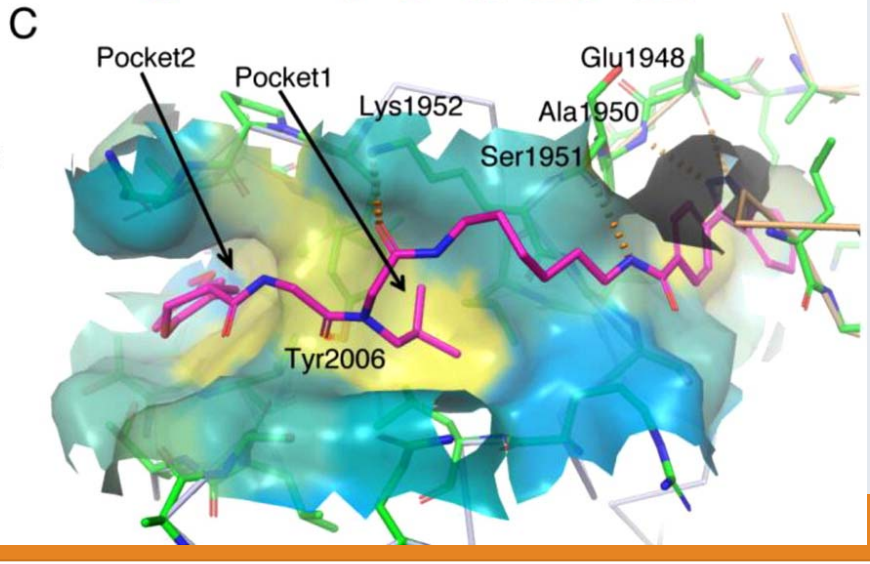
Docking Studies



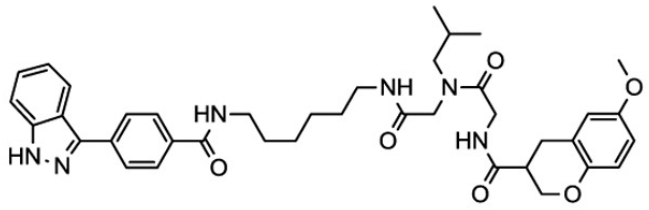
- two hydrophobic pockets exist in both JNK3 and LRRK2
  - these accommodate the isobutyl group of SR9444 and the chroman-3-carboxyl amide moiety



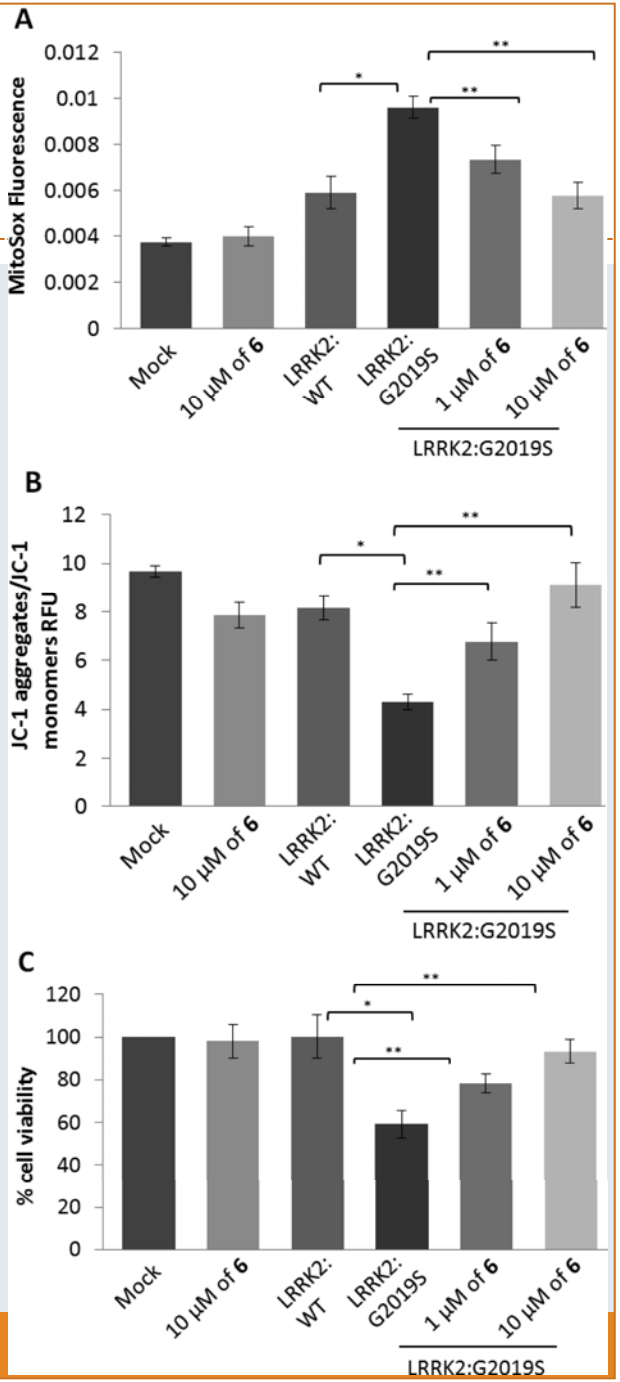




Cell Activities of SR9444

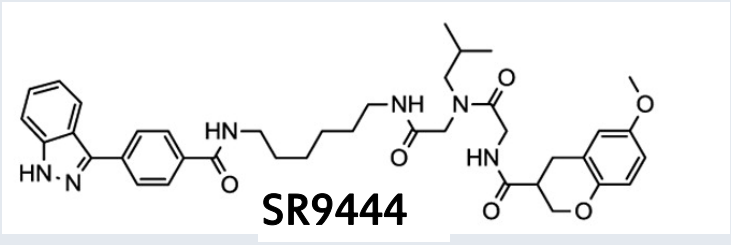


- IC_{50} of SR9444 inhibition of c-Jun phosphorylation in cells was 2.8 µM
- increases in ROS, seen with LRRK2-G2019S expression, are reduced upon 1 µM and 10 µM SR9444 addition
- mitochondrial membrane potential increases caused by mutant LRRK2 expression were returned to normal in levels with 10 µM SR9444
- cell viability returned to >95% after SR9444 treatment



Conclusions

- the use of bidentate-binding dual inhibitors is validated
- JNK and LRRK2 enzymatic inhibition is seen with SR9444
  - great loss of activity is seen when transferred into cells (12 nM vs. 2.8 µM) = poor cell permeability



SR9444

- need to lower the number of amide bonds, PSA value (155 Å), minimise MW (697 g/mol)
  - consider that increasing binding at multiple regions may allow for weaker binding moieties, increasing selectivity
- currently investigating the possibility of bidentate-binders which only target substrate binding pocket 1