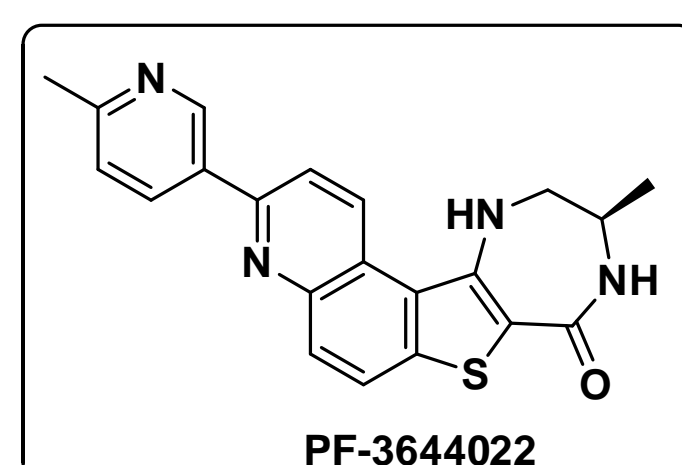


Introduction

- Mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2) plays an important role in TNF- α biosynthesis
- The p38 mitogen-activated protein (MAP) kinase pathway has been shown to play an important role in the production of TNF- α and other cytokines implicated in chronic inflammatory diseases
- MAPKAP-K2 is a direct substrate of the p38 kinase in the MAP kinase pathway and it is believed to be a potentially safer target compared to p38 for anti-inflammatory therapy
- At the time of commencing our work, there were no reported inhibitors of MAPKAP-K2 on which to base a program
- There have now been several reported programs that have identified robust inhibitors of MAPKAP-K2 e.g. PF-3644022 from Pfizer (Mourey et al. *J. Pharmacol. Exp. Ther.* 2010, 333, 797-807)

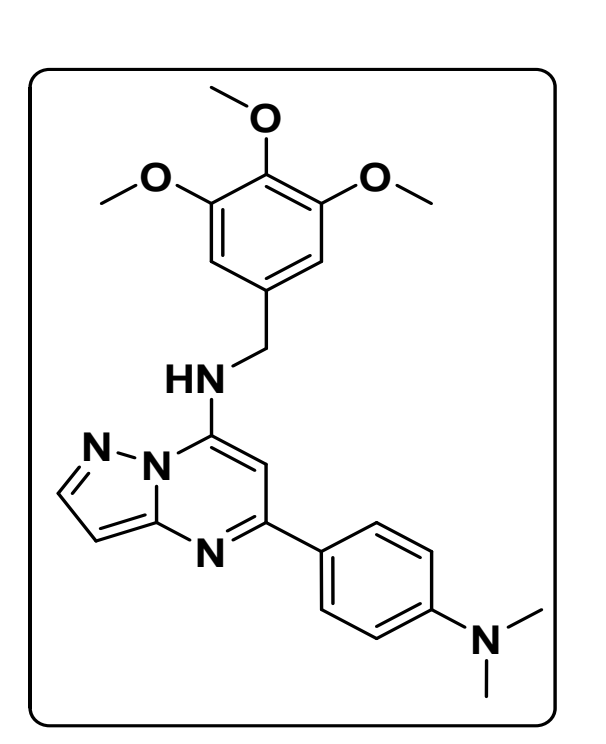
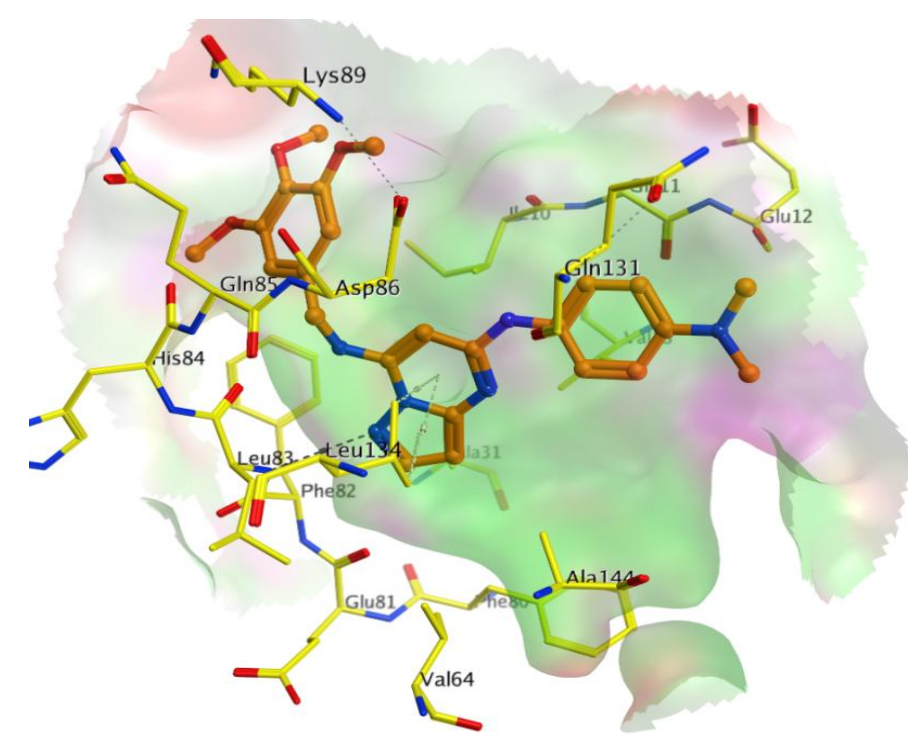
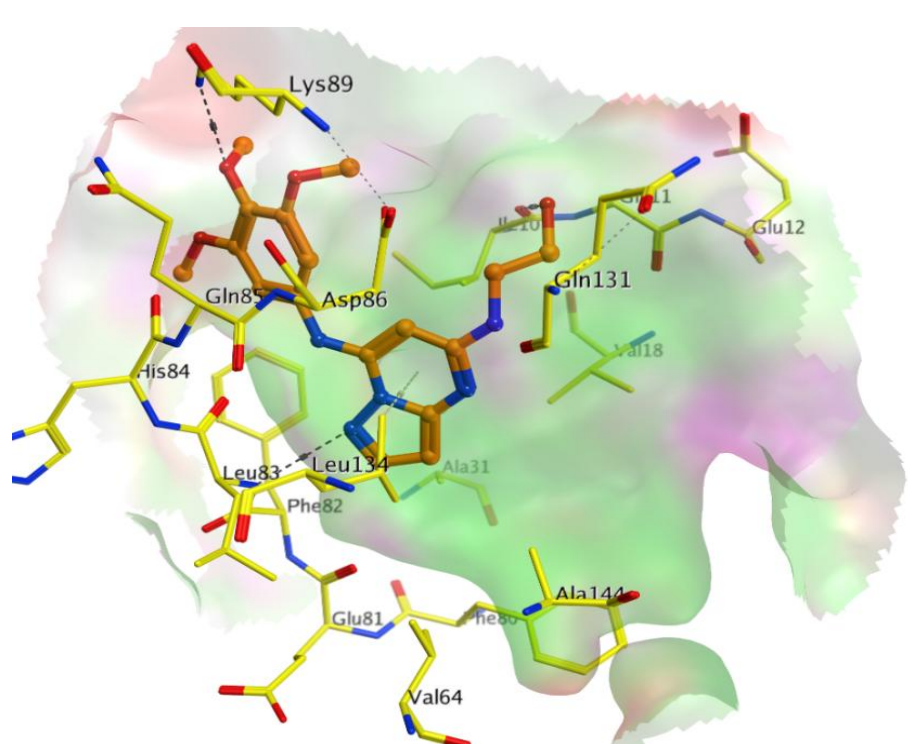
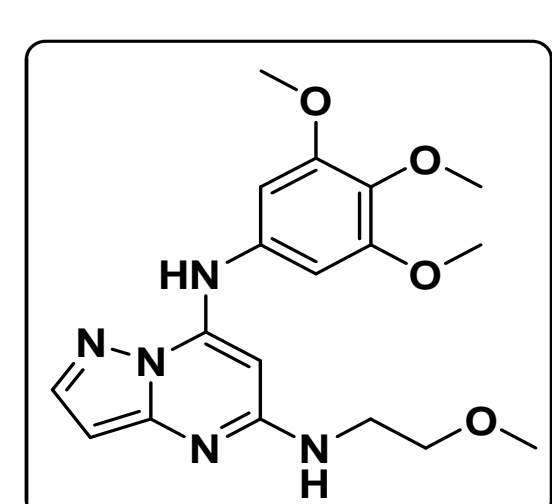
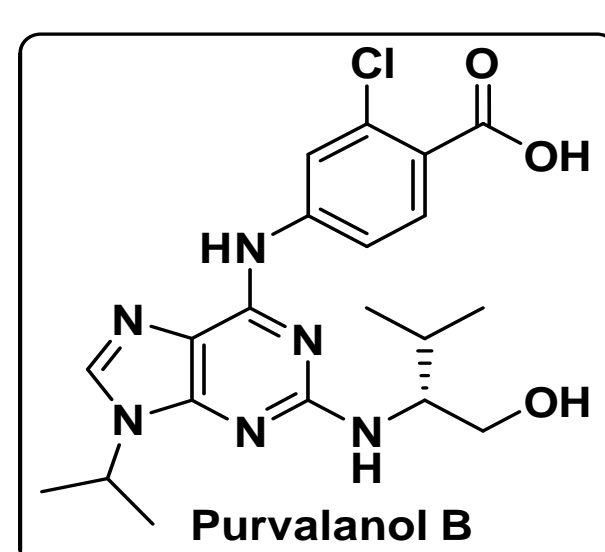


Structurally diverse screen

- We initially screened a 100,000 member diverse library and identified a small number of ATP-competitive hits with weak activity that proved not to be robust starting points for our program

Kinase library design

- SFK03 library design focused on the cyclin-dependent kinase (CDK) family
- Crystal structure of the CDK-2/purvalanol-B complex (PDB1CKP) used to model various N-bridgehead bicyclic scaffolds bound at the ATP site
- Pyrazolo[1,5a]pyrimidine scaffold selected for the library
- Key double H-bond between the purine ring of Purvalanol-B and Leu⁸³ preserved

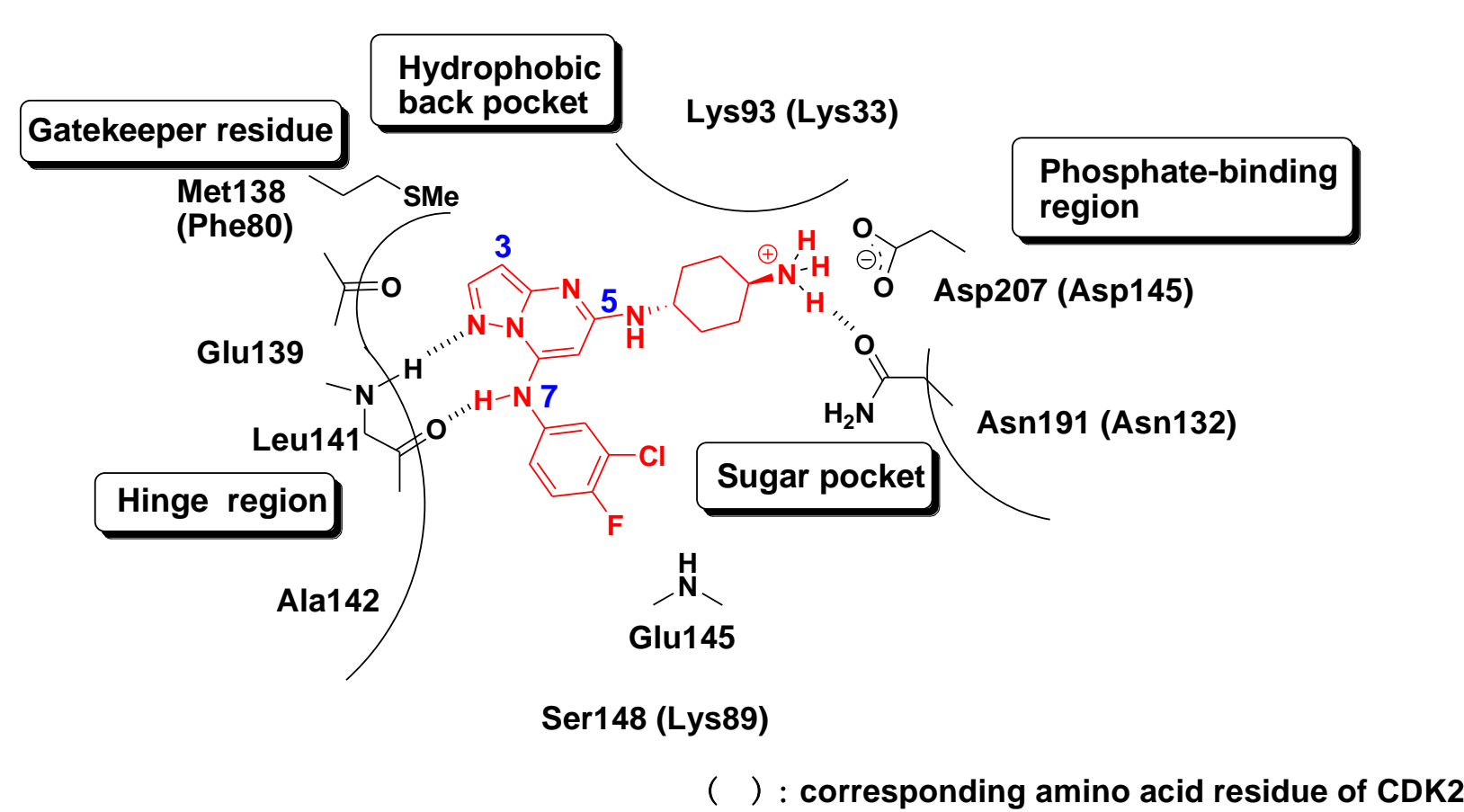


Example library compounds docked into CDK2 crystal structure

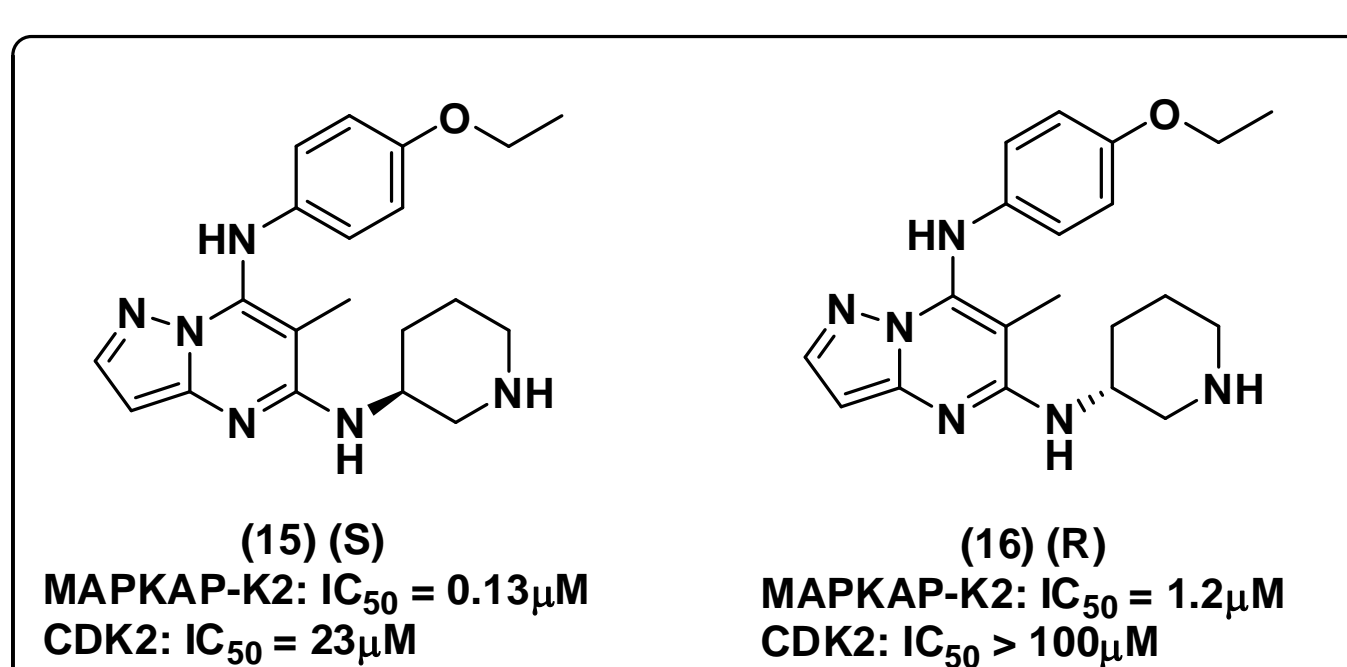
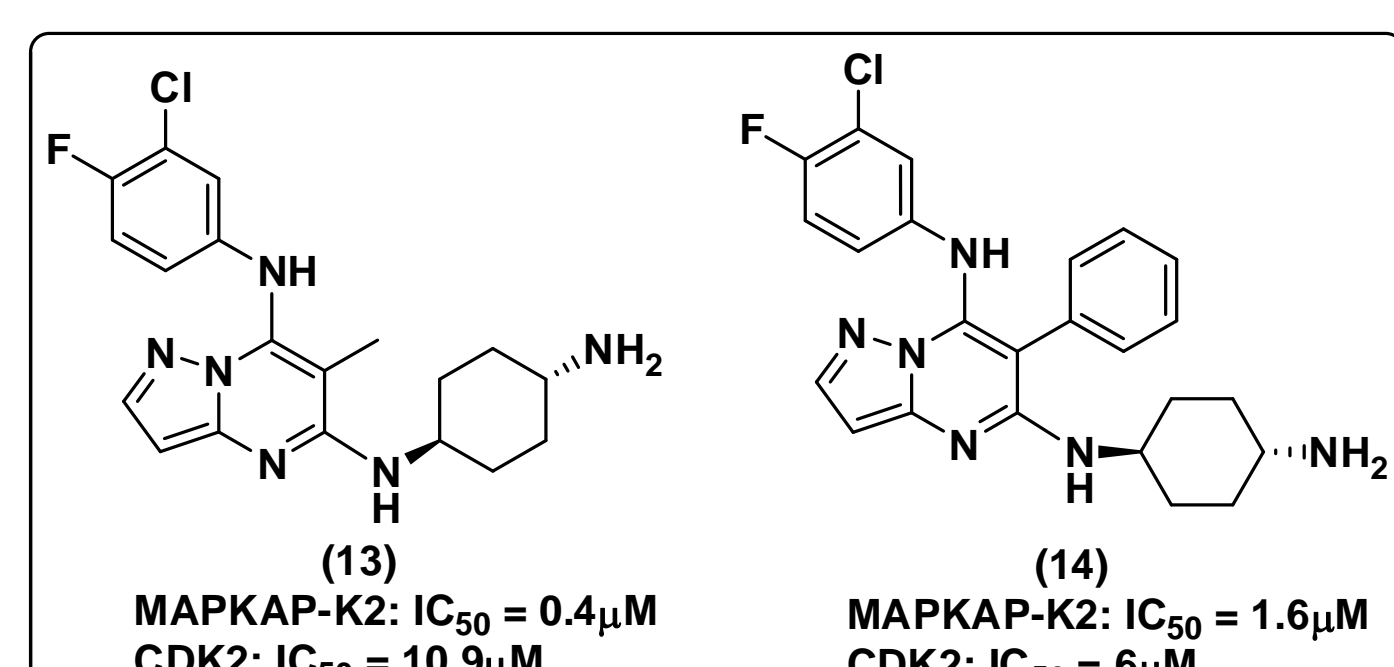
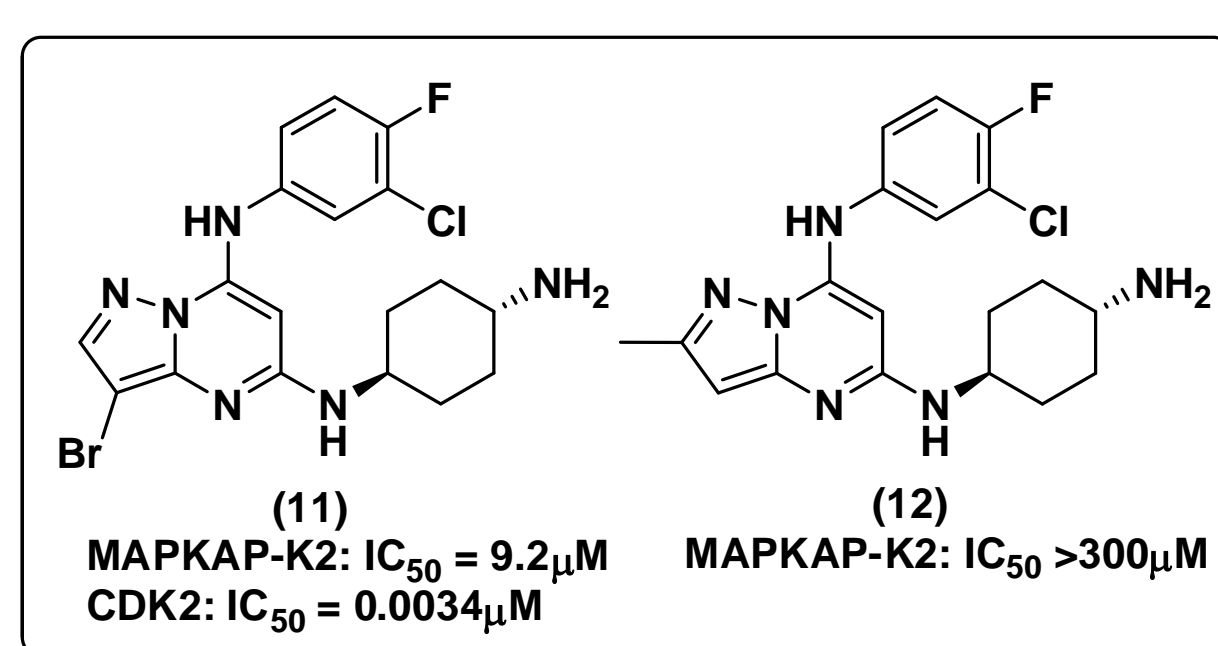
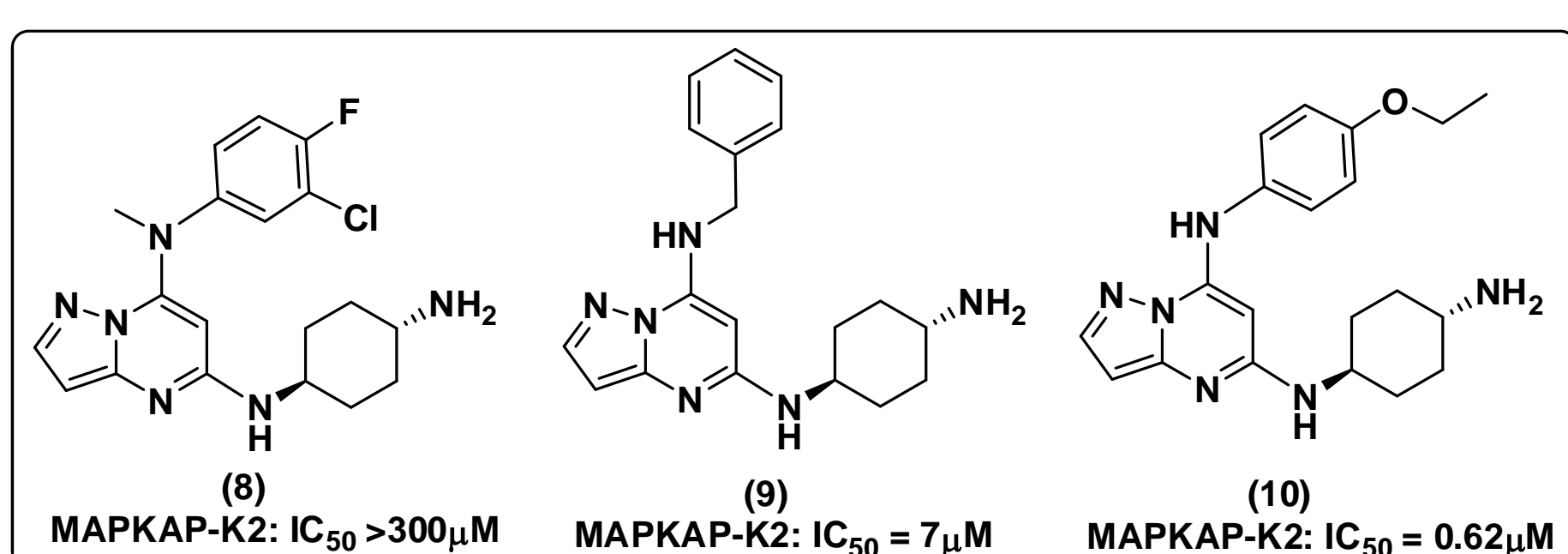
- 7-position complements hydrophobic pocket in the ATP site
- 5-position complements elongated pocket containing polar recognition groups
- Monomers validated using our modelling predictions

Structure-based optimisation

- Optimisation of the hit compounds guided by use of homology model
- Compounds designed to improve binding at MAPKAP-K2 and reduce potency at CDK2

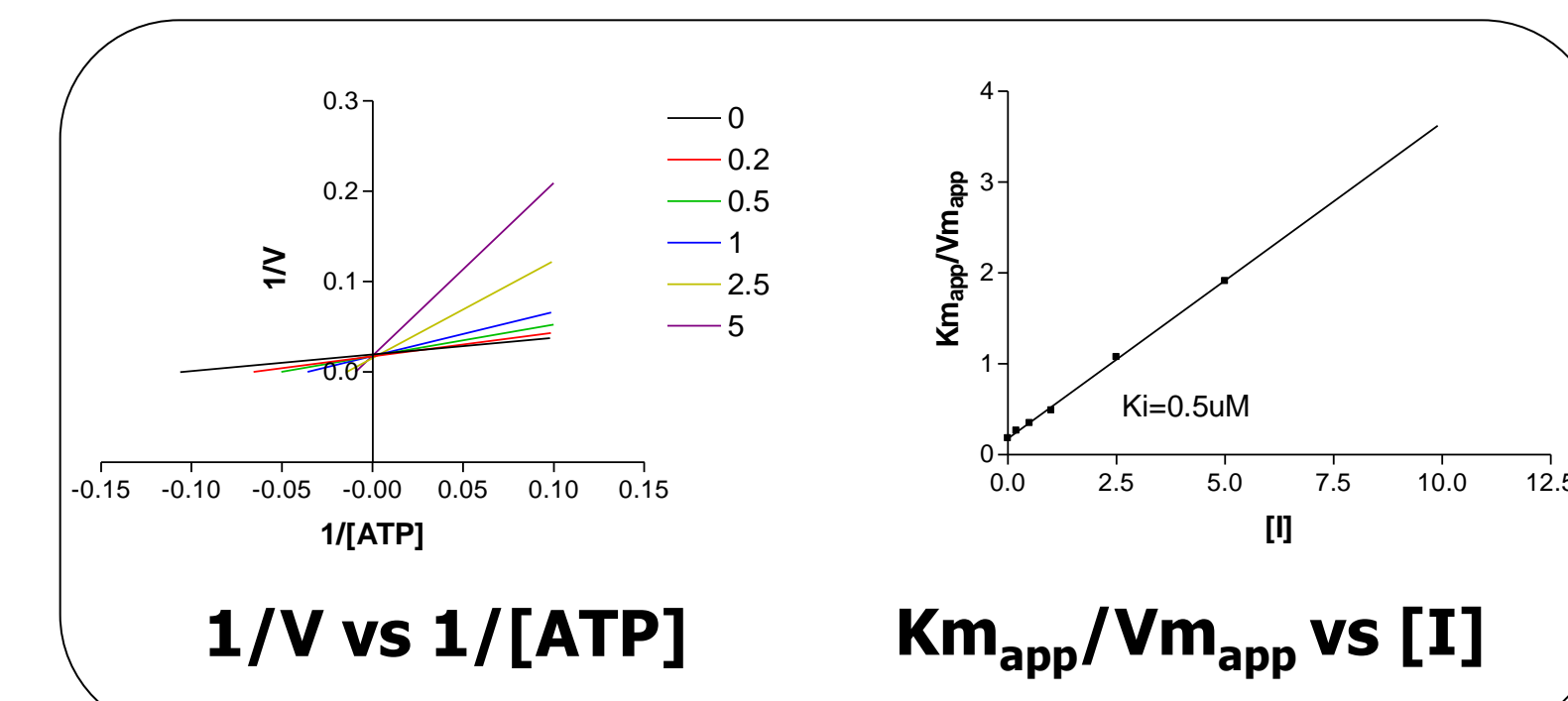
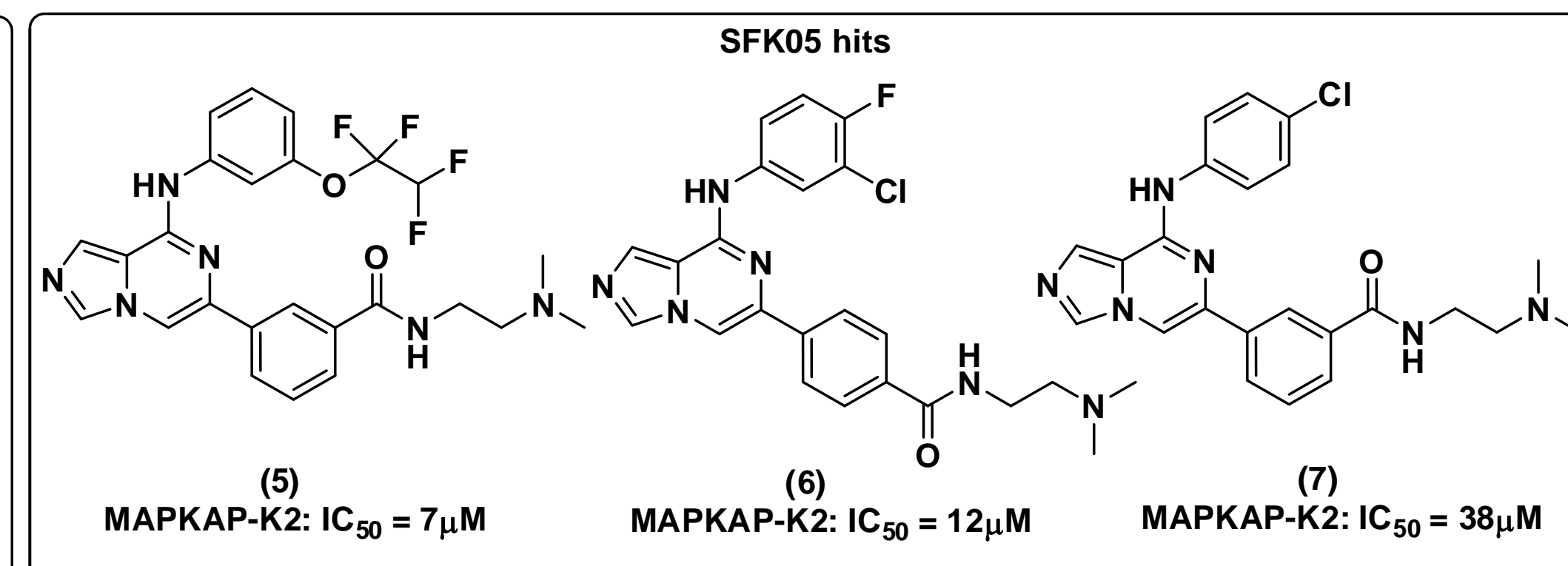
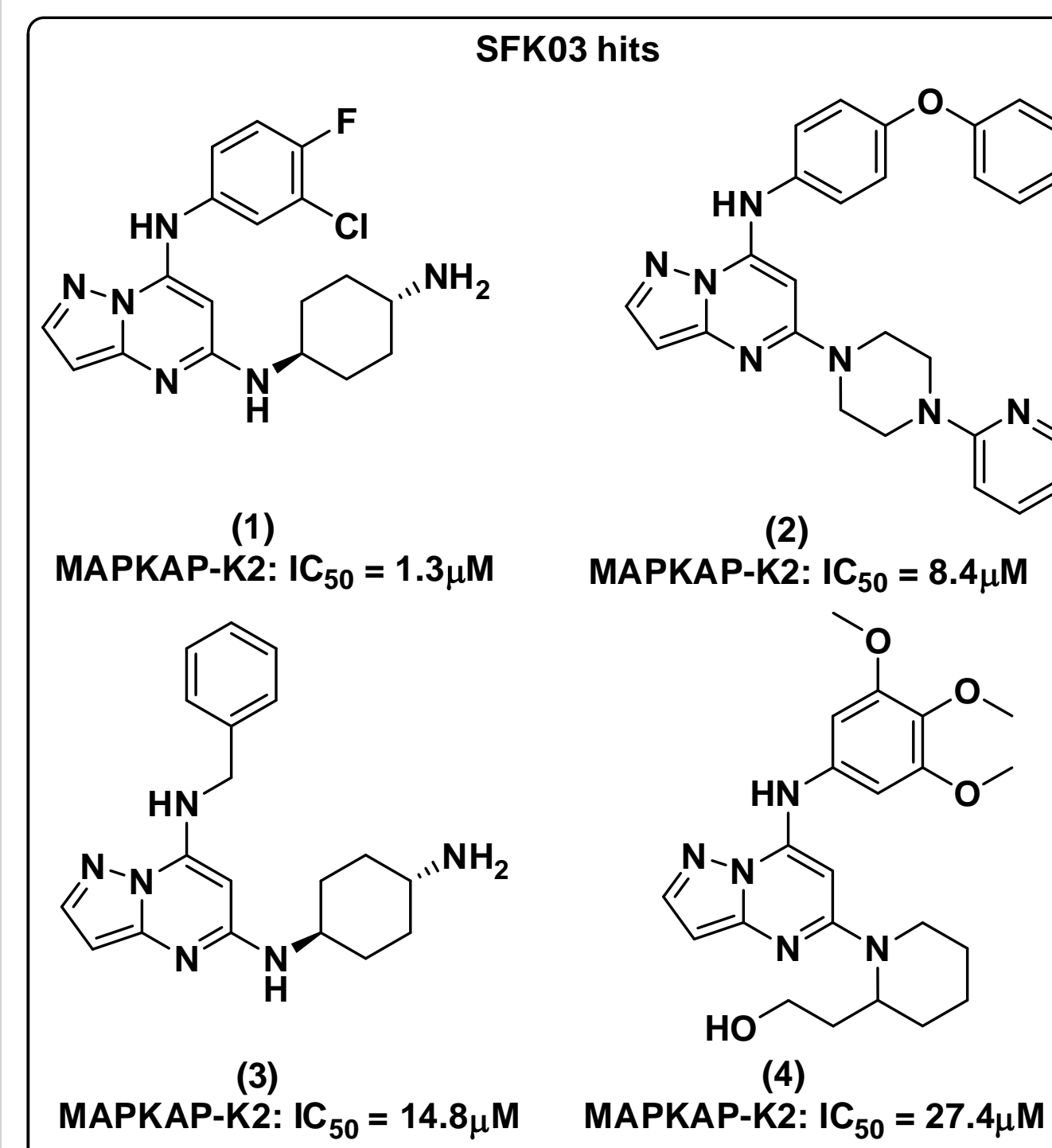


- 7-position - NH-Aryl preferred and a range of aryl substituents tolerated
- 2- and 3-position substituents detrimental to activity
- 6-position substituent drives selectivity; methyl group optimum
- 5-position piperidine - S enantiomer preferred

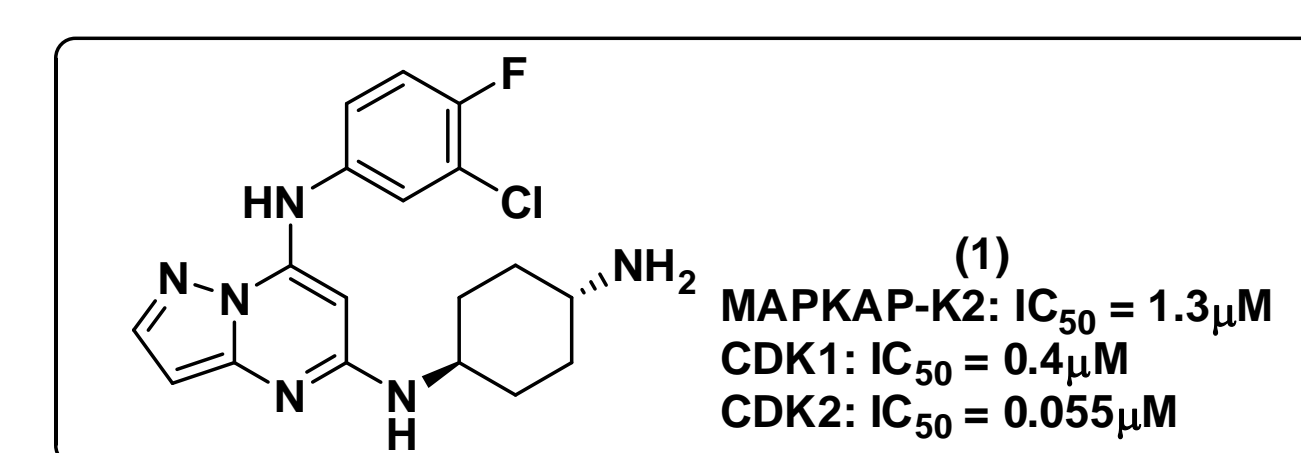


Focused screening campaign

- We screened a set of kinase-focused libraries and identified a small number of hits
- Initial hits were weakly active, but did exhibit some evidence of SAR

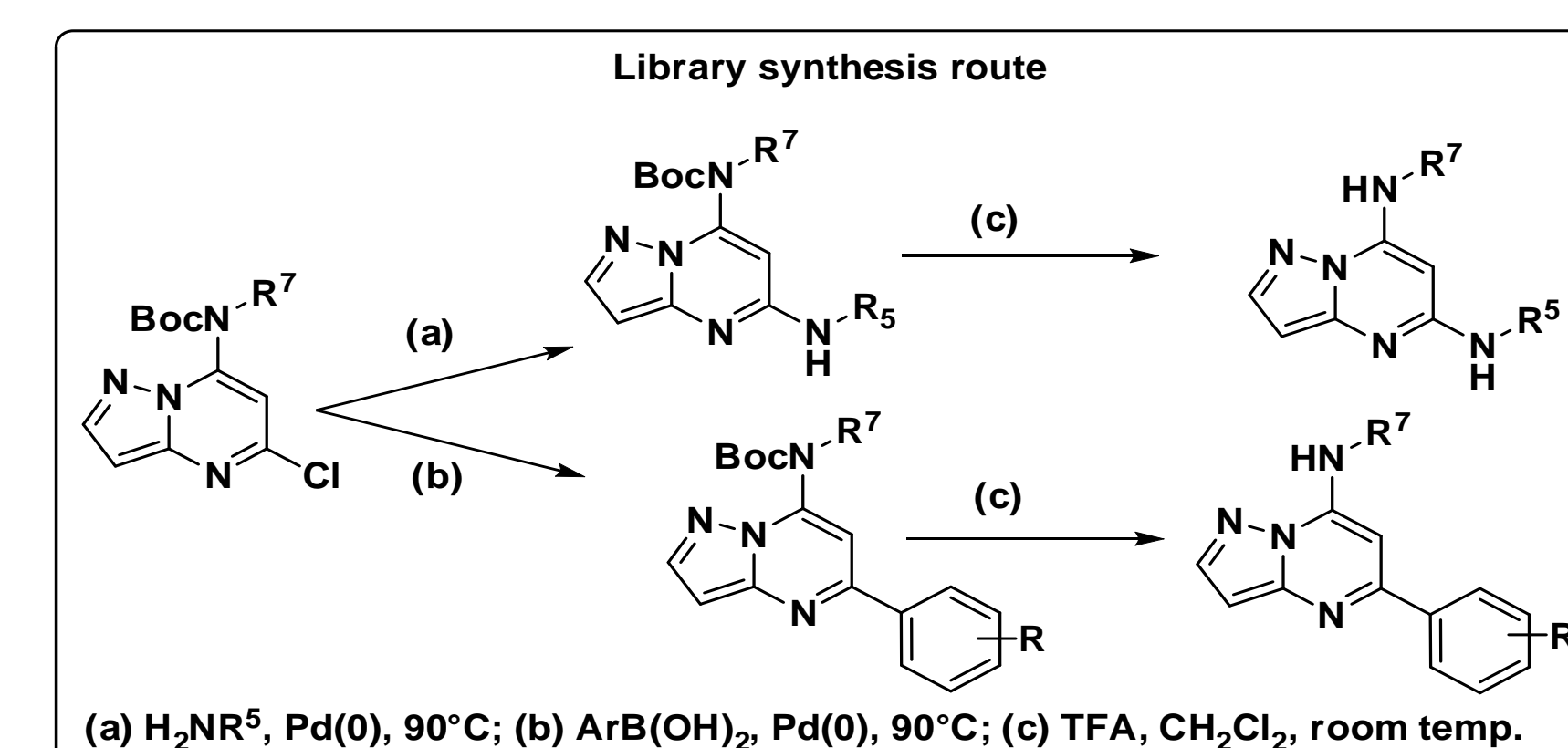


- The most promising hits were from SFK03
- Compound 1 was shown to be an ATP-competitive inhibitor and was selected as the start point for a hit-to-lead program
- Compound 1 demonstrated higher potency against CDK1 and CDK2

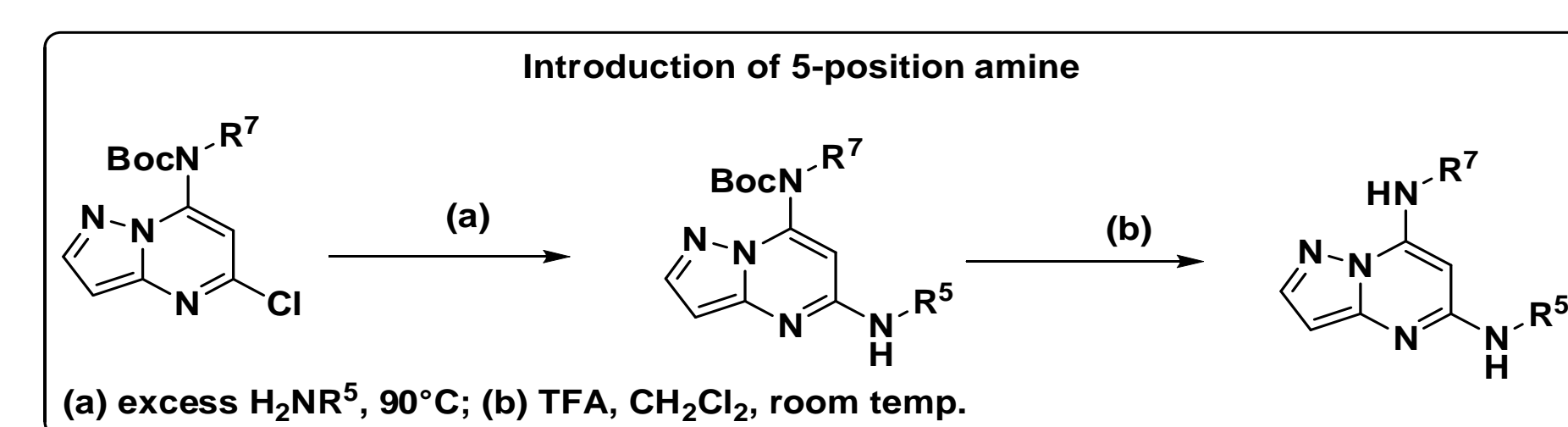


Synthesis

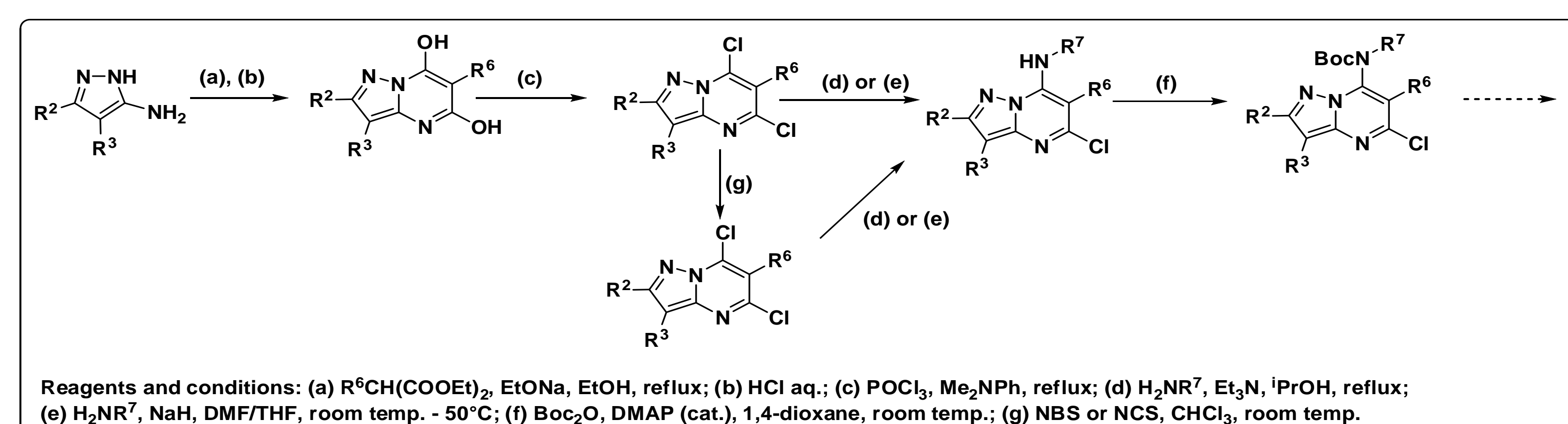
- Library synthesis route was adapted to enable a full exploration of the SAR around the initial hit compound



- Pd(0) catalysed introduction of the amine at the 5-position was replaced with a thermal displacement reaction
- Boc protection of 7-position NH required

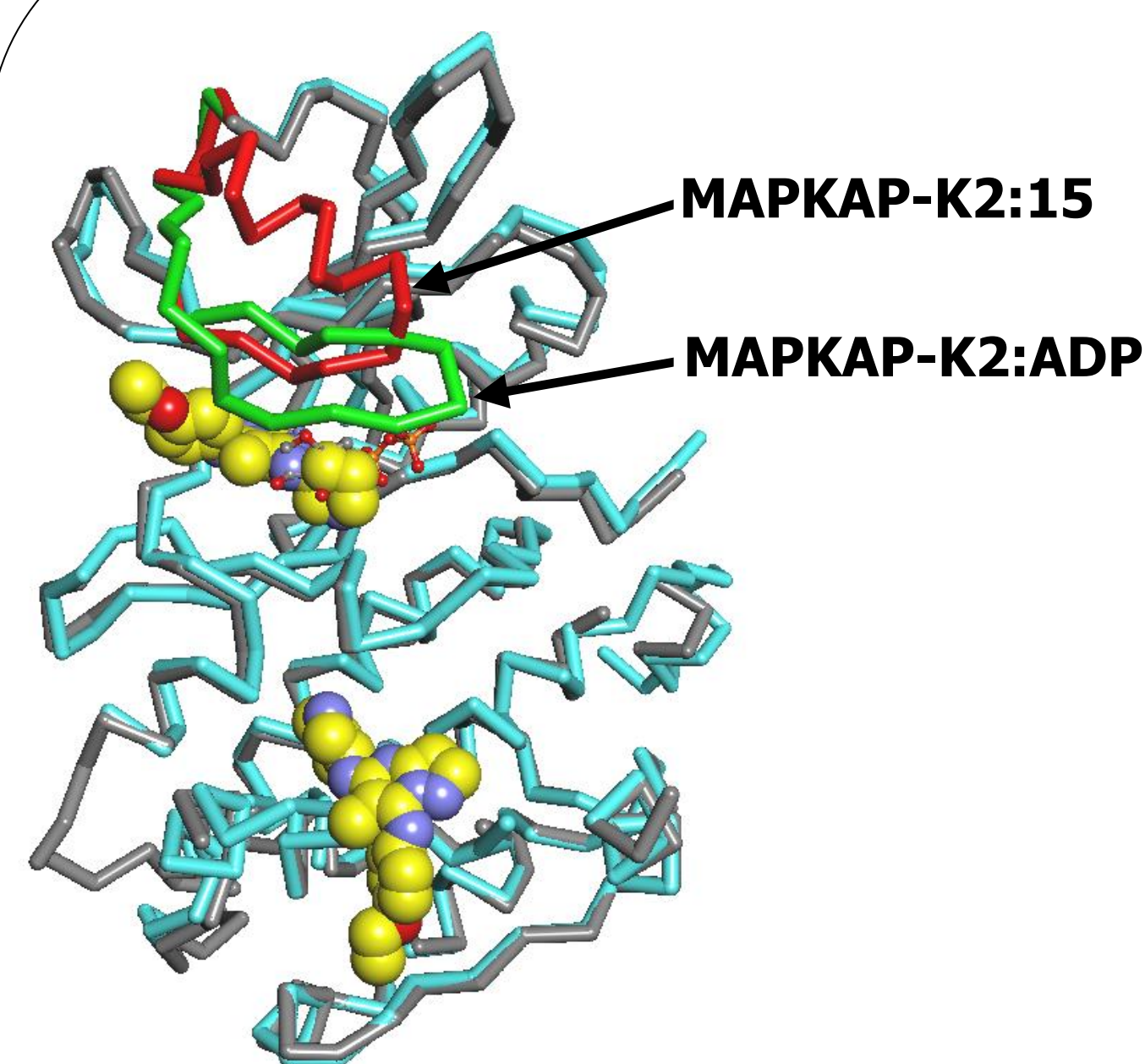


- Substituents incorporated at the various positions of the scaffold



X-ray crystal structure

- An X-ray crystal structure of a compound from the active series bound to crystalline MAPKAP-K2 was obtained and confirmed the predicted binding mode (Fujino et al. *Acta. Crystallogr.* 2010, 66, 80-87)



- Overlay of crystal structure of MAPKAP-K2 with 15 (grey and red) and MAPKAP-K2 with ADP (blue and green)
- Two molecules bound in each monomer complex
- One molecule bound to ATP-binding site and other molecule bound near the substrate-binding site
- Gly-rich loop of MAPKAP-K2 with 15 bound adopts α -helix conformation (shown in red)
- MAPKAP-K2 with ADP bound adopts a β -sheet conformation (shown in green)

- The lead compound 15 was found to exhibit good cellular potency as an anti-TNF- α agent and *in vivo* efficacy in a mouse model of endotoxin shock. A full account of the work disclosed here will be published in due course