SMALL BUT SELECTIVE: DEVELOPING CHEMICAL PROBES FOR PKN2

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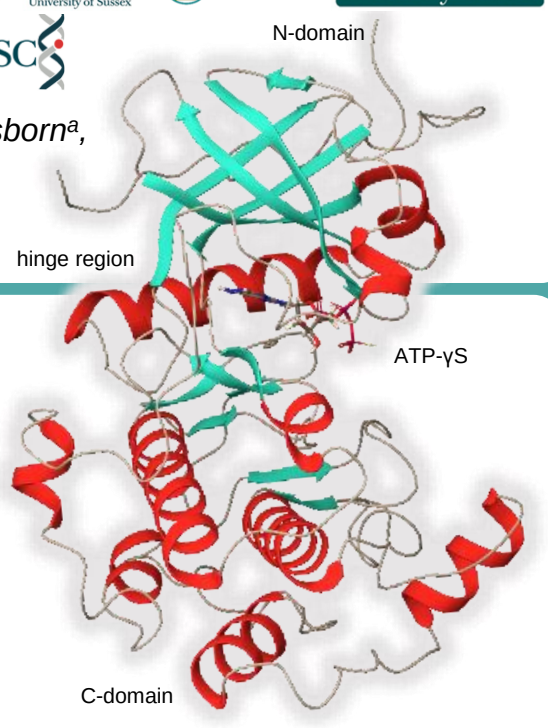
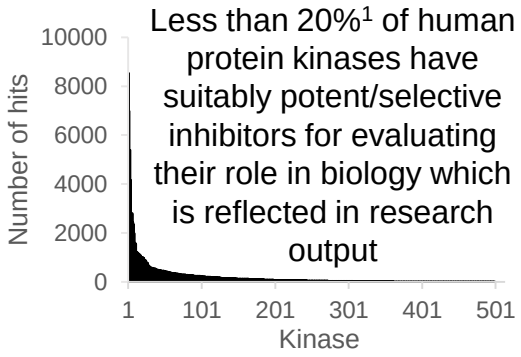


PKN2 in complex with ATPγS (PDB: 4CRS)

1. Probing the "Dark Kinome"



Plot of PubMed publication search hits for known human kinases (April 2019)

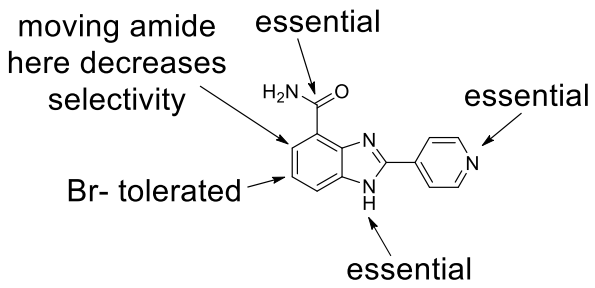
2. PKN2: an AGC kinase

- PKN2 (also PRK2) is a relatively neglected kinase in research, likely due to a lack of selective inhibitors[2]
- It is one of three homologues (PKN1/2/3)
- Reported cell signalling function in cell adhesion, transport, cytoskeleton regulation, cell cycle and apoptosis[3,4]
- Identified as a target of interest in colon[5], breast[6], renal[7], head[8], neck[8] and prostate[9] cancers
- The following work sought to optimise reported PKN2 inhibitors for use as chemical probes to validate this kinase as a potential drug target

3. Preparation of PKN2 inhibitors

Three compounds were selected from a data triage of 1200 reported PKN2 inhibitors in ChEMBL.[10] They were resynthesised and validated as PKN2 inhibitors in TR-FRET and NanoBRET assays. A library of each series was prepared to investigate structure activity relationships and improve on potency/selectivity.

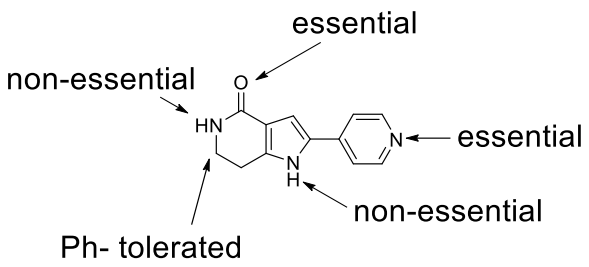
4. SERIES A: Benzimidazoles



Series A SAR summary

A series of benzimidazoles were made *via* a 4-step process.[11] The original hit was the most potent/selective.[12]

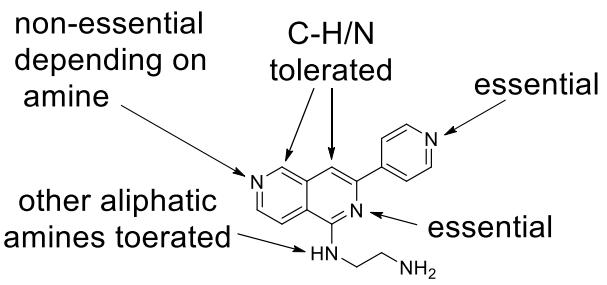
5. SERIES B: Pyrolopyridinones



Series B SAR summary

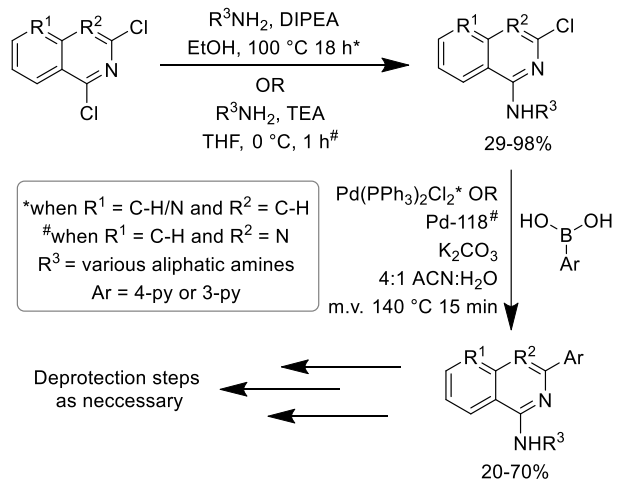
The molecule above[13] and subsequent analogues were made using pre-existing chemistry. Despite promising SAR, the best compounds were not as selective as other compounds in Series C.[12]

6. SERIES C: Naphthyridines



Series C SAR summary

The above hit[14] was prepared *via* an 8-step procedure, which was optimised to 2/3 steps by exchanging the 2,7-naphthyridine heterocycle core for a 2,6-naphthyridine/quinazoline/quinoline.[12]

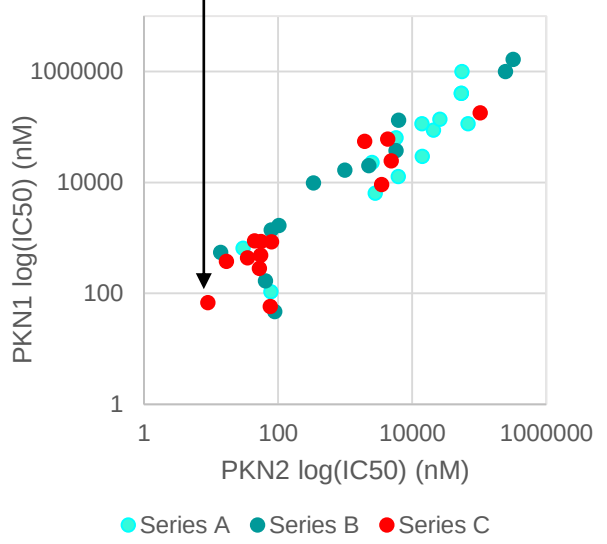


Series C general synthetic scheme

7. Biological Activity

The 70+ compounds synthesised so far show varying but promising levels of potency and selectivity towards PKN2 over PKN1 and the wider kinome.[12]

Best compound to date:
- PKN2 IC_{50} < 10 nM
- 7-fold PKN2 selective over PKN1
- Inhibits 9/400 kinases > 70%@ 1 μM (DiscoverX KINOME*scan*® panel)



Logarithmic scatter plot of a selection of synthesised compounds and their corresponding IC_{50} values for PKN2 vs. PKN1

8. Conclusions and Future Work

We have shown it is possible to make compounds that selectively target PKN2 within the PKN family and wider kinome. Further chemistry and pending crystallography studies are underway to deliver a suitably potent and selective PKN2 chemical probe to facilitate further investigation of this kinase's biological role.

9. Acknowledgements

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1) *Br J Pharmacol*, 2015, **172**(11), 2675–700; 2) *Cancer Cell*, 2017, **32**(1), 9–25; 3) *Semin. Cancer Biol.* 2017, **48**, 1–17; 4) *Nat. Rev. Mol. Cell Biol.*, 2010, **11**, 9–22; 5) *Molecular Cancer*, 2018, **17**(13); 6) *Sci Rep.* 2017, **7**(1); 7) *DNA Repair (Amst).* 2016, **46**; 8) *J. Cell Commun. Signal.*, 2018, **12**(4), 709–721; 9) *Oncotarget*, 2015, **6**(28); 10) www.ebi.ac.uk/chembl/; 11) *J. Med. Chem.*, 2010, **53**, 5400–5421; 12) *Manuscripts in preparation; 13*) *J. Med. Chem*, 2000, **43**, 4084–4097; 14) *J. Med. Chem.*, 2007, **50**, 2647–2654.