Fyn & Pyk2 Kinase Inhibition for Alzheimer's Disease

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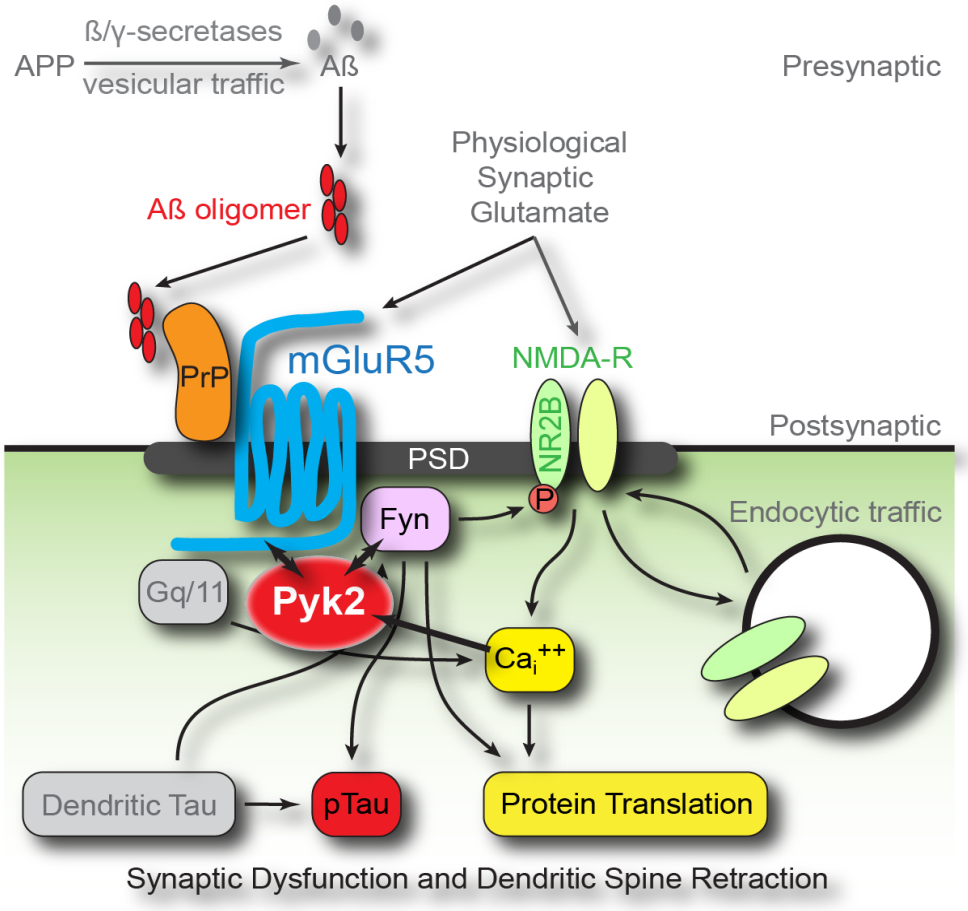
Synapse Damage Pathway in Alzheimer's Disease

- No Disease-Modifying Therapy for AD
- Pharma focus and failures relate to Aß levels *per se*

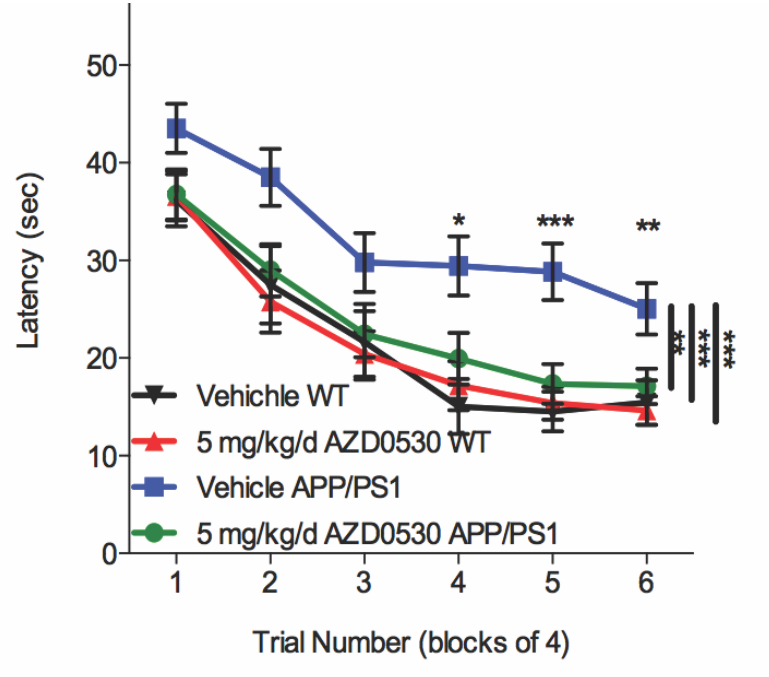
- Amyloid ß Oligomers (Aßo) damage synapses in AD to cause symptoms
- Discovered molecular cascade
- Pyk2 genetically linked to AD risk
- Fyn or Pyk2 kinase blockade restores synapses and memory to AD mice



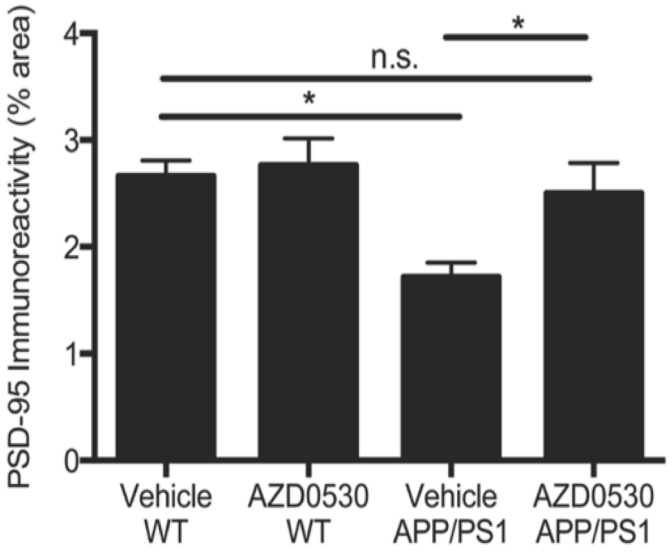
Biology documented by our lab in multiple high profile publications and supported by numerous competitive NIH grants

Efficacy of Fyn and Pyk2 Inhibition in Preclinical Models

Learning Improved
by Fyn Inhibitor



Synapses Restored
by Fyn Inhibitor



Similar rescue of memory and synapses in Pyk2 null state

(data not shown)

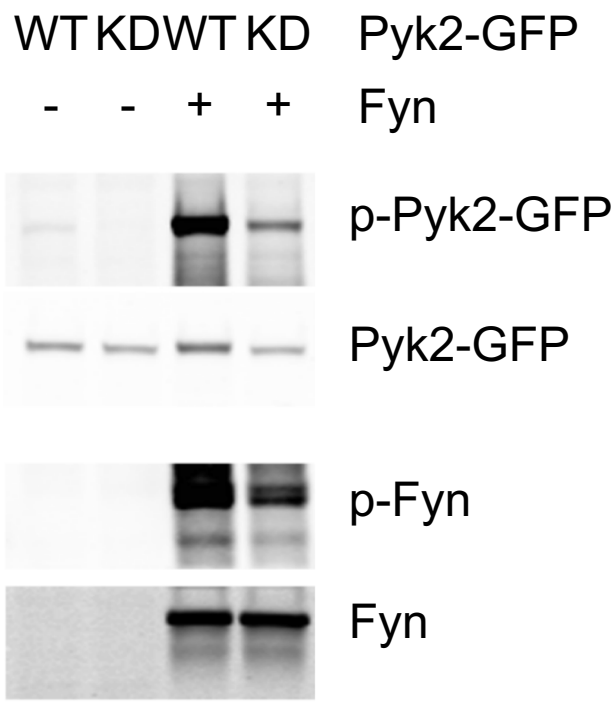
- We have just completed a Phase 2a Trial with Fyn Inhibitor for AD with AZ and NIH
- Limited dose due to platelet suppression and interstitial pulmonary fibrosis, and Fyn null phenotypes
- Therapeutic index of 2 extrapolated from mouse
- Data analyses now in progress, to be reported in July

Fyn/Pyk2 Inhibition with Robust Therapeutic Index

- Dose-limiting side-effects with complete single kinase inhibition
- Synergistic cross-phosphorylation and co-activation of Fyn and Pyk2
- Partial inhibition of both enzymes anticipated to be efficacious with broad therapeutic window

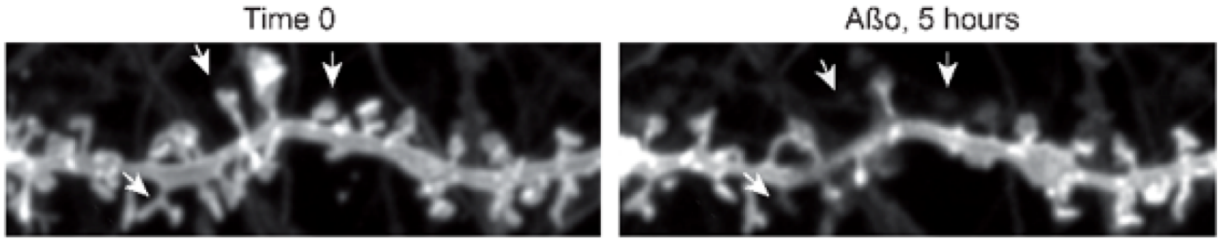
- Option A: one compound with balanced dual inhibition
- Option B: Proprietary Pyk2 inhibitor compound +/- existing Fyn inhibitors

- Blavatnik Project to Develop SAR and IP for Dual Pyk2/Fyn or Pyk2 inhibitors



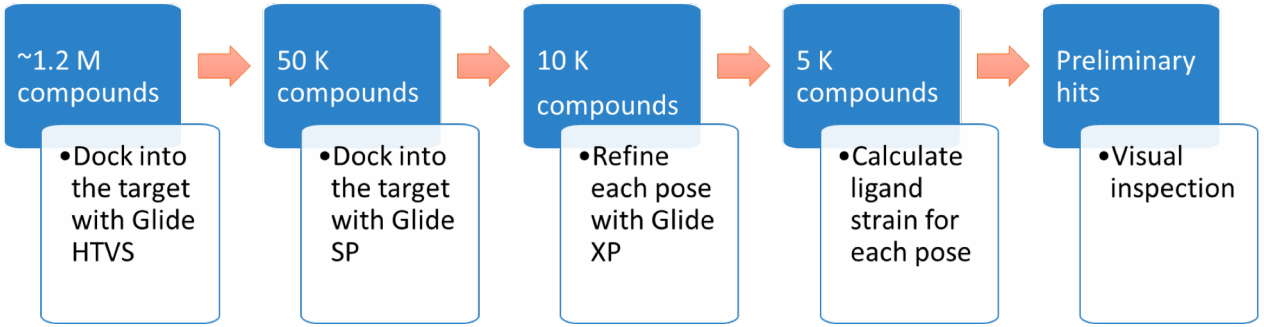
Current Blavatnik Fund & CRL Contract

Description	Complete	Location
1: Virtual screen using commercial deck against Fyn and Pyk2	Dec-17	CRL
2: Compound provision for 500 selected compounds	Feb-18	CRL
3: Biochemical assay development for Fyn and Pyk2	Feb-18	CRL
4: Compound handling	Feb-18	CRL
5: Profile 100 compounds at 1 and 10 μM in both assays	Mar -18	CRL
6: Hit expansion by purchase & synthesis of close analogues, including biochemical retest	Ongoing	CRL
7: Selectivity panel, 20-30 major kinases at 1 μM		CRL
8: Cellular assay development for Fyn and Pyk2	Initiated	CRL
9: 1 week compound profiling cellular assays		CRL
10: DMPK analysis for 10 compounds		CRL
11: Test dual Fyn/Pyk2 kinase inhibitors with highly predictive synapse-specific preclinical *in vitro* AD models		Yale



Test synapse stability by imaging and plasticity by LTP electrophysiology

Virtual Screening



Method	Hits selected
Structure based VS (2DQ7)	889
Structure based VS (3FZP)	219
Structure based VS (3FZS)	47
E-pharmacophore	77
Ligand based pharmacophore screening	495
Hit expansion	79
Bayesian classification	90
ROCS 3-D shape screening	453

REMOVED

- Duplicates
- Hits with
  1. Structural alerts
  2. LogP ≥ 4.5
  3. MW > 450 & MW < 250
  4. Polar Surface Area > 120

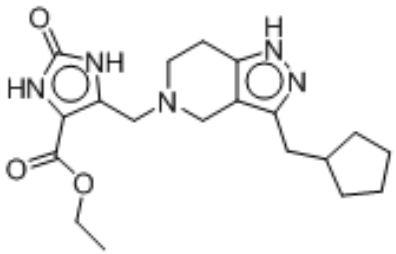
522 HITS

16 FYN-PYK2 shared

348 HITS OBTAINED from VENDORS

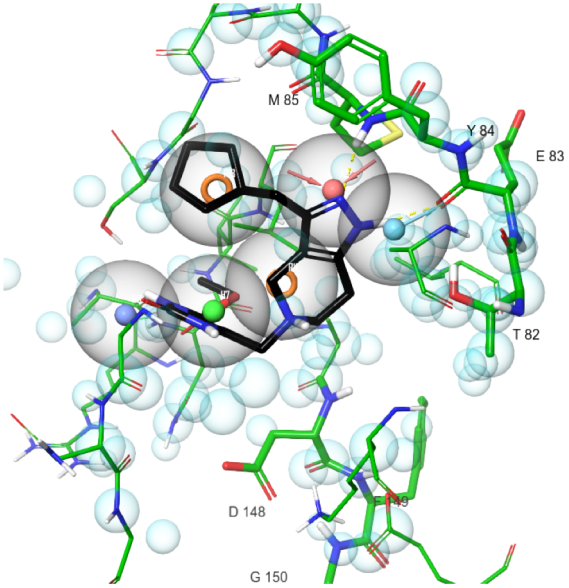
Hit Selection from Virtual Screen

FYN-PYK2 shared hit example from E-pharmacophore results



CBR_92236391

FYN (PDB ID 2DQ7$_{DFG\text{-}in}$)



PYK2 (PDB ID 3FZP$_{DFG\text{-}in}$)

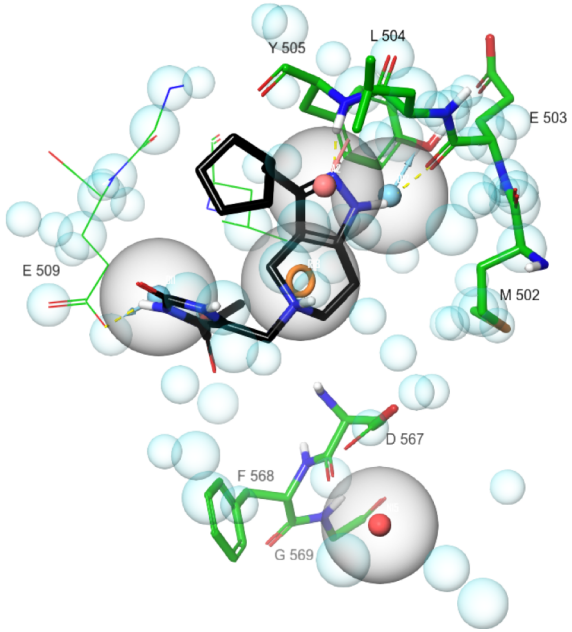
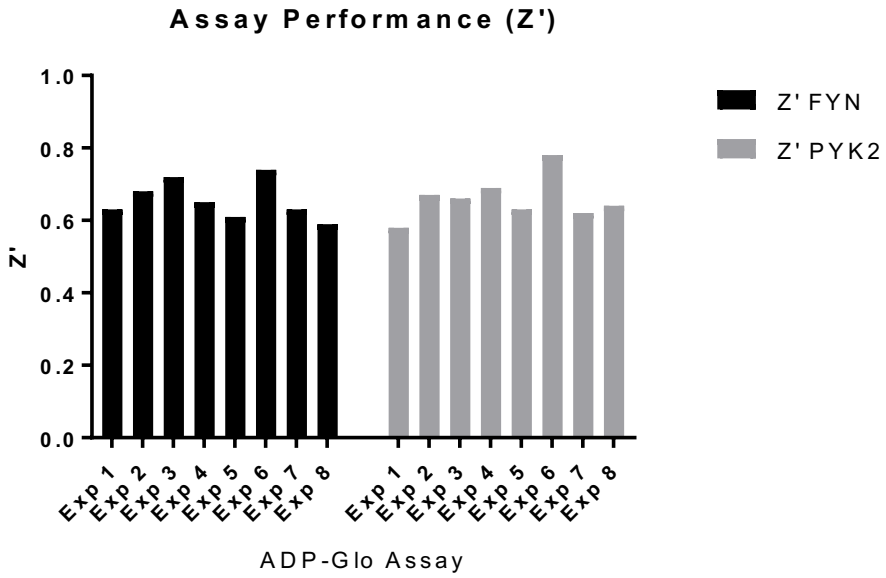


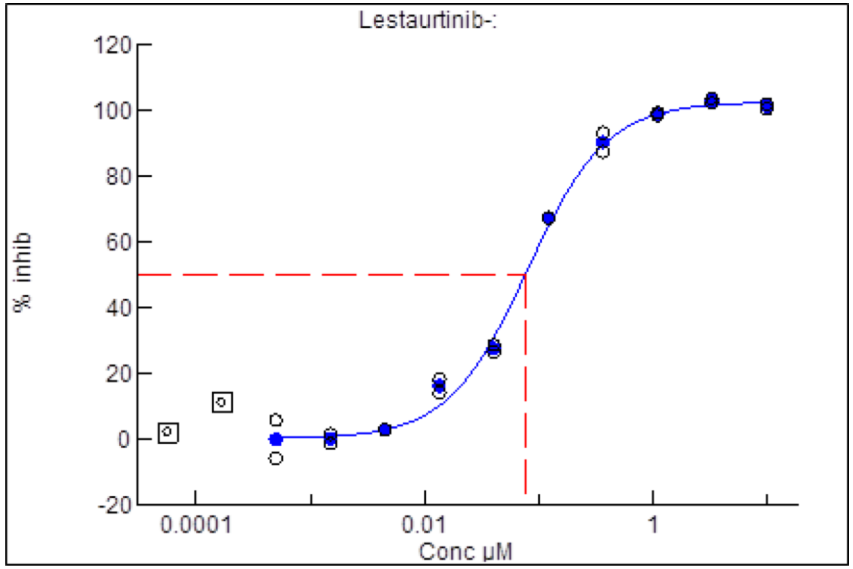
Figure legend: protein amino acids shown in green; ligand based pharmacophore features shown in green (lipophilic features), orange (aromatic moiety), blue (hydrogen bond donor group), red (hydrogen bond acceptor group); excluded volumes indicated with blue spheres and hydrogen bonds reported with yellow, dashed lines.

Assay Development: ADP Glo™ FYN & Pyk2 assays



FYN: mean +/- SD = 0.65 +/- 0.05

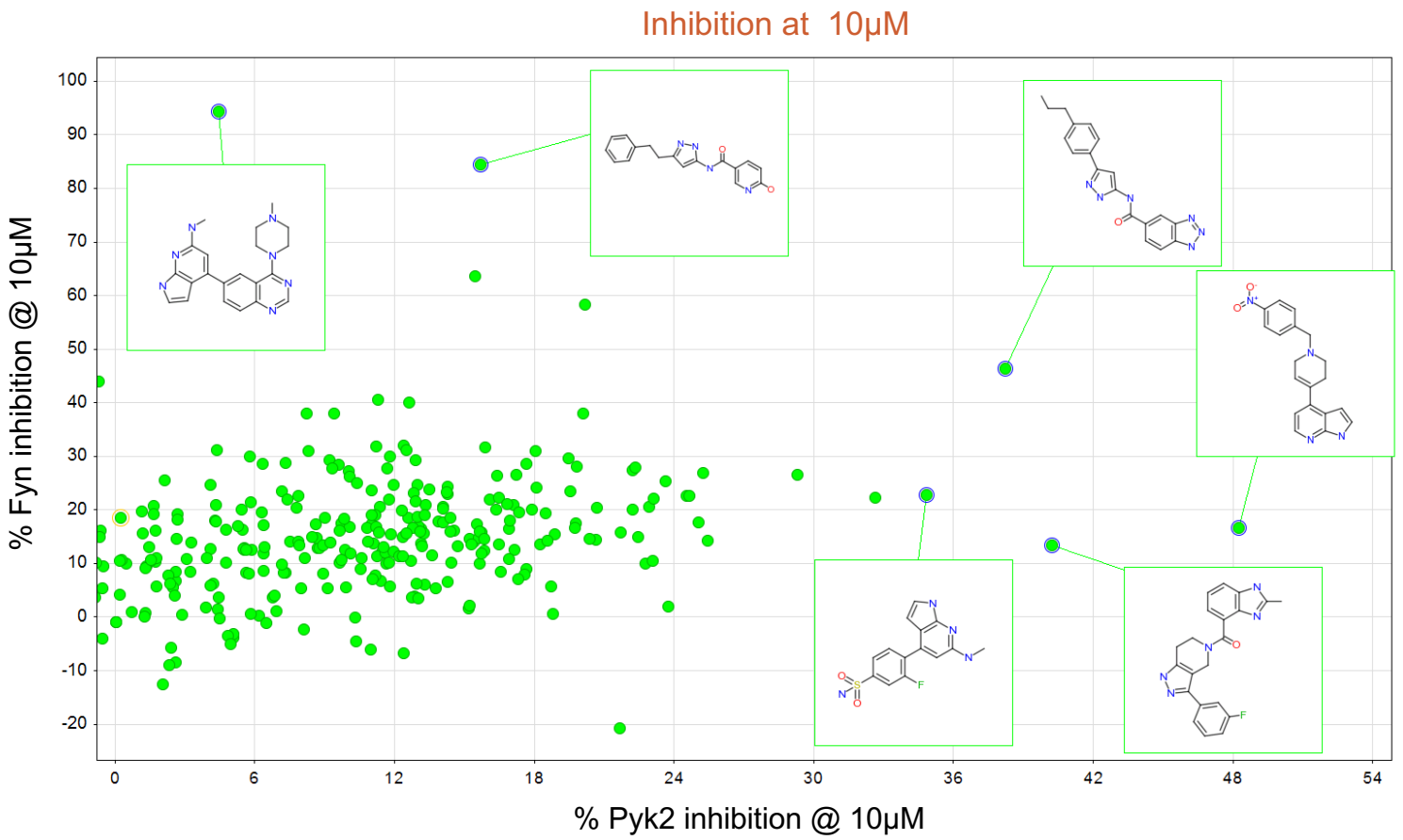
PYK2: mean +/- SD = 0.66 +/- 0.06



Expected potencies for known Fyn and Pyk2 inhibitors

Cell-based assays being developed in Jurkat cells with endogenous Fyn and Pyk2

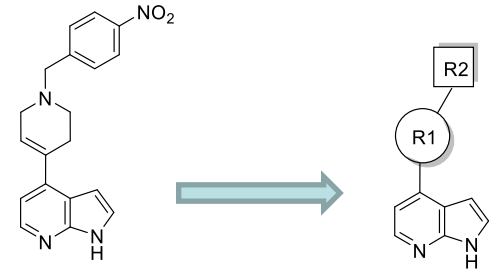
Fyn/Pyk2 Inhibitors



Hit expansion

Selected compounds for hit expansion

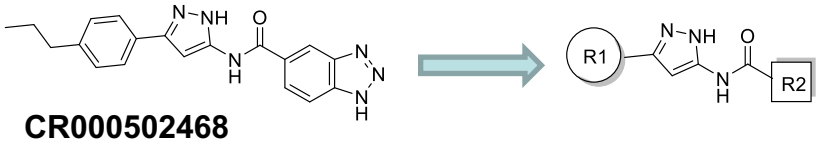
Series 1 : Aza-indole



CR000502413
FYN 16% @ 10 µM
PYK2 IC_{50} 23.8 µM

- **502413** could be developed for PYK2 inhibition and possibly dual inhibition
- Synthetic tractability:
  - Cores structures (Azaindole-R1) can be easily accessed
  - Large arrays can be generated from reductive amination to introduce R2

Series 2: Amino-pyrazole



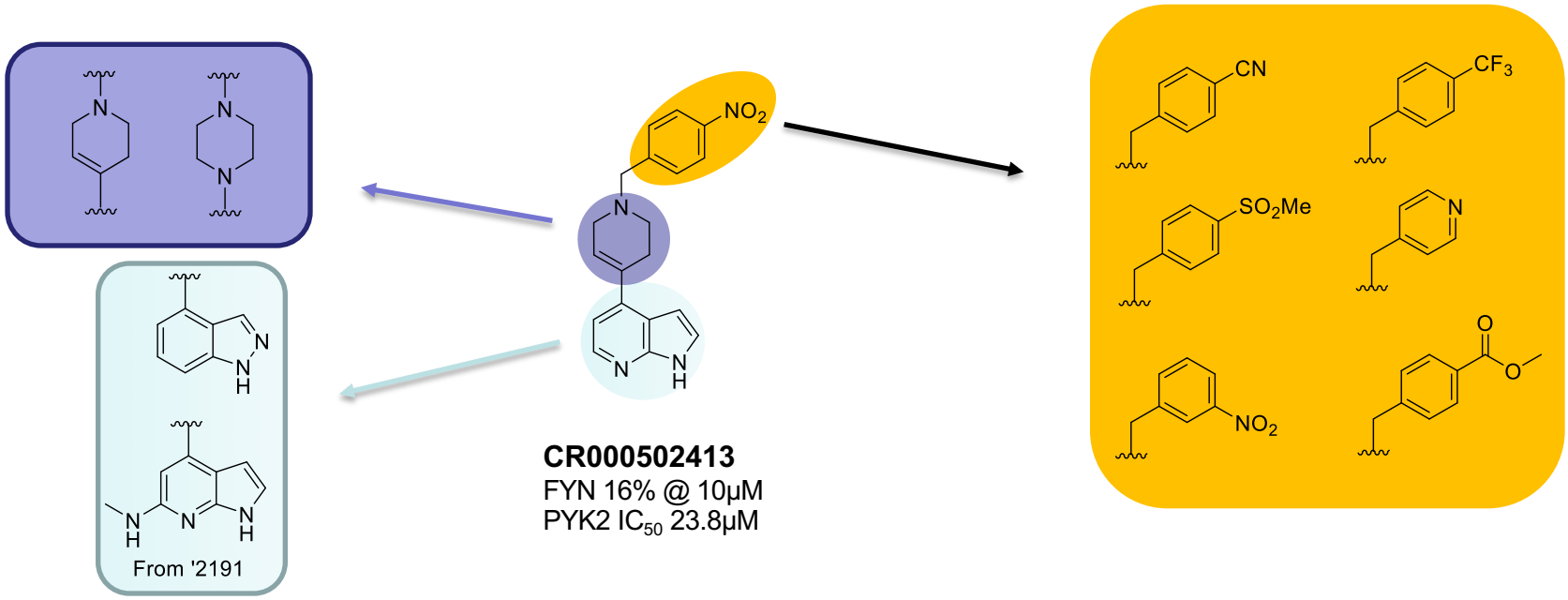
CR000502468
FYN IC_{50} 16 µM
PYK2 IC_{50} 23 µM

- **502468** could be developed as dual inhibitor
- Synthetic tractability: arrays of analogues can be generated from amide coupling

SERIES 1: CR000502413 Hit expansion

Aza-indole scaffold

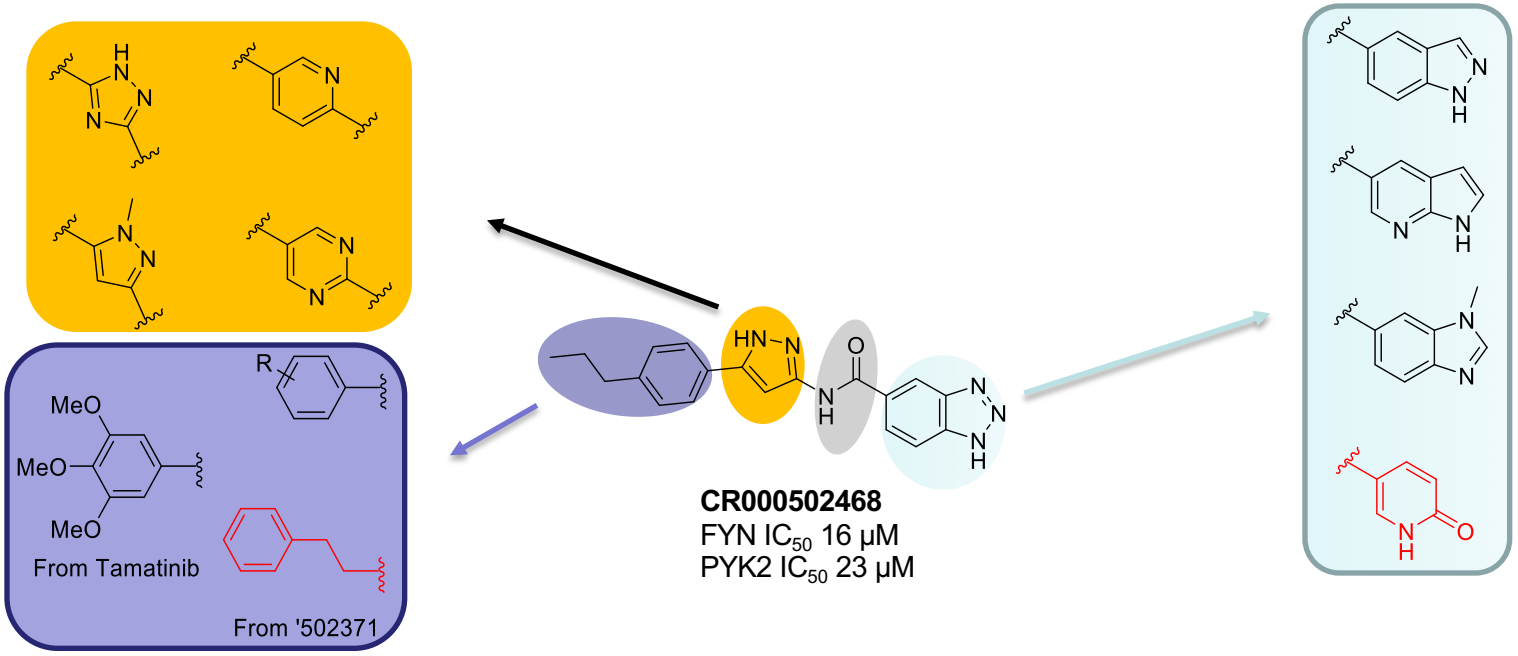
- A rapid hit expansion has been completed
- The central ring SAR was probed using unsaturated piperidine and piperazine



Series 2: CR000502468 Hit expansion

Amino-pyrazole scaffold

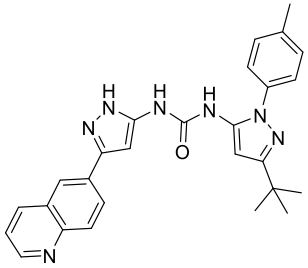
- Chemistry and purification method have been validated to synthesise the desired targets



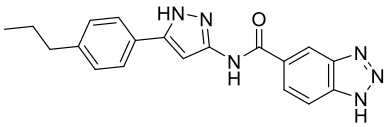
Series 2: CR000502468 Hit expansion

Amino-pyrazole scaffold

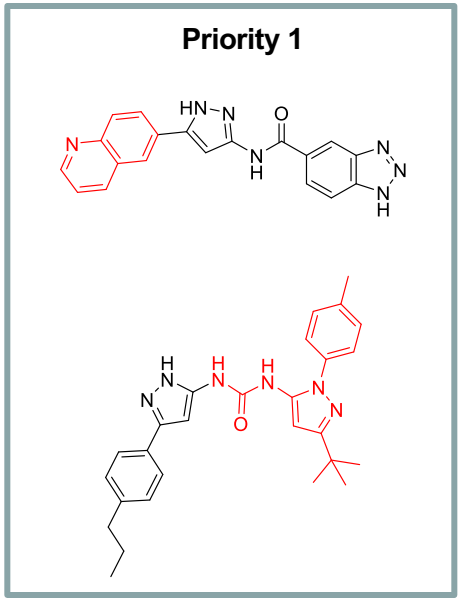
- A focused set of compounds based on SAR knowledge of the pyrazole urea scaffold will also be synthesised

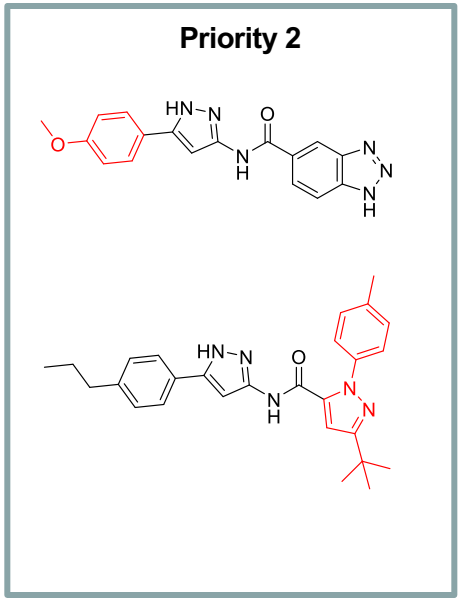


PYK2 IC_{50} 6 nM



CR000502468
FYN IC50 16 µM
PYK2 IC50 23 µM

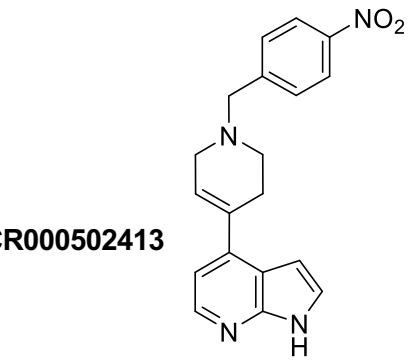




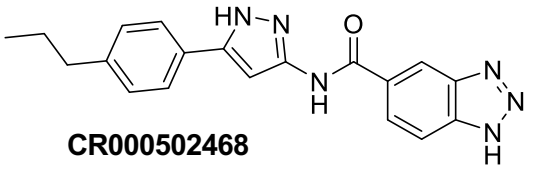
Hit Expansion Summary

- **Series 1:**
  - 45 compounds were synthesised
  - Unsaturated piperazine is critical to retain PYK2 potency
  - Small structural changes on the phenyl substituents can alter potency against PYK2 and FYN

CR000502413

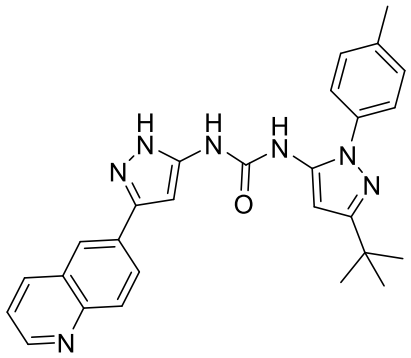


- **Series 2:**
  - 10 compounds have been synthesised and characterised
  - This array will be tested shortly against FYN and PYK2



CR000502468

- 502468 will be modified based on structure activity knowledge of pyrazole ureas



PYK2 IC_{50} 6 nM

Anticipated Results by September

- Evaluate focused compound library and analogues for potency and selectivity to develop SAR profile
- Kinase selectivity results
- Compound activity in cellular assay
- Pharmacologic ADME properties of inhibitors
- IP protection

Proposed Blavatnik Continuation

- Decision point before September
  - Option A (Dual) *versus* Option B (Proprietary Pyk2)
  - Advance to DMPK work *versus* further SAR

- Use of Second Year ($300K) Funds
  - PK/PD, dose-ranging toxicology for lead(s)
  - Further medicinal chemistry, as indicated
  - Continued contract with CRL

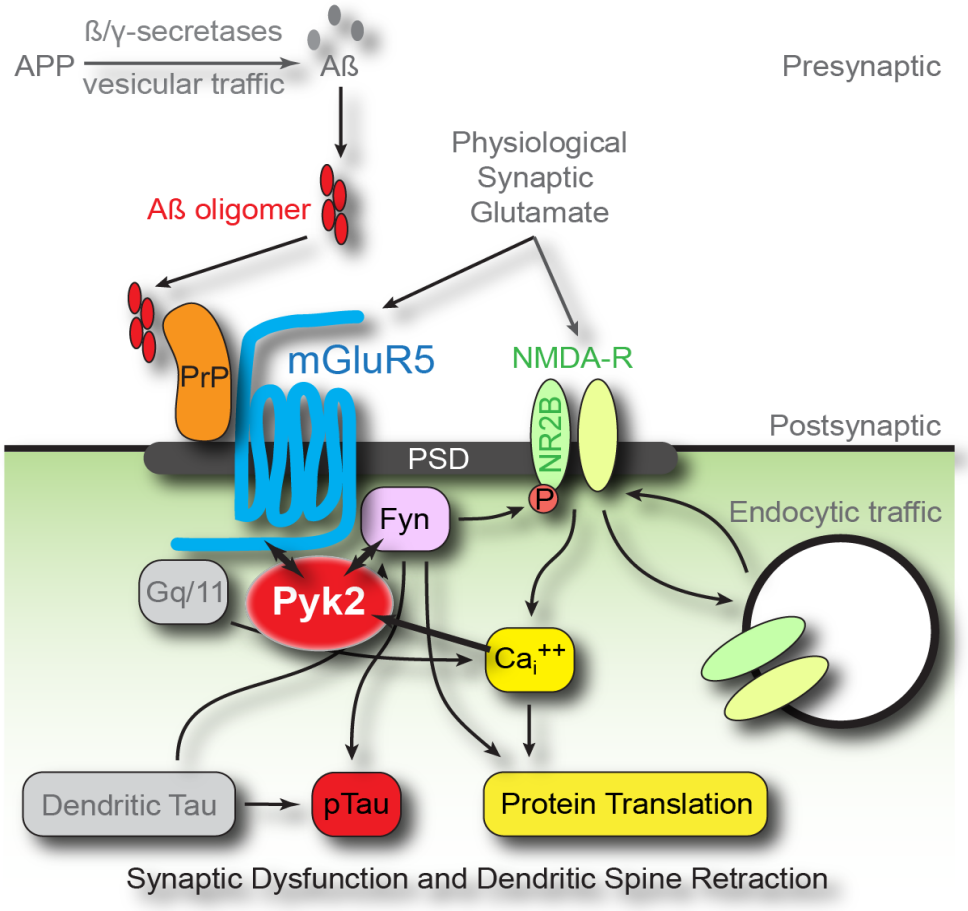
  - Test synapse and AD mouse memory at Yale (no additional cost)

Synapse Damage Pathway in Alzheimer's Disease

- First Disease-Modifying Therapy
- Protect synapses in AD
- Pyk2 genetically linked to AD risk
- Fyn or Pyk2 kinase blockade restores synapses and memory to AD mice

- Develop SAR and IP for Dual Pyk2/Fyn or Pyk2 inhibitors
- Progress during last 6 months

- Continue advance of chemistry and IP to partnership



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