Discovery of Fyn inhibitors using the SoftFocus® screening library

Kevin Doyle[1], Andrew Sharpe[1], Daniel Todd[1] & Warren Lau[2]
[1]Discovery, Charles River, Chesterford Research Park, Saffron Walden, CB10 1XL
[2]Nexus BioPharma Inc., New Jersey, USA

discovery from charles river

1 BACKGROUND

Fyn kinase is a member of the Src-family of non-receptor tyrosine kinases. It has been reported to have several physiological roles across differing therapeutic indications (cancer, obesity, Alzheimer's disease). For example:

- Yamada *et al.* provide compelling evidence for Fyn-dependent regulation of energy expenditure and body weight mediated via phosphorylation of LKB1 and activation of the AMPK energy sensing pathway. These studies suggest that pharmacological inhibition of Fyn kinase will lead to activation of AMPK and enhanced loss of fat without affecting lean mass
- Kaufman *et al.* report that Fyn inhibition prevents both Aßo-induced Fyn signalling and downstream phosphorylation of the AD risk gene product Pyk2, and of NR2B Glu receptors in brain slices. These data suggest a potential role for Fyn inhibitors in the treatment of Alzheimer's disease

To investigate the role of Fyn kinase further, a programme was initiated to discover selective Fyn inhibitors suitable for use as tool compounds for a PoC study.

A focussed screen of the Charles River SoftFocus® kinase (SFK) compound collection was undertaken and actives were subsequently optimized as part of an active-to-hit phase to improve potency, assess selectivity and develop preliminary SAR knowledge.

References:
Yamada E *et al.* Fyn-dependent regulation of energy expenditure and body weight is mediated by tyrosine phosphorylation of LKB1 *Cell Metab.* **2010** *11* 113-124
Kaufman AC *et al.* Fyn inhibition rescues established memory and synapse loss in Alzheimer mice *Ann Neurol.* **2015** 77 953-971

2 SCREENING DATA

The screening work flow below highlights the strategy undertaken; a Promega ADP-Glo™ luminescence-based assay using full-length recombinant human Fyn A was utilised. The screen yielded 600 active compounds that had their Fyn IC_{50}'s determined. Based on these data, an active-to-hit phase was initiated.

Screening Work Flow

20,644 SFK compounds screened @ 10 µM — Primary screen

Criteria: >44% inhibition

1536 compounds screened @ 10 µM (n=2) — Hit confirmation & counter-screen

Potency determination

600 compounds progressed to IC_{50} determination

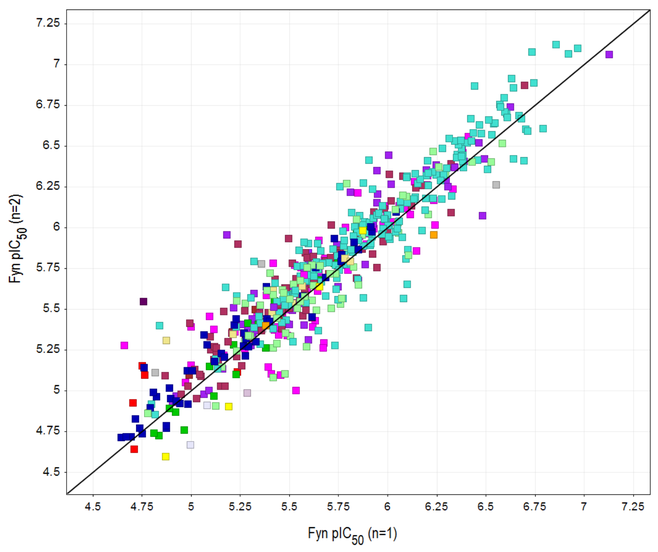

Figure 1: Primary potency data for 600 compounds. Colour coded by SFK scaffold. ~150 compounds with Fyn IC_{50} < 1µM (pIC_{50} >6)

3 ACTIVE-TO-HIT RESULTS

The scope/aim for the active-to-hit phase was to validate the actives discovered from screening programme as well as to expand preliminary SAR and improve Fyn biochemical potency. The determination of some early stage ADME properties as well as assessment of kinase selectivity and cellular activity was included within this Phase.

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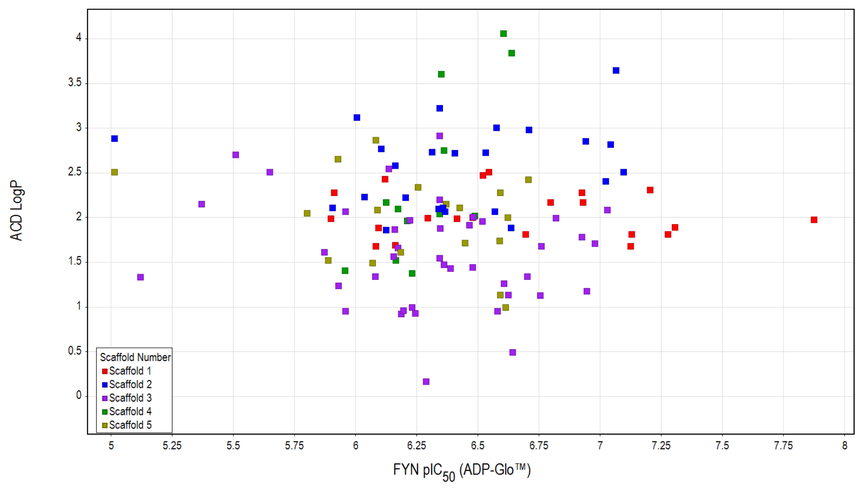

Figure 2: Potency data for ~120 compounds, spread over five different SFK scaffolds, prepared during active-to-hit phase. Compounds coloured by SFK scaffold

Compound ID	Scaffold	Fyn IC_{50} (nM)	Kinetic solubility (µM)	HLM, $t_{1/2}$ (min)	LLE	PFI	CNS MPO Score
'7613	Scaffold 1	14	142	N/A	5.90	5.0	4.7
'7279	Scaffold 2	68	184	60	4.58	3.8	4.6
'7080	Scaffold 3	84	5	8	5.27	5.0	4.5
'4031	Scaffold 4	220	20	N/A	2.80	7.0	3.9
'4923	Scaffold 5	295	35	35	4.85	4.6	5.8

Table 1: Lead exemplars from the five SFK scaffolds investigated during active-to-hit phase
LLE = pIC_{50} - ACDLogP; PFI = #Ar + chromLogD
For explanation of CNS MPO Score, see *ACS Chem. Neurosci.* ***2010****, 1,* 435–449 (Properties calculated in ACD Labs Percepta Portal)

24 exemplars profiled in selectivity assays

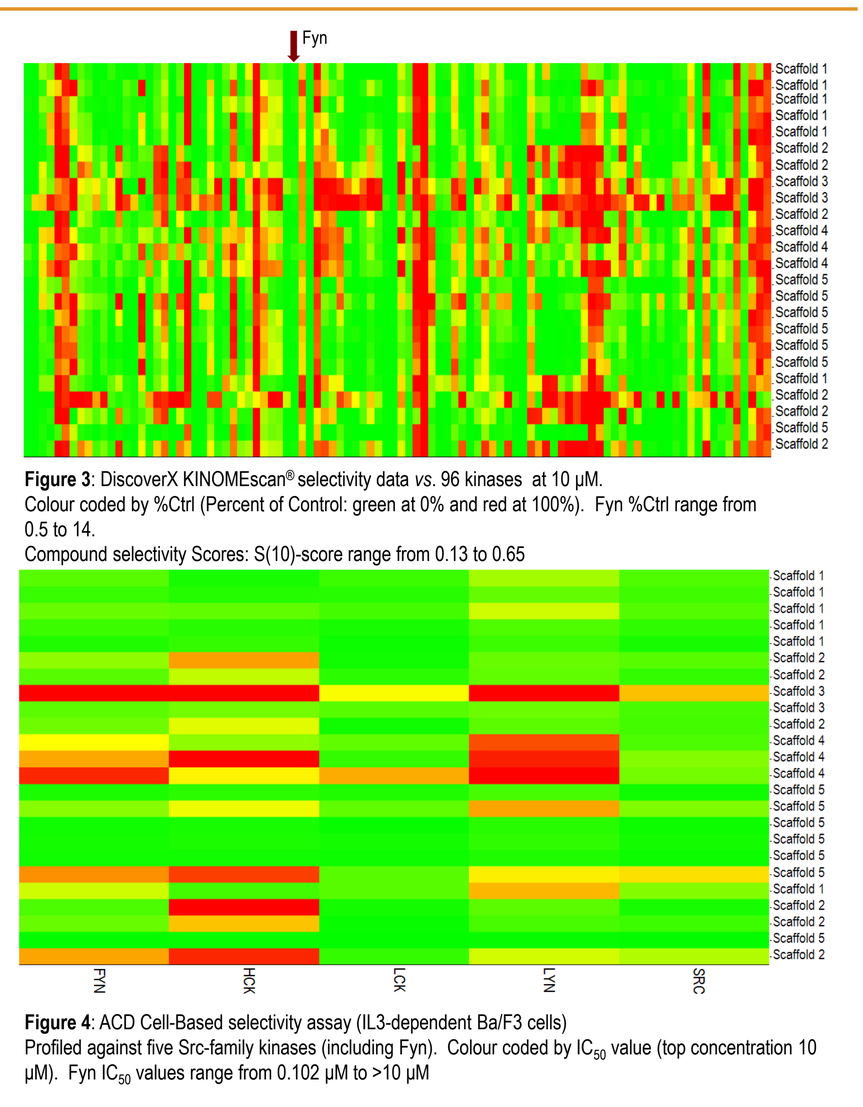

Figure 3: DiscoverX KINOMEscan® selectivity data *vs.* 96 kinases at 10 µM.
Colour coded by %Ctrl (Percent of Control: green at 0% and red at 100%). Fyn %Ctrl range from 0.5 to 14.
Compound selectivity Scores: S(10)-score range from 0.13 to 0.65

Figure 4: ACD Cell-Based selectivity assay (IL3-dependent Ba/F3 cells)
Profiled against five Src-family kinases (including Fyn). Colour coded by IC_{50} value (top concentration 10 µM). Fyn IC_{50} values range from 0.102 µM to >10 µM

4 SUMMARY

As part of a project to discover suitable Fyn inhibitor tool compounds, a focussed screening programme was undertaken using the Charles River SoftFocus® kinase (SFK) compound collection. From a screen of ~20k compounds, this gave a selection of actives (~150 compounds with IC_{50} < 1uM) spread over a range of SFK scaffolds. The positive outcome of this initiated a rapid active-to-hit phase.

The successful active-to-hit phase provided a series of compounds, spread over several SFK scaffolds, with low nM Fyn biochemical potencies and preliminary SAR that was transferrable across Scaffolds (data not shown). Early stage ADME data (kinetic solubility and human microsomal stability) was collected and coupled with their physiochemical profiles, suggests the compounds occupy acceptable property space (*e.g.* PFI score). Coupled with a range of CNS MPO scores, this proposes that these compounds could be further developed as either peripheral or CNS-focussed Fyn tool compounds for potential PoC studies.

From these compounds, a selection of 24 exemplars were profiled in a selectivity panel of 96 kinases at DiscoverX as well as five Src-family kinase cellular assay, including Fyn, in the Carna ACD Cellular assay. Selectivity, across both platforms, was observed for the compounds.

In summary, the focussed Screening Programme followed by the Active-to-Hit Phase has developed series of compounds that are capable of being developed further to assist the investigations of the many physiological roles of Fyn kinase.

nexus BioPharma Inc.

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