Early drug discovery: looking beyond potency

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INTRODUCTION

The process of identifying small molecule hits suitable for subsequent optimisation into leads, and eventually candidates, is a critical stage of the drug discovery workflow.
Historically, the early stages of drug discovery have been based on finding compounds with either the highest activity or the highest affinity against the target of interest, with little consideration for the mechanism of action or the forces driving the binding event.
Understanding the kinetics, thermodynamics and how the compound causes its effect in biochemical, biophysical and cellular settings is now viewed as important in identifying the most promising hits that could lead to differentiated lead series. Furthermore it allows the identification and prioritization of not just a single mechanism, but potentially a range of thoroughly characterized diverse mechanisms, to produce a number of differentiated options to ultimately achieve the desired biological effect *in vivo.*
This poster will describe how the assay development and screening group at Concept Life Sciences sets up screening cascades to obtain as much information as possible on compound-target interactions in order to drive better SAR decisions in early drug discovery programs.

INFORMATION-RICH SCREENING CASCADE

Program progression and well-informed decision making is dependent on an effective and information-rich screening cascade (Figure 1). At Concept, our expert bioscientists can offer full screening cascade development as part of an integrated discovery program or identify and develop suitable assays to answer specific questions.

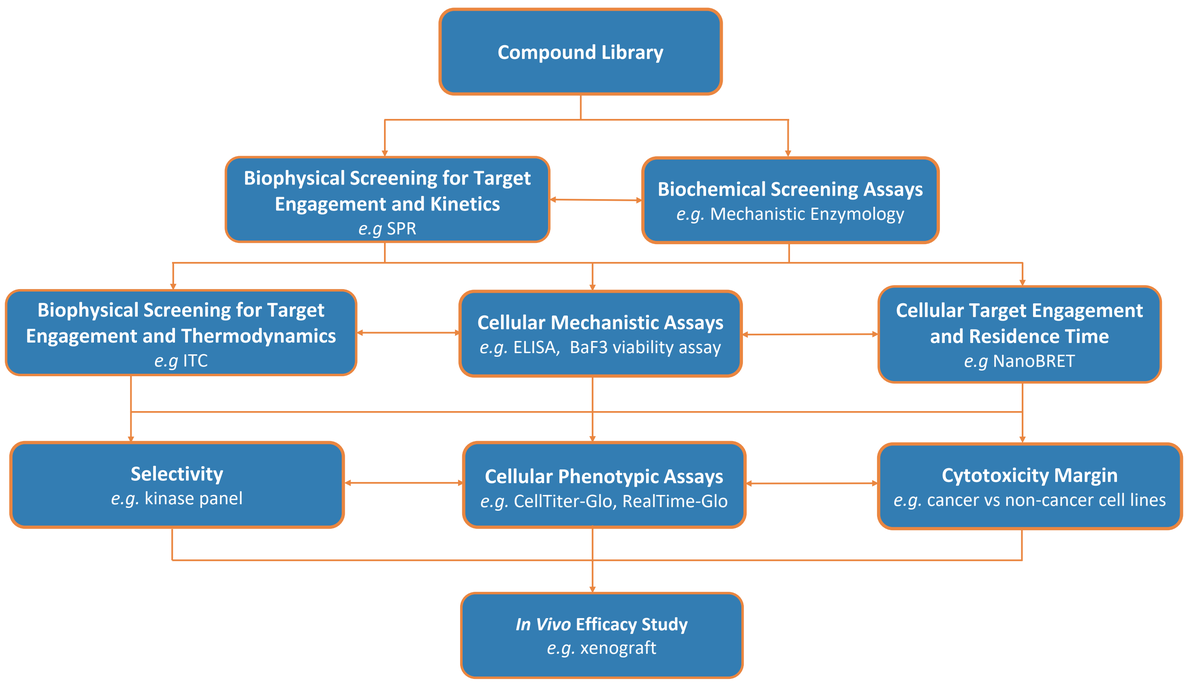


Figure 1: A typical early discovery information-rich screening cascade; example taken from an oncology project

BIOCHEMICAL /BIOPHYSICAL ASSAYS

Biochemical mode-of-action studies are used to determine whether an inhibitor is competitive, non-competitive or uncompetitive.

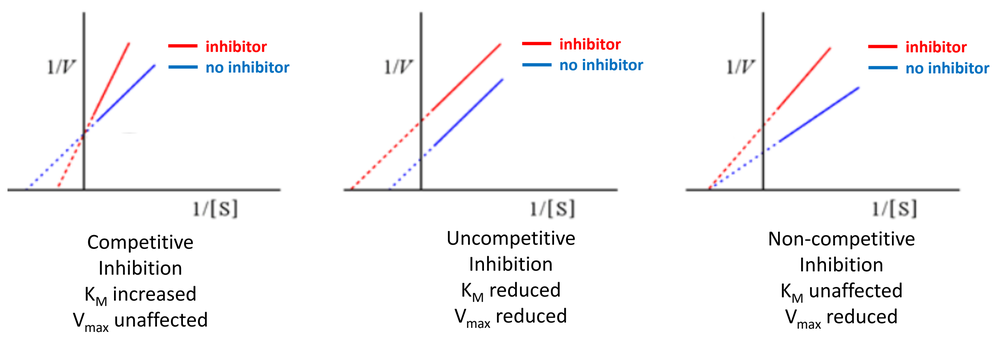


Figure 2: Scatchard Plots of three different types of enzyme inhibitors

We utilise SPR technology to measure in vitro affinity, binding kinetics (k_{on} & k_{off}) and stoichiometry

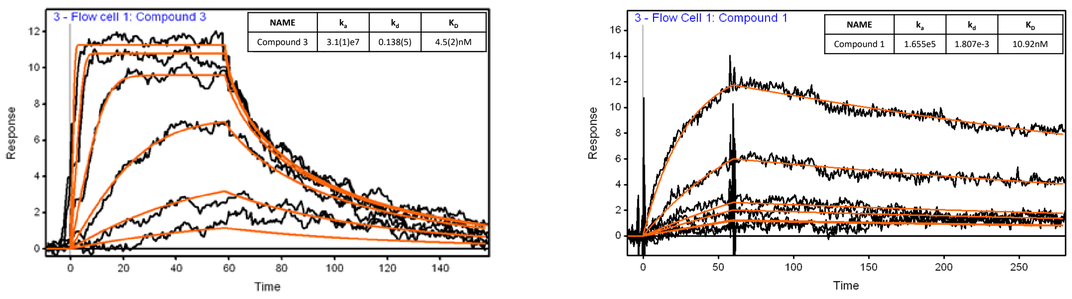


Figure 3: SPR sensorgrams showing similar affinity binders with significantly different binding kinetics

We utilise ITC to measure in vitro affinity, binding thermodynamics (ΔH & ΔS) and stoichiometry

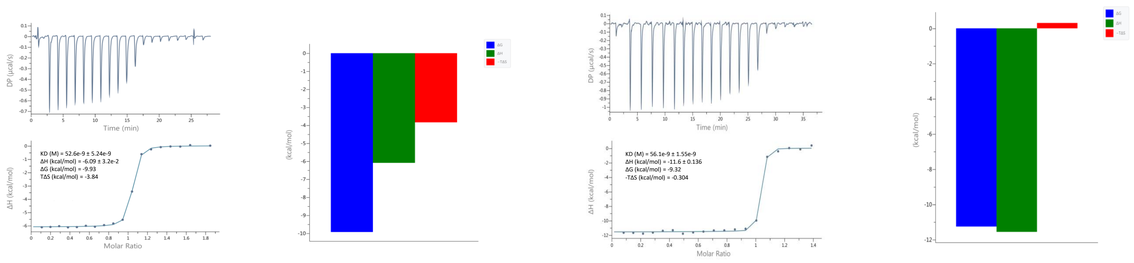
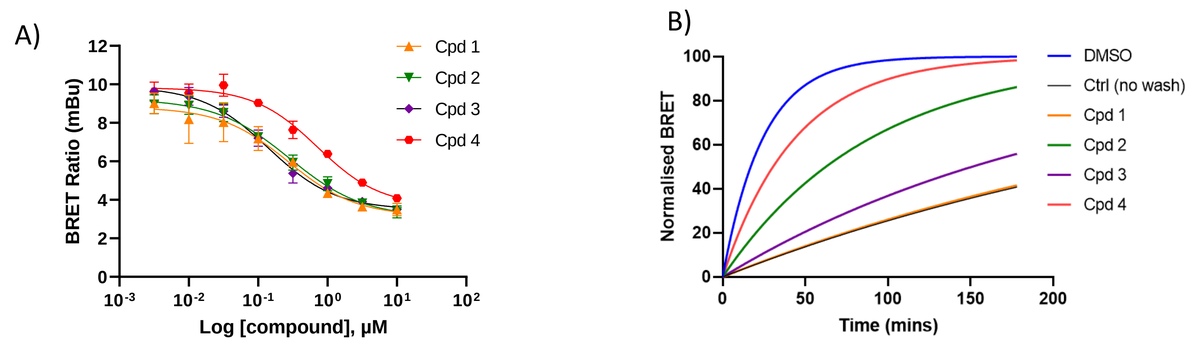


Figure 4: ITC plots showing similar affinity binders with significantly different thermodynamic signatures

CELLULAR TARGET ENGAGEMENT

We utilise NanoBRET technology to measure target engagement in cells. This assay is not limited to target class or cellular background and allows us to:

- Quantitate compound affinity (how tightly it binds to a protein) and target protein occupancy (how much compound binds to a protein) in live cells.
- Assess how long a compound binds to the target protein (its residence time) under physiological conditions.



	Cpd 1	Cpd 2	Cpd 3	Cpd 4
IC50 µM	0.271	0.282	0.140	0.714

	DMSO	Ctrl (no wash)	Cpd 1	Cpd 2	Cpd 3	Cpd 4
K_{off}	0.0408	0.0029	0.0030	0.0111	0.0046	0.0227
Tau	24	340	332	90	218	44
Half-time	17	236	230	62	151	31

Figure 5: NanoBRET (A) IC50 and (B) residence time curves demonstrating that compounds with very similar potencies may have significantly different off-rates.

CELLULAR MECHANISTIC ASSAYS

The selection of suitable cellular models and the use of mechanistic cellular assays to measure target engagement, helping translate biochemical potency and mode of action into cellular function, is key to an efficient screening cascade. At Concept, we select the most appropriate cell model and assay readout, ensuring delivery of robust, decision-making data.

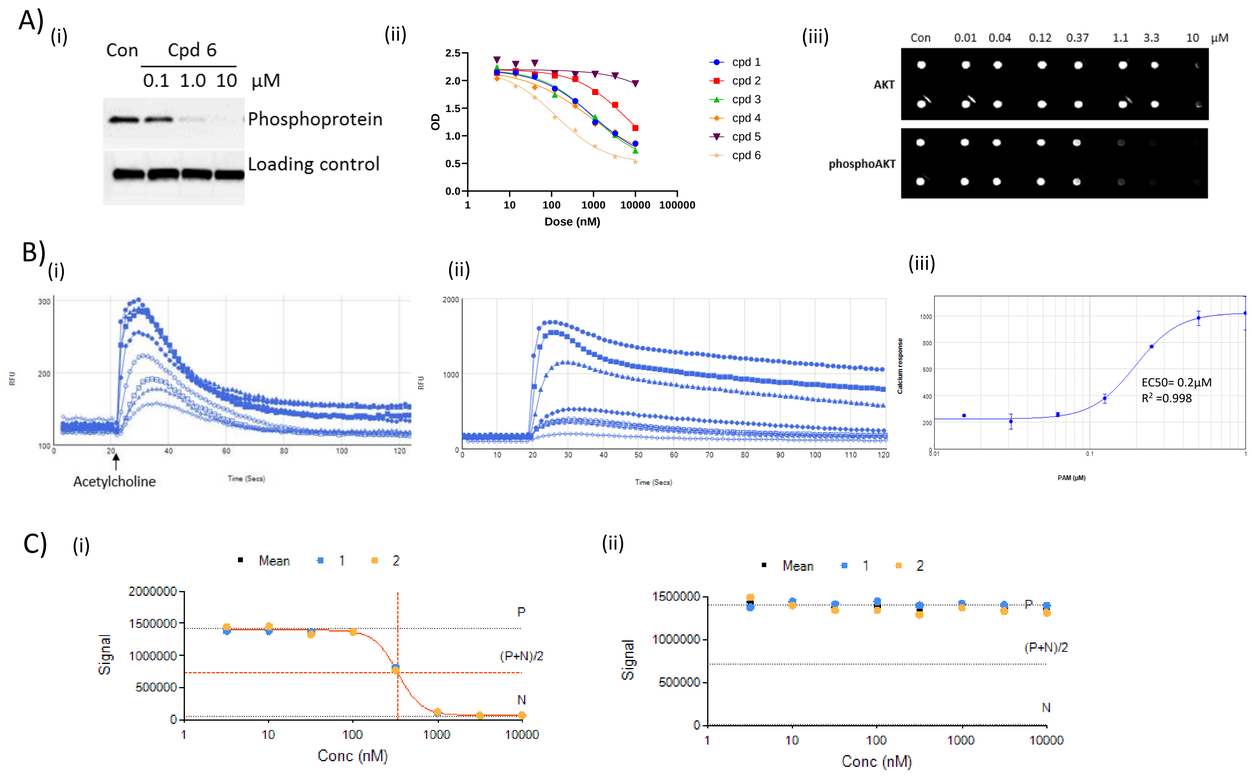


Figure 5: Examples of different ways to measure the mechanistic effects of compounds in cells. (A) Protein levels by Western (i) and ELISA (PathScan (ii) and MSD (iii)). (B) Ligand binding activity using calcium flux assay; agonist activity (i), positive allosteric modulator activity (PAM; ii) and dose response curve for PAM (iii). (C) kinase activity and non-specific toxicity by measuring viability in BaF3 kinase-expressing cells (i) and BaF3 control cells (ii).

CELLULAR PHENOTYPIC ASSAYS

Informative functional cellular assays depend upon selection of suitable disease-relevant models to demonstrate compound efficacy. At Concept, we confirm suitability of cellular models by, for example expression profiling, and select the most relevant readout.

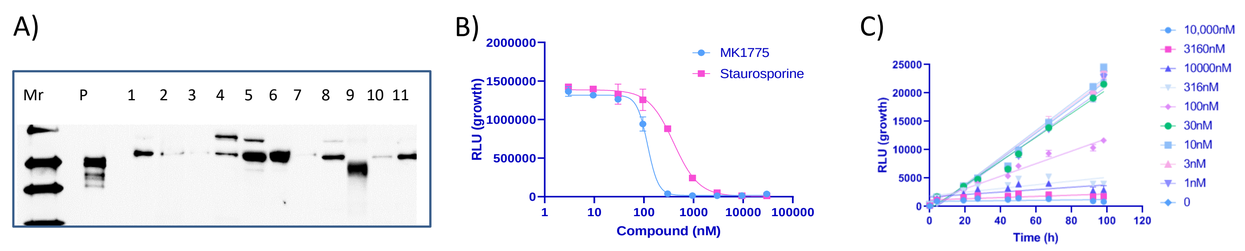


Figure 6: Functional cellular assays. (A) Profiling protein expression in cell lines; RNA and protein expression data do not always correlate. P= pure protein, 1-11= different cell lysates. Viability assays measuring endpoint and (C) real-time measurements in a cancer cell line model.

SUMMARY

Detailed understanding of the molecular interactions of test compounds enables the selection of truly differentiated series. Early prioritization of these studies thus facilitates the identification of unwanted mechanisms, allowing specific de-prioritization of compounds that would otherwise delay development due to issues such as lack of true target engagement or action via mechanisms that are not suitable for progression. This mechanism-based, informed decision-making process ultimately leads to better quality compounds with a greater chance of clinical success, enhances the quality of regulatory submissions and produces higher-quality publications.