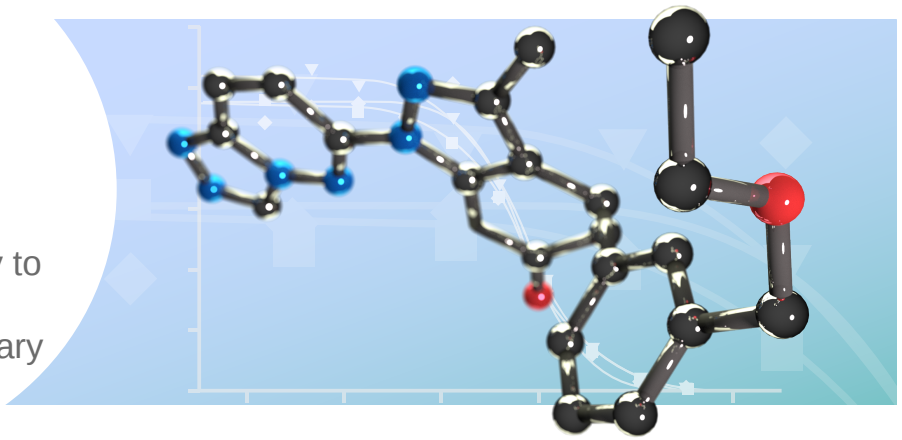
CASE STUDY

CYCLICA

Rapid Discovery of a Sirtuin 2 Inhibitor Using Ligand Design



Ligand Design leverages polypharmacology to find both specific and multi-targeted hits through the exploration of a commercial library

Overview

- We used our Ligand Design platform to identify binders to a panel of protein targets (SIRT1, SIRT2, SIRT3, PTP1B, MCL1)
- Within 20 business days we identified 22 molecules from a commercial library of 100,000 compounds, purchased them, and had them tested *in vitro*
- Ligand Design identified molecules that were active, selective, and chemically distinct from known binders, including a selective hit for SIRT2, CYC-1858, that demonstrated an IC_{50} of 2.0 µM
- Ligand Design uses our MatchMaker engine, a deep learning model without any additional target-specific training, to rapidly identify hits

A POLYPHARMACOLOGY-BASED APPROACH TO *IN SILICO* SCREENING

It has been estimated that drugs can interact with 30-300 proteins *in vivo*, rather than exclusively with their intended target; this concept is referred to as polypharmacology[1]. Despite this finding, typical *in silico* drug discovery approaches focus on a single target, leaving polypharmacology largely unexplored, particularly within the early stages of discovery. Understanding a molecule's polypharmacological profile is useful from both a positive and negative drug design perspective: either to harness synergistic interactions with multiple proteins or to engineer selectivity. Both of these aspects represent significant added value during the drug development process—accelerating timelines while reducing costs.

Cyclica's MatchMaker engine provides an ideal tool for predicting both on-target efficacy and polypharmacology. MatchMaker is a deep learning algorithm that leverages millions of known drug-target interactions in addition to 3D protein structures to predict binding of a small molecule against the entire human proteome. MatchMaker is effective on proteins that have little or no data on known binders because it is trained on structural features across the entire structurally characterized proteome, enabling model generalizability across proteins. MatchMaker's generalizability contrasts with most other AI based methods for drug design, which work with target specific, ligand-based models that require thousands of existing data points and have a difficult time extrapolating to scaffolds other than those already explored for the target.

The discovery of high-quality, hit molecules to seed drug discovery programs is critical for successful outcomes and represents one of the earliest problems tackled by computational methods. Conventional *in silico* hit discovery has typically relied on docking libraries of small molecules to a high quality structural model of a single protein. These docking experiments are often supplemented with more extensive pharmacophore modelling and expert assessment of proposed molecular poses[2]. Recently, knowledge-based approaches like convolutional neural nets have been applied to augment the speed and throughput of classical virtual screening; however, these approaches suffer from single protein target tunnel-vision.

Advantageously, Cyclica's Ligand Design platform, powered by MatchMaker, constructs polypharmacological profiles for each compound interrogated and generates hit molecules that are predicted to have preferential activity for target(s) of interest. The platform can be executed in three modes: (1) in a fully generative manner, where precomputed virtual fragment libraries are combined iteratively to explore druglike chemical space (2) in a semi-generative fashion that combines the 73,000 synthons that define the Enamine *REAL* Space to explore a virtual library of over 10^{10} purchasable molecules and (3) in a screening approach that explores fixed libraries ($<10^8$). The latter represents the fastest of the three methods and is capable of generating results in a few hours.

Here we employ the Library Screening mode on a collection of commercially available molecules to rapidly discover hits for a given panel of protein targets. **Within 20 business days, we screened all 100,000 molecules against the human-proteome, analysed and selected top molecules, and tested molecules within biochemical assays.** This case study highlights how drug discovery teams can use Ligand Design to rapidly identify hit molecules to seed their drug discovery programs.

SCREENING AGAINST A FUNCTIONALLY DIVERSE TARGET PANEL

As part of a larger study to understand the applications and limitations of polypharmacology-driven Library Screening, a panel of five proteins was selected: the protein deacetylases sirtuin 1, 2, 3, the apoptosis regulator MCL1, and the non-receptor protein tyrosine phosphatase PTP1B. We designed a series of virtual screening experiments, each with a different collection of desirable targets that included functionally similar or distinct members of the panel. The DIVERSet library (ChemBridge Corporation, San Diego, CA, USA), consisting of 10^5 compounds, was chosen as the fixed library due to its efficient exploration of drug-like chemical space.

In this preliminary study, we chose molecules based solely on high MatchMaker ranks for some or all targets; selection did not consider target selectivity. Of these top molecules, twenty two were purchased and assayed for inhibition of each protein in targeted biochemical assays performed by Reaction Biology (Malvern, PA, USA). By running all compounds against all protein targets there is the added benefit of implicitly evaluating pan-assay activity to identify bad actors. Activity was evaluated in triplicate at a 10 µM compound concentration. As a positive control, IC_{50} curves for known inhibitors were run alongside the assays for each target. The *in vitro* results were then compared to the *in silico* MatchMaker ranks of the proteins to evaluate the accuracy of the predictions. Compounds that reduced target activity by 50% or more were characterized as hits and further tested to obtain IC_{50} measurements and information on selectivity.

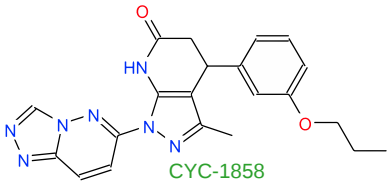


Figure 1. CYC-1858 is a selective low µM inhibitor of sirtuin 2.

CHARACTERIZATION OF NOVEL ENZYME INHIBITORS

Eight of the 22 molecules predicted to bind with a target ranking in the top 25 of all proteins assessed (top 0.3 % of the MatchMaker proteome screening results) showed measurable inhibition for at least one protein. Three compounds reduced the activity of the target below 50% and were characterized as hits; their chemotypes do not resemble those of any published compounds for these targets.

CYC-1858 is a chemically novel inhibitor of sirtuin 2 (**Figure 1**). Despite being selected out of an experiment where all three sirtuins were targeted, CYC-1858 demonstrates excellent selectivity at 10 µM and represents a novel scaffold for the selective inhibition of sirtuin 2. The determined IC_{50} of 2.0 ± 0.1 µM is comparable to current best-in-class selective sirtuin 2 inhibitors (**Figure 2**)[3]. The successful discovery of this inhibitor supports the approach of using a panel of related protein targets to maximize hit discovery rates. The two other compounds classified as hits are being further validated experimentally.

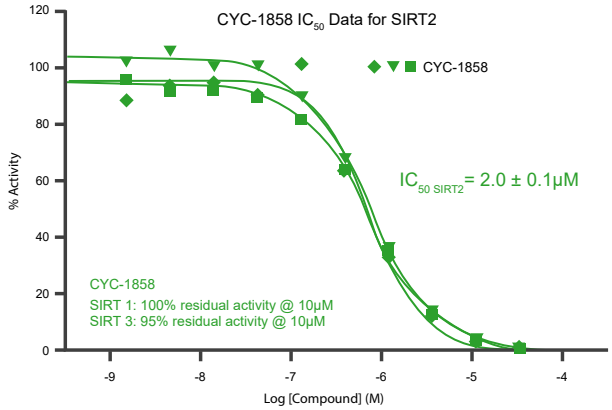


Figure 2. IC_{50} curves for CYC-1858, along with related data.

CONCLUSIONS

Our results demonstrate that Cyclica's Ligand Design, using its polypharmacology Library Screening mode, can discover molecules with low micromolar potency. Molecules were selected with the highest enrichment of all target proteins based on their MatchMaker ranks; interestingly, this approach found hits that were selective for a single target. The hit rates for sirtuin 2 are consistent with what is commonly observed with other *in silico* screening techniques[3]. This is particularly encouraging as the library we screened is an order of magnitude smaller than the libraries interrogated in typical virtual screening campaigns[4]. Following this initial study, we expanded Ligand Design Library Screening to search through ~4×10^6 molecules and consider nearly 9,000 human proteins to uncover hits that can be readily ordered and tested within 4 weeks. Further, Ligand Design's semi-generative (Enamine *REAL* Space) and fully generative (fragment-based) approaches, which have the added bonus of combining multi-parameter optimization with polypharmacology evaluation, can be run concurrently with Library Screening to further improve hit rates, hit-quality, and molecular diversity.

REFERENCES

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