Discovery of Novel Heterocycle Inhibitors: Hit to Lead Compound in <10 Months

Kendall M. Muzzarelli, Zahra Assar, Raniya Abdel-Haq, Sarah Lee, Sean Konopka, Colin McHugh, Carly Norris, Judy P. Hines, Laura E. Kostrzewa, Olivia Haskin, Tom Owen, Ines Morano, and Stephen D. Barrett

Cayman Chemical Company, Ann Arbor, MI

KEY FINDING

Our Integrated Drug Discovery Platform accelerates drug design: Hit discovery to lead optimization in less than 10 months.

INTRODUCTION

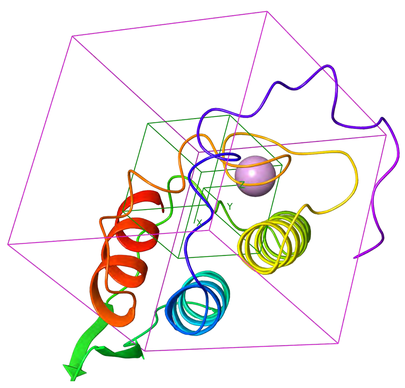
Cayman Chemical identified a certain lipid-based protein target of interest implicated in various disease states including cancer and inflammation. In less than ten months, we developed and deployed our primary and secondary biochemical and biophysical screening assays and carried out *in silico* screening of multiple commercial compound libraries to identify and confirm various virtual hits. We furthermore executed an iterative structure-activity relationship (SAR) program utilizing computer-aided drug design (CADD) and synthetic and medicinal chemistry to produce over 100 novel compounds targeting the lipid-based biological target. During this exceptionally successful program, we deployed, in addition to our activity-based primary screen, multiple biophysical methods, including the use of our thermal shift assay (TSA), surface plasmon resonance (SPR), and X-ray crystallography techniques.

METHODS

Computer-aided Drug Design (CADD)

Ligand- and structure-based drug design were performed using Schrödinger software and the Enamine Stock Screening Compounds Collection. Against our selected lipid-based target, we carried out the following:

- Hit Identification: 3 million compounds were used for shape-based screening (GPU-based). Shape similarities were between 44%-62%.
- Hit-to-Lead Optimization
- *In silico* Enumeration



Recombinant Protein Expression and Purification

Human recombinant protein was expressed as inclusion bodies in *Escherichia coli* BL21 (DE3). Inclusion bodies were solubilized and purified by ion exchange chromatography. The protein was then refolded for 5 days at 4 °C. After refolding, protein material was further purified by hydrophobic interaction chromatography and then buffer exchanged.

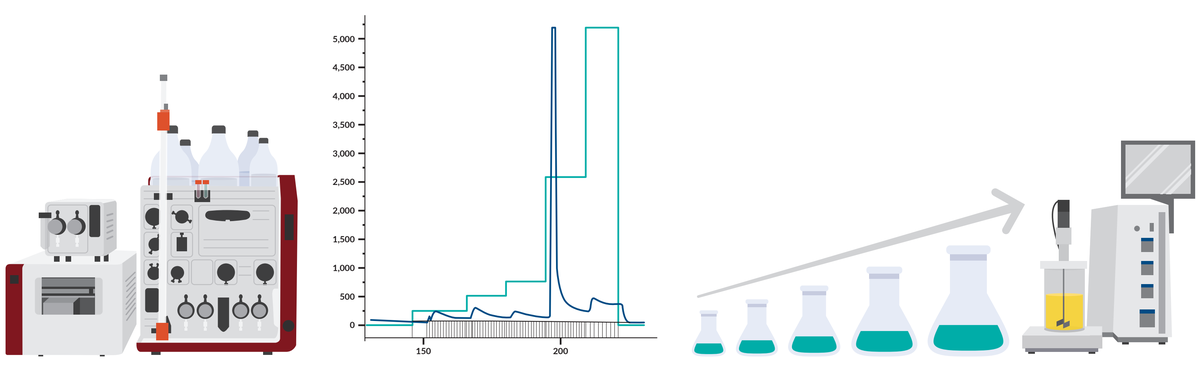
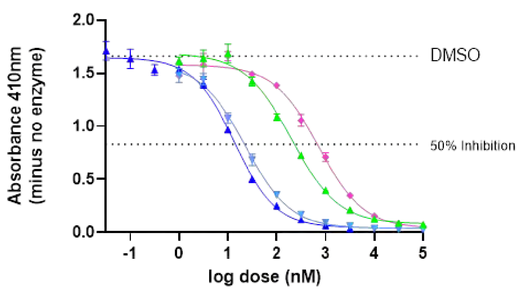


FIGURE 1 – In-house expression optimization, development, and production.

Inhibition Assay

In quadruplicate, inhibitor (100 µM-1 nM), protein (420 ng/well or 14 mM), and DTNB (1.4 mM) are incubated in a reaction well for 15 minutes before adding substrate. An endpoint 410 nm absorbance read is taken after 30 minutes. Absorbance is plotted against inhibitor concentration in GraphPad to determine IC_{50}.



Surface Plasmon Resonance

Surface plasmon resonance (SPR) single cycle kinetics (SCK) experiments were carried out with a Biacore™ T200 instrument. Protein was immobilized through an antibody capture method. Compounds were injected at various concentrations (0.2-200 nM) in assay buffer. The K_D values were analyzed with BIA evaluation software (GE Healthcare Life Science).

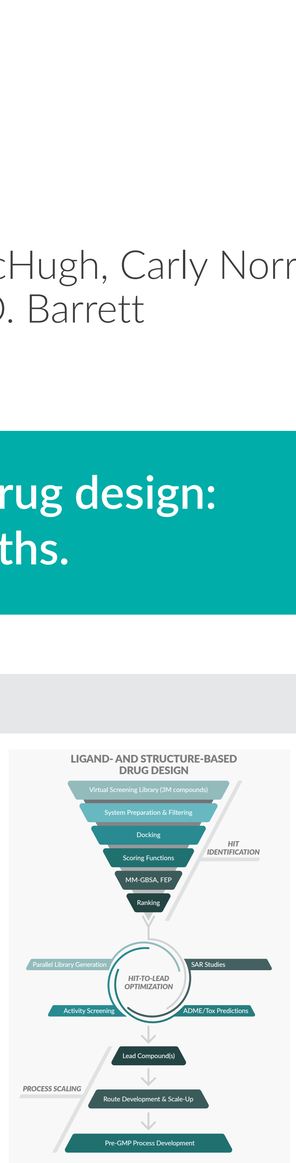
X-ray Crystallography

Macromolecular and protein-complex crystal structures were identified after three days. Crystals were soaked in mother liquor supplemented with cryoprotectant and flash frozen in liquid nitrogen and sent to Argonne National Laboratory and Diamond Light Source for data collection. Data was indexed, processed, and scaled and structures were solved by molecular replacement.

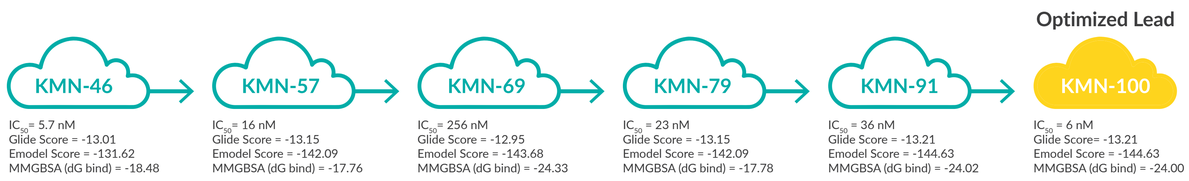
RESULTS

Selected Lipid Target–CADD, Biophysics, and *in vitro* Data

Iterative drug design-SAR carried out by Cayman medicinal chemists, computational chemists, and chemical biologists with activity screening expertise utilizing a vHTS platform led to our discovery of novel, selective heterocyclic inhibitors from both a literature-precedented scaffold (Lead Series) and an Enamine library representing a new chemotype (Backup Lead Series).



Literature-precedented Scaffold (Lead Optimization)



Enamine Library (Hit-to-Lead Identification)

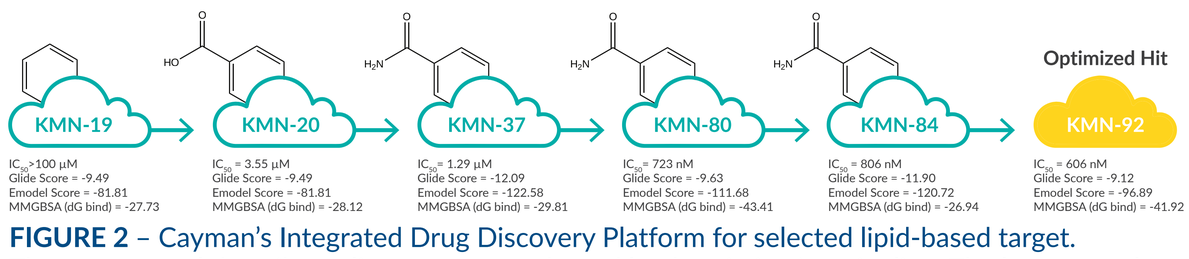
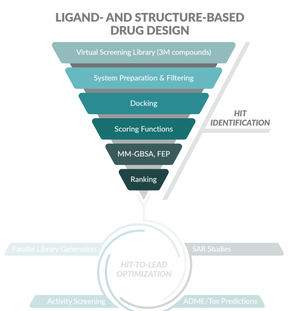


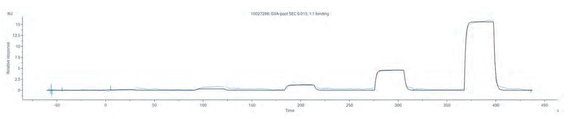
FIGURE 2 – Cayman's Integrated Drug Discovery Platform for selected lipid-based target. The upper panel describes a literature-precedented lead optimization pipeline. The lower panel describes a hit-to-lead identification with a starting compound identified by our vHTS platform utilizing the Enamine library.

Structural Biology Studies

Enamine Library

Glide Score = -8.90
Emodel Score = -81.05
MMGBSA (dG bind) = -37.54
K_D = 730 nM





Control Compound

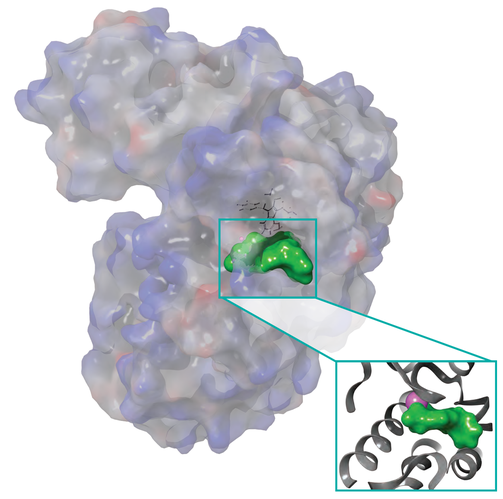
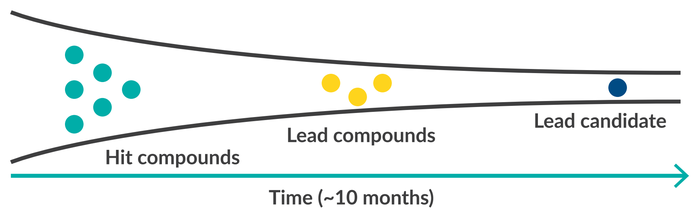


FIGURE 3 – Cayman's Integrated Drug Discovery Platform for a selected lipid-based target. The left panel describes the pipeline with which we identified a novel hit from the Enamine compound library. The right panel describes an unprecedented structure of the protein target bound to a compound comprising a literature-precedented scaffold.

CONCLUSIONS

Our Integrated Drug Discovery Platform accelerates drug design

Hit Discovery, resulting in the identification of a novel chemical series from a commercial compound library, and Lead Optimization led to novel heterocyclic inhibitors with nanomolar potencies in under 10 months.



References

1. Sastry, G.M., Dixon, S.L., and Sherman, W. Rapid shape-based ligand alignment and virtual screening method based on atom/feature-pair similarities and volume overlap scoring. *J. Chem. Inf. Model.* **51(10)**, 2455-2466 (2011).
2. Friesner, R.A., Murphy, R.B., Repasky, M.P., *et al.* Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J. Med. Chem.* **49(21)**, 6177–6196 (2006).

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