Integrated Drug Discovery – PD-1/PD-L1 Inhibitors

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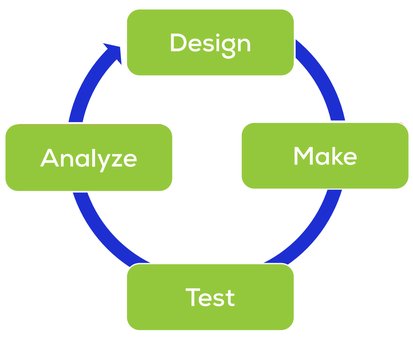
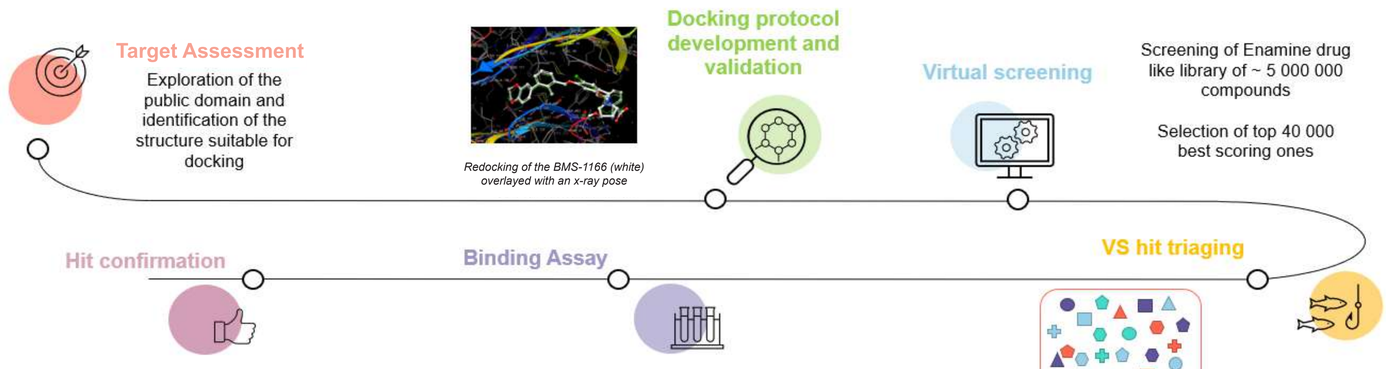
1 Introduction

Programmed cell death 1 protein (PD-1) is a co-inhibitory receptor expressed on the surface of T-cells. PD-1 terminates T-cell mediated anti-tumour responses by binding with PD-L1. Blocking the PD-1/PD-L1 interaction has been proven to reactivate the T-cell mediated anti-tumour immunity. Consequently, generating durable clinical responses, and prolonging patient survival rate [1,2], with several mAbs approved in oncology indications. [3]

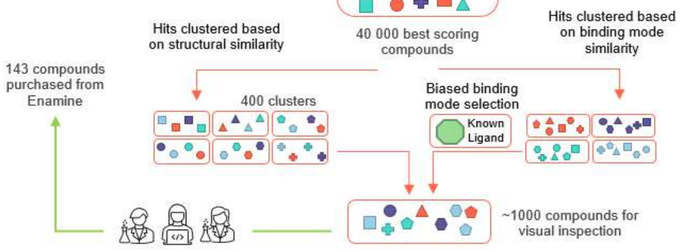
The aim of this project was to deploy Charnwood Discovery's integrated drug discovery platform to identify and develop novel small molecule PD-L1 modulators.



2 Hit Finding - vHTS Methodology



Enamine's drug like library was docked into a crystal structure of the PD-L1 dimer (PDB id 6R3K). To expedite the hit triage, only the top-scoring ligands were considered for further analysis. Subsequently, hits were filtered on 3 criteria: chemical similarity, interactions with the target, and how well they reproduced interactions of known binders. 1000 compounds were selected and visually assessed based on key interactions in the binding pocket, for synthetic feasibility and chemical diversity.



3 PD-L1 SPR Screen

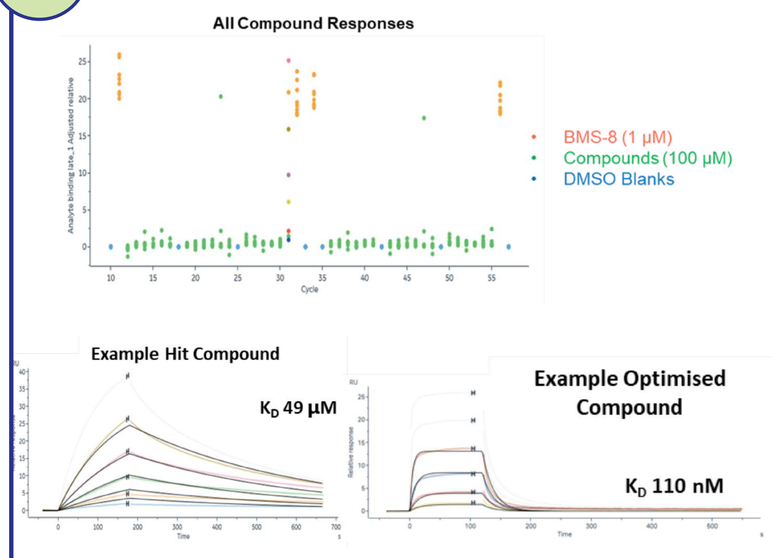


Figure 1: (Top) Reference and blank-subtracted response levels for all compounds in 100 µM SPR spot test. Some sensorgrams were excluded after failing Insight Evaluation software's QC checks. (Bottom) Two examples of sensorgrams used to measure kinetic affinities from the start of the project (left) and towards the end of the project (right).

A *de novo* SPR assay was developed to measure compound binding to amine-coupled PD-L1. It was validated with several tool compounds such as BMS-8.

We screened all purchased vHTS compounds in a spot-test format (100 µM, technical n=2 , biological N=2), excluding poorly-behaved PAINS-like compound results from further analysis.

We measured (*where possible*) the kinetic affinities for all hits and then subsequently developed compounds to help guide the SAR and medicinal chemistry efforts.

4 PD-L1 Hit-to-Lead Campaign

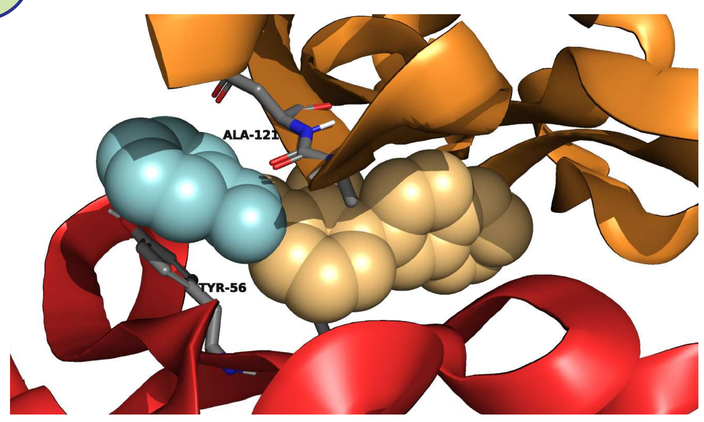


*Figure 2: Docked pose of compound **CMP-00002794**. Monomer 1 shown as orange cartoon, Monomer 2 shown as red cartoon. Ligand represented in bubble form, biphenyl motif and linker shown in yellow, terminal non-aryl binding group shown in cyan.*

Utilizing knowledge gained during our vHTS campaign, we identified subtle changes to the hit scaffold which allowed the identification of compounds such as **CMP-00002794**.

CMP-00002794 Profile

IC_{50} TR-FRET = 9100 nM
CHILogD = 2.72
LLE = 2.32
PFI (chromlogD) = 6.8
Kinetic solubility = 169 µM
Mics (µL/min/mg) r/h = >396 / 264

Targeting (ASP-122) and/or Lysine (LYS-124) at the exit of the deep hydrophobic tunnel created by PD-L1 homodimerization with polar binding motifs, afforded compounds such as **CMP-00003824**, with increased potency, allied to a maintenance of favorable PhysChem properties (LogD, solubility, aromatic ring count). During this process metabolic clearance has also been dramatically lowered, particularly in human microsomes.

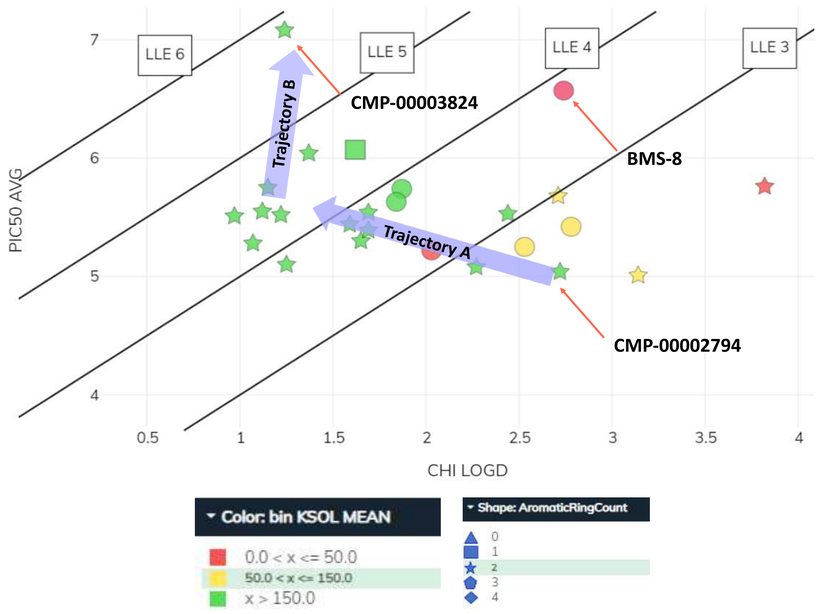


*Figure 3: LLE plot depicting the optimisation journey of hit molecule **CMP-00002794**. A rigorous focus on efficient binding and physiochemical property optimisation facilitated rapid optaimisation to deliver a highly efficient lead molecule **(CMP-00003824)**.*

CMP-00003824 Profile

IC_{50} TR-FRET = 80 nM
CHILogD = 1.24
LLE = 5.84
PFI (chromlogD) = 4.4
Kinetic solubility >200 µM
Mics (µL/min/mg) r/h = 181 / <8
Caco-2 (AtoB/BtoA/ER) = 6.8 / 11.8 / 1.7

LLE Plot [4] **(Figure 3)** highlights the rapid and efficient optimization of **CMP-0002794** into **CMP-00003824**.

The optimization trajectory can be broken down into 2 key stages:

1) Introduction of polar groups forming favorable interactions with PD-L1 (trajectory A)
2) Subtle core changes to maximize interactions between bi-aryl core and PD-L1 pocket (trajectory B)

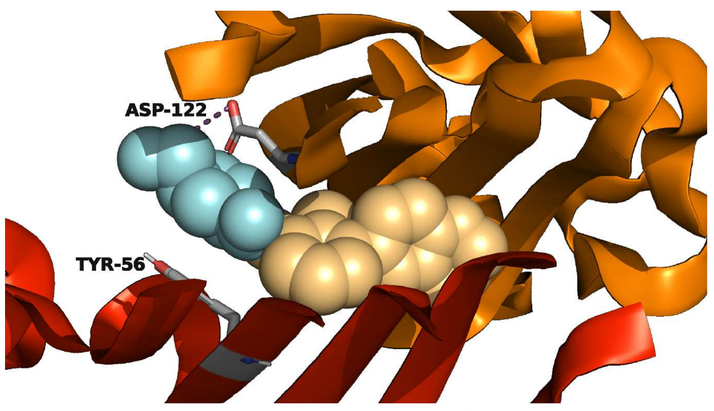


*Figure 4: Docked pose of compound **CMP-00003824**. Monomer 1 shown as orange cartoon, Monomer 2 shown as red cartoon. Ligand represented in bubble form, biphenyl motif and linker shown in yellow, terminal non-aryl binding group shown in cyan. Polar contact depicted as magenta dashes.*

Together these strategies delivered **CMP-00003824** as a promising lead molecule, which is well differentiated from classical small-molecule PD-L1 modulators, which are commonly low LLE, high aromatic ring count ligands. **(Figures 4&5)**

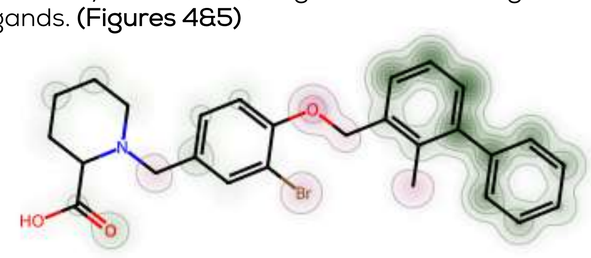


*Figure 5: Similarity map of BMS-8. **CMP-00003824** shares Tanimoto similarity of 0.4 with BMS-8. Atoms coloured green contribute to calculated similarity the most, while atoms coloured pink are the negative difference.*

5 Summary and Conclusions

This project illustrates the efficient optimization of hit molecules into lead-like structures. The key learnings were:

- Utilizing our in-house integrated drug discovery platform, we rapidly optimized **CMP-0002794** into **CMP-00003824**
- Focusing on multiparameter optimization and utilizing metric driven approaches afforded lead molecules with a well-rounded profile
- **CMP-0003824** with an LLE of ~6, moderate permeability and good aqueous solubility represents an attractive PD-L1 lead, well differentiated from existing series
- Microsomal clearance is blq in human, however rat Clint remains very high. Future work will focus on obtaining clearance levels suitable for efficacy studies in animal models
- Key to this success was focusing on maintaining the aromatic ring count at two or fewer [5] and focusing on achieving potency boosts *via* specific-directional interactions with PD-L1

References: 1. J Cell Physiol. 2019; 234:16824–37. 2. Trends Mol Med. 2015; 21:24–33. 3. Front Pharmacol. 2017; 8:561. 4. J. Med. Chem. 2018, 61, 15, 6421–6467 5. Drug Discov Today. 2011 Feb;16(3-4):164-71

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