Structure-Based Virtual Screening to Identify Novel BRD4 Inhibitors

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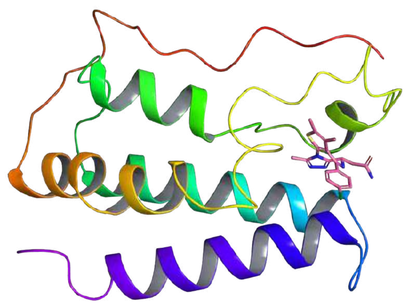
Introduction

Bromodomain-containing protein 4 (BRD4) is a member of the Bromodomains and Extraterminal (BET) family of proteins, a family critically involved in regulation of transcription.[1] BRD4 has gained a lot of attention as an oncology target due to its correlation with pro-oncogenic transcription factors and regulators, such as the Myc family.[2]

Within this study, structure-based virtual screening was applied to BRD4, specifically the N-terminal bromodomain (BD1), to identify novel small molecule inhibitors.

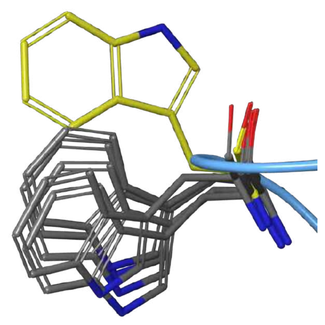


Crystal Structure of BRD4-BD1 in complex with (+)-JQ1 (pdb: 6YQN)

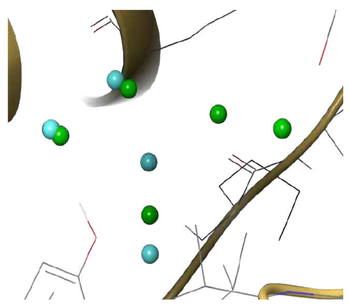
Target Analysis of BRD4-BD1

Thirty-six crystal structures of BRD4-BD1 were visually assessed to identify a structure suitable for docking.

Crystal structures showing shifted side chains were eliminated (e.g., Trp81, right).[3] Consideration was also given to crystal structures with a simplified water network (e.g., blue spheres, below).



Crystal structures showing shifted Trp81 within 5A5S X-Ray (yellow)



Water networks with 5 water molecules (green) or 4 (blue)

It was anticipated that a simplified water network would be beneficial for docking[4] and five crystal structures were chosen for further study, including two with disrupted water networks.

Data Triage and Compound Selection

To rapidly assess compounds covering a range of chemical space, the Enamine druglike library was used for virtual screening.

Docking with Glide yielded 4.5 million hit ligands. To expedite the hit triage, only the top-scoring ligands were considered for further analysis. Subsequently, hits were filtered on 2 criteria:

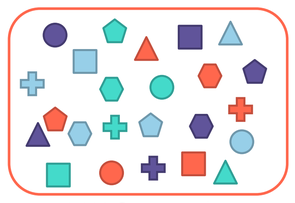
Enamine Druglike Library
5 million compounds

Docked Ligands
4.5 million compounds

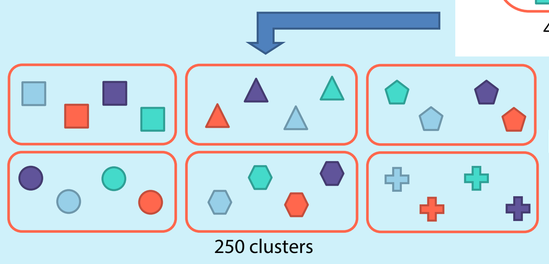
Top scoring ligands
40,000 compounds

Structure Filter

Ligands were clustered into 250 groups based on chemical similarity (shown as shape).



40,000 Compounds



250 clusters

Where possible, two compounds were selected from each cluster: the best docking compound and the cluster centroid .

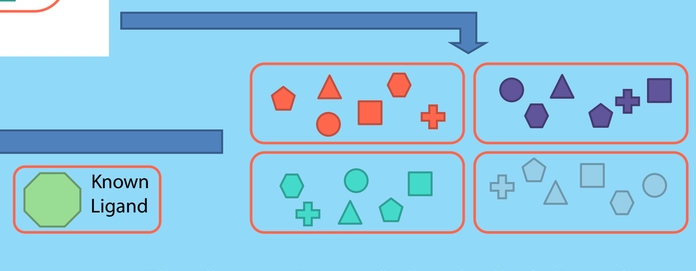
This structure filter provided 448 chemically diverse compounds .

Combined and duplicates removed

1,323 Compounds

BindingPose Filter

Eve ry molecule forms a unique set of interactions with the protein . We can think of these as fingerprints (shown by colour).



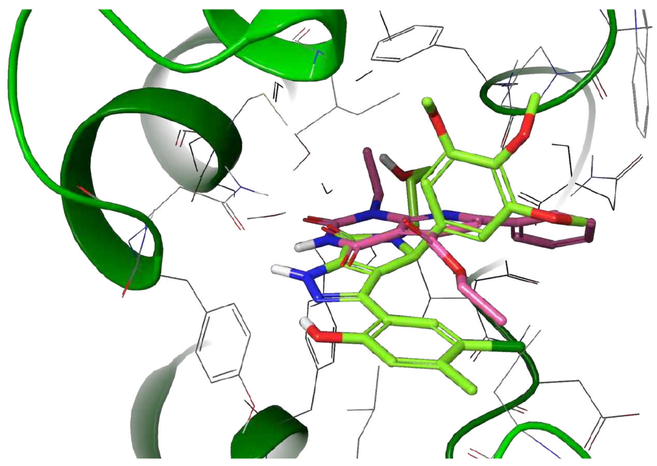
The fingerprints of the docked ligands were programmatically compared to that of a known ligand and scored (0-1), where 1 would be identical interactions with the binding pocket of BRD4.

Selection from a range of the scored ligands resulted in 1,127 compounds .

The selected 1,323 compounds were then visually inspected to identify a subset for investigation. Throughout the visual assessment, key interactions in the binding pocket, synthetic feasibility and chemical diversity were prioritised. Ultimately, 50 compounds were selected for investigation with 20 purchased for initial assays.

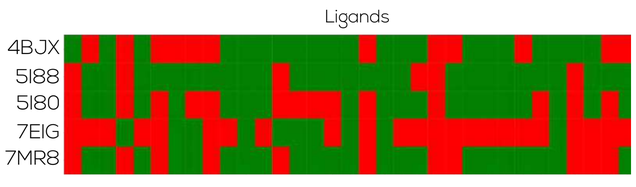
Filtered Ligands - 1,323 Compounds

20 Purchased

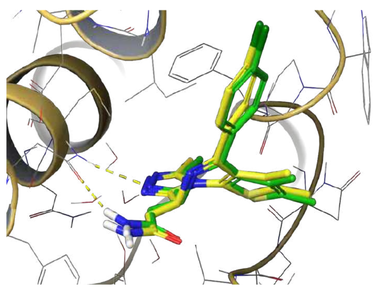


Docking Protocol Validation

To ensure the docking procedure would yield relevant poses for the ligands of interest, validation of the protocol was carried out. Known ligands were docked using Glide into a subset of crystal structures to assess replication of published binding poses.



As well as successfully docking most of the known ligands, 5I88 contained a disturbed water network.[4] We envisaged that the smaller network would allow us to sample a larger volume of space within the pocket, leading to a more flexible docking protocol.

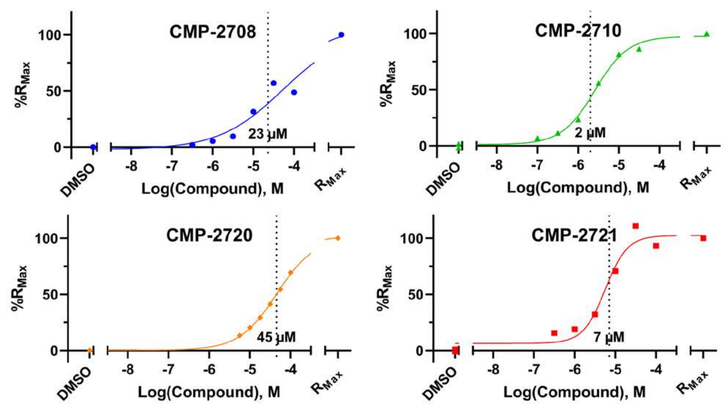


Docked (+)-JQ1 in BRD4-BD1 binding pocket (yellow) with crystallised pose (green)

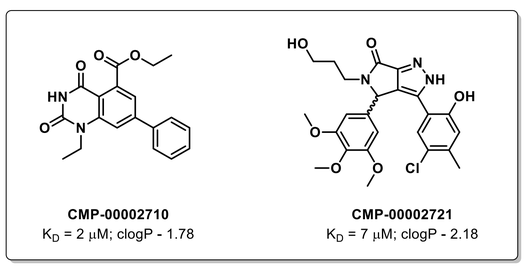
Notably, relevant poses were returned for 82% of the ligand test set. With the docking protocol validated in 5I88, we proceeded to dock the Enamine druglike library.

Binding Assay - SPR

SPR screening was conducted to rapidly assess the binding of the new ligands to BRD4-BD1. Of the 20 compounds screened, we identified four hits.[5]



In-house synthesis, followed by SPR validation confirmed the two hits shown below. SAR studies are underway to develop our understanding of these series



CMP-00002710
K_D = 2 µM; clogP - 1.78

CMP-00002721
K_D = 7 µM; clogP - 2.18

Summary and Conclusions

We have shown our structure-based virtual workflows are capable of rapidly identifying hit molecules across a broad chemical space against a target of interest.

In this work we demonstrated several of our computational capabilities at Charnwood Discovery:

- Target Assessment – using literature resources and modelling suites, we identified the ideal protein crystal for docking
- Virtual Screening – libraries of millions of compounds were screened rapidly using force field-based methods
- Cheminformatics and Data Science – tailor-made workflows were generated in Python to decrypt the docking results and identify hit molecules across a range of chemical space

From an initial library of 5 million compounds and a desired target protein, we were able to screen the library and triage down to 1,323 compounds. Of these, 20 were purchased and 2 showed single-digit micromolar affinities as identified by SPR.

1 - Cheung, K. L. *et al.* (2021), *Front. Mol. Biosci.*, 8:728777 2 - Devaiah, B. N. *et al.* (2020), *PNAS*, **117** (24): 13457-13467 3 - Demont, E. H. *et al.* (2015), *J. Med. Chem.*, **58** (14): 5649-5673 4 - Lukac, I. *et al.* (2021), *J. Comput. Aided Mol. Des.*, **35** (10): 1025-1036
5 - Dose-response curves represent 'Late Binding' response points from SPR sensorgrams (n=1), all under steady state conditions