Identification of novel inhibitors of bromodomain BRD4 by virtual screening and experimental validation

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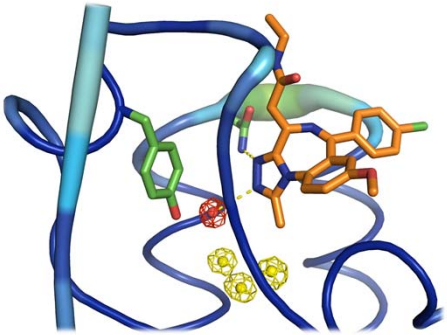
Introduction

Bromodomain-containing proteins are of biological interest as substantial components of transcription factor complexes and determinants of epigenetic control. They specifically identify acetylated Lysine amino acids on histone tails, thus influencing the expression level of key genes. Not surprisingly, the therapeutic relevance of these protein-protein interactions has been shown for several disorders[1]. For example, the BET bromodomain family member BRD4 has been proposed as a promising pharmacological target in cancer[2].
To date, only a few binders and active compounds have been described for this protein[2,3]. Several crystallographic structures are available, allowing for the rational structure-based discovery of novel inhibitors of BRD4. Here, we present the results of a virtual screening experiment followed by *in vitro* validation performed on this protein.

Our virtual screening workflow

- Library preparation: • Selection of ***more than 9M drug-like compounds***
- Docking I: • Docking of all ligands using the HTVS (fast) docking scheme from Glide (Schrödinger Inc.) –> ***5-6 days of computation*** in 256 CPUs
- Filter I: • Selection of the first ranked 500,000 compounds
- Docking II: • Docking of the ligands using the eXtra Precision docking scheme from Glide –> ***3-4 days of computation*** in 256 CPUs
- Filter II: • Selection of the first ranked 500 compounds
- Post-process I: • Remove compounds with inappropriate physico-chemical/pharmacological properties using ***QikProp and SEA***
- Post-process II: • Perform a selection of ligands based on a ***diversity clustering***

Crystallographic analysis



Definition of the binding pocket used during the virtual screening campaign: 4 water molecules were kept (in red and yellow). The interaction with the universally conserved Asn140, that recognizes specific acetylated Lysine residues in histone tails, and Tyr97 are highlighted.

Binding affinity measurement

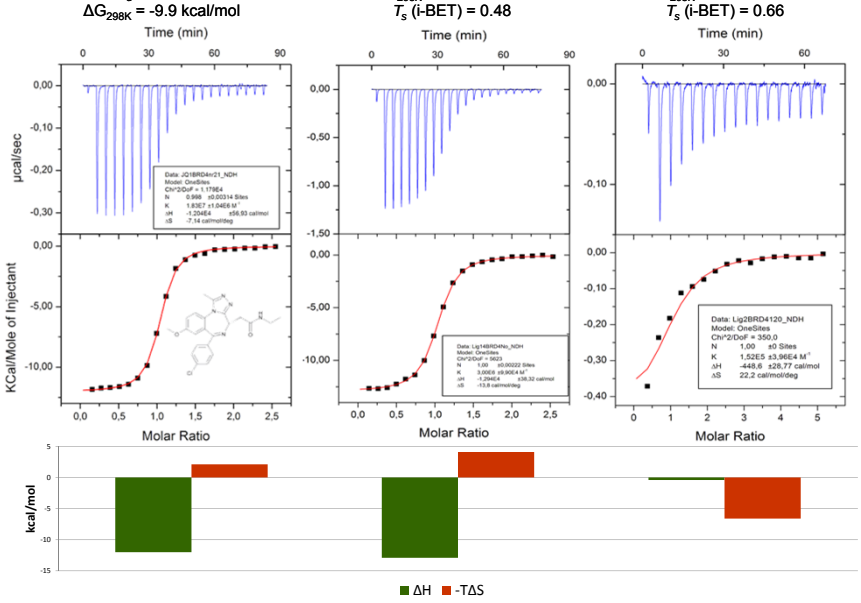
We performed ITC measurements to determine the experimental *in vitro* binding affinity of our candidates:

$$\Delta G = -RT \ln K_{eq} = \Delta H - T\Delta S$$

i-BET
K_d = 50.5 nM
ΔG_{298K} = -9.9 kcal/mol

Ligand #14
K_d = 333 nM
ΔG_{298K} = -8.8 kcal/mol
T_s (i-BET) = 0.48

Ligand #2
K_d = 6.6 µM
ΔG_{298K} = -7.0 kcal/mol
T_s (i-BET) = 0.66



Conclusions

- From the virtual screening campaign on BRD4, we have identified:
  - ***A binder with affinity 333 nM***
  - ***Several binders in the low-µM range***

- **Co-crystals** of the best binders are being currently obtained, in order to further understand the binding process.
- The identified binders will be tested in **competition and selectivity assays**, and their *in vitro* and *in vivo* activity determined experimentally.
- Interesting candidates may be submitted for **patenting** in future.

References

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