Exploring the bromodomain of SMARCA4 (BRG1) by Weak Affinity Chromatography (WAC™)

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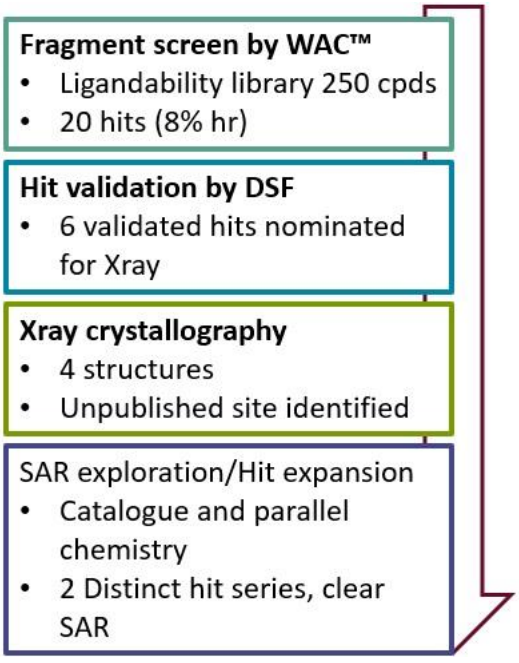
Background

SMARCA4 (BRG1), an ATP-dependent helicase, is a subunit of the SWI/SNF chromatin remodeling complex which regulates genes involved in repair of damaged DNA, replication of DNA, control of cell growth, division, and maturation. [1]

The role of BRG1 in sensitivity to anti-cancer drugs had been highlighted by elucidating the mechanism of action of darinaparsin, which has been shown to induce phosphorylation of BRG1 leading to its exclusion from chromatin. As a result, BRG1 can no longer act as a transcriptional co-regulator leading to the inability of cells to express HO-1, a cytoprotective enzyme. [2] At the same time it is established that a kinase, CDK9, directly binds to and phosphorylates BRG1 in vitro. CDK9 inhibition was demonstrated to dephosphorylate BRG1 reactivating tumour-suppressor genes. [3]

Considering the biological significance of BRG1 phosphorylation and the fact that known SMARCA4 ligands [4-6] were not shown to play a role in the process, we set out to explore the protein with the aim to identify new modulators.

Results and Discussion



Using Weak Affinity Chromatography (WAC™) as the primary screening method a number of hits for the bromodomain of SMARCA4 were identified. The advantages of WAC™ for fragment screening are the detection of weak binders at low concentrations (< 5 µM) and immediate ranking of hits. Our ligandability fragment set (250 compounds) was used for the screen – designed to rapidly assess the ability of biological targets to bind small organic molecules.

Subsequent hit validation by DSF (thermal shift assay) afforded confidence in several hits which were progressed into X-ray crystallography. This resulted in four structures of protein-ligand complexes, where two hits bound to the orthosteric site while the other two fragments were found at a new site which, to the best of our knowledge, is not described in the literature.

At this point, the activities were directed towards exploring and characterizing the newly identified binding site by hit clustering, SAR analysis, hit expansion (by SAR-by-catalogue and parallel chemistry) as well as computational chemistry (docking, hit evolution).

References

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