Targeting the Todalam site on α-Tubulin: toward novel protein immobilization systems

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Microtubules (MTs) are highly dynamic polymers that regulate numerous cellular functions, such as intracellular cargo trafficking and cell proliferation. Tubulin αβ-heterodimer is the fundamental structural unit of MTs and small molecules' binding to this protein can regulate dynamically growth and shrinkage of MTs, with important therapeutic implications [1]. Indeed, over the last decades many Microtubules Targeting Agents (MTAs) with diverse chemical scaffolds have been investigated and up to now, eight binding sites have been experimentally characterized [2]. Among them, the latest identified site is located on the α-subunit and has been recently discovered in a comprehensive analysis of potential binding pockets on tubulin [3]. Immediately afterwards, a first generation of MTAs binding the newly identified site has been designed, and Todalam, the most active compound, gave its name to the binding site [4].

Starting from these findings, we designed a new set of compounds targeting the Todalam site, with the final aim of developing novel tubulin ligands with a simple structure and synthesis process that can be used to immobilize a tubulin dimer for high-throughput biophysical assays. We performed virtual screening of the Enamine library and of an in-house designed library of compounds. The Enamine library, that contains ~3M purchasable molecules, was screened through several filters: (1) substructure search, (2) docking with the PLANTS [5] software, and (3) re-docking of the best candidates with S4MPLE [6]. Computational methods were used to identify a new set of promising chemically diverse Todalam site binders, which were later experimentally validated through X-ray crystallography. Of the 15 molecules proposed by computational methods, 12 were experimentally observed to bind to the Todalam site, resulting in a hit rate of 80%.

Stemming from the X-ray crystallography data, we developed an iterative process of model-guided experimentation and experiment-guided modeling to strengthen the predictive power of computational models. Using the established pipeline, small molecules specifically targeting the Todalam site were rationally designed and developed into warhead-containing compounds with the goal of targeting residue αCys4. Of the 14 designed potential covalent binders, nine were found to bind to the Todalam site by crystallography experiments. Further analyses are on the way to assess the possible formation of a protein-ligand covalent bond.

Bibliography:

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