Application of ensemble pharmacophore-based virtual screening to the discovery of novel antimitotic tubulin inhibitors

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Abstract

Tubulin is a well-validated target for herbicides, fungicides, anti-parasitic, and anti-tumor drugs. Many of the non-cancer tubulin drugs bind to its colchicine site but no colchicine-site anticancer drug is available. The colchicine site is composed of three interconnected sub-pockets that fit their ligands and modify others' preference, making the design of molecular hybrids (that bind to more than one sub-pocket) a difficult task. Taking advantage of the more than eighty published X-ray structures of tubulin in complex with ligands bound to the colchicine site, we generated an ensemble of pharmacophore representations that flexibly sample the interactional space between the ligands and target. We searched the ZINC database for scaffolds able to fit several of the subpockets, such as tetrazoles, sulfonamides and diarylmethanes, selected roughly ~8000 compounds with favorable predicted properties. A Flexi-pharma virtual screening, based on ensemble pharmacophore, was performed by two different methodologies. Combining the scaffolds that best fit the ensemble pharmacophore-representation, we designed a new family of ligands, resulting in a novel tubulin modulator. We synthesized tetrazole 5 and tested it as a tubulin inhibitor in vitro. In good agreement with the design principles, it demonstrated micromolar activity against in vitro tubulin polymerization and nanomolar anti-proliferative effect against human epithelioid carcinoma HeLa cells through microtubule disruption, as shown by immunofluorescence confocal microscopy. The integrative methodology succedes in the design of new scaffolds for flexible proteins with structural coupling between pockets, thus expanding the way in which computational methods can be used as significant tools in the drug design process.

Author keywords

Antimitotic; Colchicine; Drug design; Ensemble pharmacophore; Tubulin; Virtual screening