

## SHOTGUN AND TARGETED MS ANALYSES PINPOINT THE ZAMPANOLIDE-TUBULIN INTERACTING SITE

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Microtubule Stabilizing Agents (MSA) are one of the most successful antitumour drugs used in clinical treatment of neoplastic diseases. These compounds mimic the paclitaxel mechanism of action, which preferentially binds to microtubules the assembled form of tubulin, displacing the assembly equilibrium between dimeric and polymeric tubulin. Since this assembly and disassembly equilibrium is essential to cell division, compounds that bind tubulin target rapidly dividing cells (as tumoural ones), arresting them in mitosis, and finally killing them through apoptosis.

The screening of compounds with a similar mechanism of action as paclitaxel but with improved chemical (easy of synthesis) or pharmacological properties (better bioavailability, lower toxicity, less prone to cause resistance) lead to the searching of chemically different molecules with apparently the same biological mechanism of action.

Zampanolide, a 20-membered macrolide from a Tongan marine sponge, and its synthetic derivative Dactylolide are potent new covalent MSA targeting the pore site. These MSA block cells in G2/M of the cell cycle, induce microtubule bundles in cells and present cytotoxic activity in the low nanomolar range. The advantages of these compounds include the amenability to large-scale synthesis and its activity in multidrug-resistant cells, since Zampanolide and Dactylolide are not substrates for P-glycoprotein efflux pump.

Using combined MS analyses based on targeted precursor ion scanning (4000 Q-Trap) and shotgun (LTQ-Orbitrap) approaches, we determined Y-224 within the paclitaxel binding domain of beta-tubulin as the Zampanolide and Dactylolide interaction site.