

## A SMALL MOLECULE WITH TAXOL-LIKE MECHANISM

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Thalidomide and its analogues have generated interest for the treatment of inflammatory and autoimmune diseases and also cancer. It is currently in clinical trials for treatment of multiple myeloma and some solid tumors. We have identified a thalidomide analogue (5HPP-33) with potent anti-proliferative activity (low micromolar range) *in vitro* against several tumor cell lines derived from six tissue types. The tumor cell lines include the estrogen-dependent (MCF-7), estrogen-independent (MDA-MB-231) breast cancer cell lines, androgen-independent (PC-3 and DU145), androgen-dependent (LNCaP) prostate cancer cell lines, colon (HT29), ovarian (1A9), bladder (TCCSUP) and Burkitt's lymphoma (Hs Sultan) cell lines. The anti-proliferative effect was independent of p53 status (wild type in LNCaP vs. mutant in DU-145) and hormone dependence in both prostate and breast cancer cell lines. The IC<sub>50</sub> values ranged from 1.7-10 M/L. Thalidomide, on the other hand, had no anti-proliferative activity with IC<sub>50</sub> value >100

M on all 9 cell lines. Cells treated with 5HPP-33 exhibited a breakdown of nucleus into micronuclei. Flow cytometric analysis revealed that 5HPP-33 induced cell cycle arrest in G<sub>2</sub>/M phase, with the arrest mainly occurring in the mitotic phase as observed by incubating with an anti-mitotic antibody. Many anti-mitotic agents perturb the microtubules dynamics by either hyperstabilizing them (like taxol) or destabilizing them (like colchicine). Effects of 5HPP-33 on microtubules were investigated by two methods. Immunofluorescence visualization of 5HPP-33 treated cells demonstrated production of more abundant and shorter microtubules and incubation of mammalian tubulin in a cell free assay with 5HPP-33 caused increase in tubulin polymerization in a concentration-dependent manner. Moreover, the microtubule polymers formed were resistant to cold. Thus we concluded that 5HPP-33 stabilized microtubules and favored tubulin polymerization similar to taxol. 5HPP-33 is not the first totally synthetic small molecule shown to induce tubulin polymerization. Three other small molecules have been reported to have a similar paclitaxel-like effect. However, in comparison to these compounds, 5HPP-33 has the simplest structure and is the first thalidomide analog reported to have such activity. Despite the clinical success of taxol, drug resistance has been demonstrated in the laboratory and clinic. Sensitivity to 5HPP-33 was tested on taxol resistant NIH3T3 cells overexpressing multidrug-resistant (MDR) efflux pump and 1A9 cells with mutant -tubulin. 5HPP-33 was equally active on taxol resistant vs. sensitive cell lines. Thus, 5HPP-33 represents a novel anti-tumor agent<sup>1</sup>.

### REFERENCE

1. Li PK, Pandit B, Sackett DL, Hu Z, Zink J, Zhi J, Freeman D, Robey RW, Werbovetz K, Lewis A, Li C. A Thalidomide analog with *in vitro* anti-proliferative, anti-mitotic and microtubule stabilizing activities *Molecular Cancer Therapeutics* 2006; 5(2): 450-456