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Novel Molecules that Interact with Microtubules and have Functional Activity Similar to Taxol

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Taxol has been approved by the FDA for the treatment of ovarian, breast and lung carcinomas. Its therapeutic activity, unusual chemical structure and unique mechanism of action have made Taxol a particularly interesting compound. In spite of its positive results in the clinic, Taxol has distinct problems such as its aqueous insolubility that has made formulation difficult, and a propensity for the development of drug resistance. For these reasons, many analogs of Taxol have been synthesized and new molecules with Taxol-like activity are being sought. Taxol acts in the tubulin/microtubule system, specifically to hyperstabilize microtubules thereby protecting them from the destabilizing effects of Ca++ and cold and inhibiting cell division. Two different photoaffinity analogs of Taxol have been used to obtain information about the molecular contacts between Taxol and its target protein, the microtubule. The 3'-(p-azidobenzamido) Taxol analog photolabels the N-terminal 31 amino acids of β-tubulin and the 2-m-azidobenzoyl Taxol analog photolabels amino acids 217-231 in β-tubulin. Although we present clear evidence that the side chain at C-13 interacts with β-tubulin and extensive published structure-activity studies have identified the side chain at C-13 as a requirement for biological activity, we have found that 2-m-azido baccatin III, a compound without the C-13 side chain, has Taxol-like activity. This is in contrast to baccatin III that lacks the 2-m-azido substituent. In cytotoxicity studies, 2-meta-azido baccatin III demonstrated Taxol-like activities, inhibiting the proliferation of three cancer cell lines maintained in tissue culture, although the compound was 30- to 100-fold less active than Taxol. FACS analysis indicated that cells treated with 2-m-azido baccatin III were blocked at the G2/M phase of the cell cycle and developed distinct bundles of microtubules, a typical morphological change induced by Taxol. In an *in vitro* microtubule assembly assay, 2-meta-azido baccatin III, like Taxol, induced the polymerization of stable microtubules in the absence of GTP that is normally required for microtubule assembly. A drug binding competition assay indicated that 2-meta-azido baccatin III inhibited the binding of [3H]-Taxol to the microtubule, indicating that the binding site was the same or overlapping with that of Taxol, although the affinity was weaker. These data provide important information for understanding the interaction between Taxol and its target protein, tubulin. Our studies suggest that the presence of the C-2 meta azido substituent is able to compensate for the loss of the C-13 side chain. It is proposed that the presence of the 2-m-azido group strengthens the association between the taxane ring and

tubulin, thereby overcoming the loss of the C-13 side chain. Such information should be considered during the design of novel Taxol-like molecules.

During the past few years, three new compounds, each with a distinct chemical structure, have been isolated from diverse sources and shown to have a mechanism of action similar to that of Taxol. Epothilone was isolated from a Myxobacterium fermentation, eleutherobin from a soft coral found off the coast of Western Australia and discodermolide from a Caribbean sponge. All three compounds, like Taxol, enhance the assembly of stable microtubules in the absence of GTP that is normally required for *in vitro* tubulin assembly. Studies with these compounds have been aided by a cell line that requires low concentrations of Taxol (2-6nM) to function normally. By taking advantage of this cell line, we have demonstrated that although these new drugs have major similarities to Taxol, at least one can be differentiated from the others. Because of these differences and their distinct pharmacological properties compared to Taxol, epothilone, eleutherobin, and discodermolide have the potential to be effective new antitumor agents.