Structure Determination and Verification by NMR of Potent Microtubule Stabilizing Compounds Isolated from the Medicinal Plant *Tacca chantrieri*: Possible Use in Tumors Resistant to Paclitaxel and Doxorubicin

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Abstract:

Tumors resistant to the potent anti-cancer drugs Paclitaxel and Doxorubicin are becoming increasingly problematic in the successful treatment of certain patients. A new class of microtubule stabilizing compounds isolated from plants of the genus Tacca have been found to have excellent anti-cancer activity *in vivo* against cell lines resistant to drugs of the taxane class. We report the structures of the most active compounds as Taccalonolide A, E, B and N as determined by NMR and comparison with published results as well as the structure of additional active components of differing structural classes.

Introduction: In the treatment of certain types of cancer the drugs of choice have been the taxane family of compounds (Paclitaxel) which work by causing cellular tubulin to polymerize and hence stabilize the resulting microtubules. The taxanes however are highly susceptible to cells which overexpress P-glycoprotein (Pgp), multidrug resistance protein 7 (MRP7) and the βIII isotype of tubulin.[1] The recently discovered Taccalonolides[2] not only circumvent these defense mechanisms but cells which overexpress βIII tubulin were found to be more susceptible to microtubule stabilization and subsequent apoptosis.

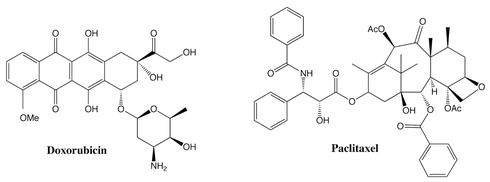


Figure 1. Structures of Doxorubicin and Paclitaxel

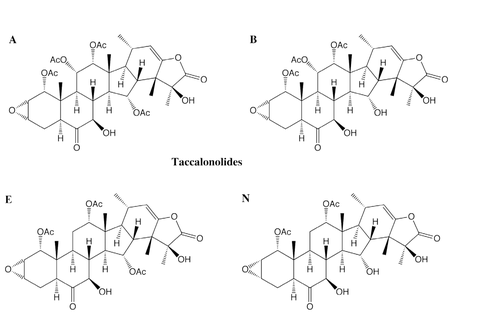


Figure 2. Structures of Taccalonolides A, B, E and N

Figure 3

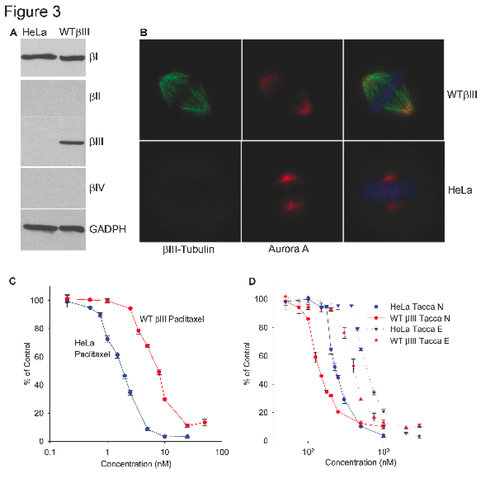


Figure 3. A) Cell line HeLa was used to produce a cell line that overexpresses βIII tubulin. B) Immunoflorescence was used to visualize the location of βIII tubulin to the mitotic spindle in the overexpression cell line. C) The βIII tubulin overexpressing cells show resistance to Paclitaxel relative to the parent cell line. D) The βIII tubulin cells show increased sensitivity to Taccalonolides E and N relative to the parent cell line.

NMR Data for Taccalonolide N

Figure 4

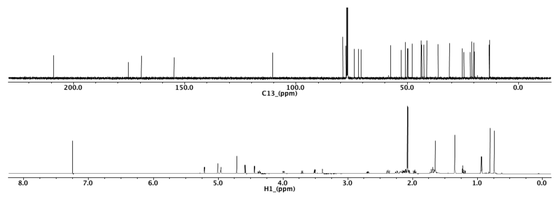
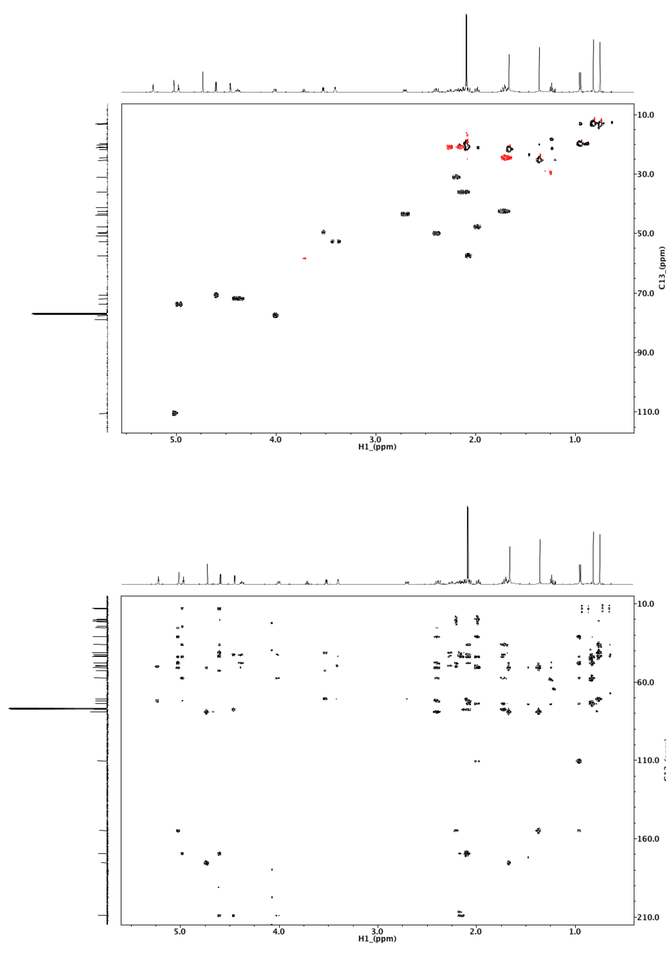


Figure 4. 1-Dimensional ^{1}H and ^{13}C spectra of Taccalonolide N recorded at 600 MHz and 150 MHz respectively. The sample was 5 mg of Taccalonolide N in 650 μl of $CDCl_3$ at 22 deg C.

Figure 5



Results and Discussion: The structures of taccalonolides A, B, E and N used in this study were confirmed by 1-dimensional and 2-dimensional NMR spectroscopy and comparison to values reported in the literature[2]. The general procedure was to prepare samples of 5 mg of each taccalonolide in $CDCl_3$ and to record ^{1}H and ^{13}C spectra (Figure 4) at 600 and 150 MHz respectively. The resulting line lists were checked against and literature values and the literature assignments were subsequently validated by using 2-dimensional NMR. The experiments used were the ^{1}H-^{13}C HSQC (Figure 5 top) and ^{1}H-^{13}C HMBC (Figure 5 bottom) which correlate the 1-bond ^{1}H-^{13}C chemical shift pairs and the 2-bond, 3-bond and occasionally 4-bond ^{1}H-^{13}C chemical shift pairs respectively.

In addition to the samples which were suspected to be known taccalonolides we received several other samples which did not compare via HPLC with known taccalonolide standards. Several of these samples had activity equal to taccalonolide N and are subsequently being studied by NMR. Preliminary analysis of the most promising new compounds indicates a structure class different from any previously reported microtuble stabilizing agent. Further work is being done to arrive at a structure of this and other samples that show promise in both *in vitro* and *in vivo* assays.

Conclusion and Future Direction:

We have confirmed the identity of the 4 compounds used in the study of drug resistant cancer cells lines to be Taccalonolide A, B, E and N (Figure 2) by NMR spectroscopy. In addition we have begun the structure elucidation of a compound with exceptionally good activity against drug resistant tumor cells which indicates a structure class not previously known to stabilize microtubule structure. Whereas we have a tentative structure completed we are conducting further tests using mass spectrometry and quantum mechanical calculations to validate the assignment of several very unusual structural motifs. A full structure will be reported upon completion of these studies.

References:

1) "The Taccalonolides: Microtubule Stabilizers that Circumvent Clinically Relevant Taxane Resistance Mechanisms" Risinger, A.L.; Jackson, E.M.; Polin, L.A.; Helms, G.L.; LeBouef, D.A.; Joe, P.A.; Hopper-Borge, E.; Luduena, R.F.; Kruh, G.D. and Moobery, S.L. **Cancer Res., 2008,** 68, pp 8881-8888.

2) "Steroidal Bitter Principles from *Tacca plantaginea*. Structures of Taccalonolide A and B." Chen, Z-L.; Wang, B-D.; Chen, M-Q. **Tetrahedron Lett., 1987**, 28, pp 1673-1678. "The Pentacyclic Steroidal Constituents of *Tacca plantaginea*. Taccalonolide E and F." Shen, J.; Chen, Z.; Gao, Y. **Chinese J. Chem.**, **1991**, 9, pp 92-94. "Some Chemical Reactions of Taccalonolide A, a Bitter Substance from *Tacca plantaginea*." Chen, -L.; Shen, J-H.; Gao, Y-S. **Heterocycles**, **1989**, 29, pp 2103-2108.

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