

## Supporting Information

for

### **Microtubule-Stabilizing Activity of Zampanolide, A Potent Macrolide Isolated from the Tongan Marine Sponge *Cacospongia mycofijiensis***

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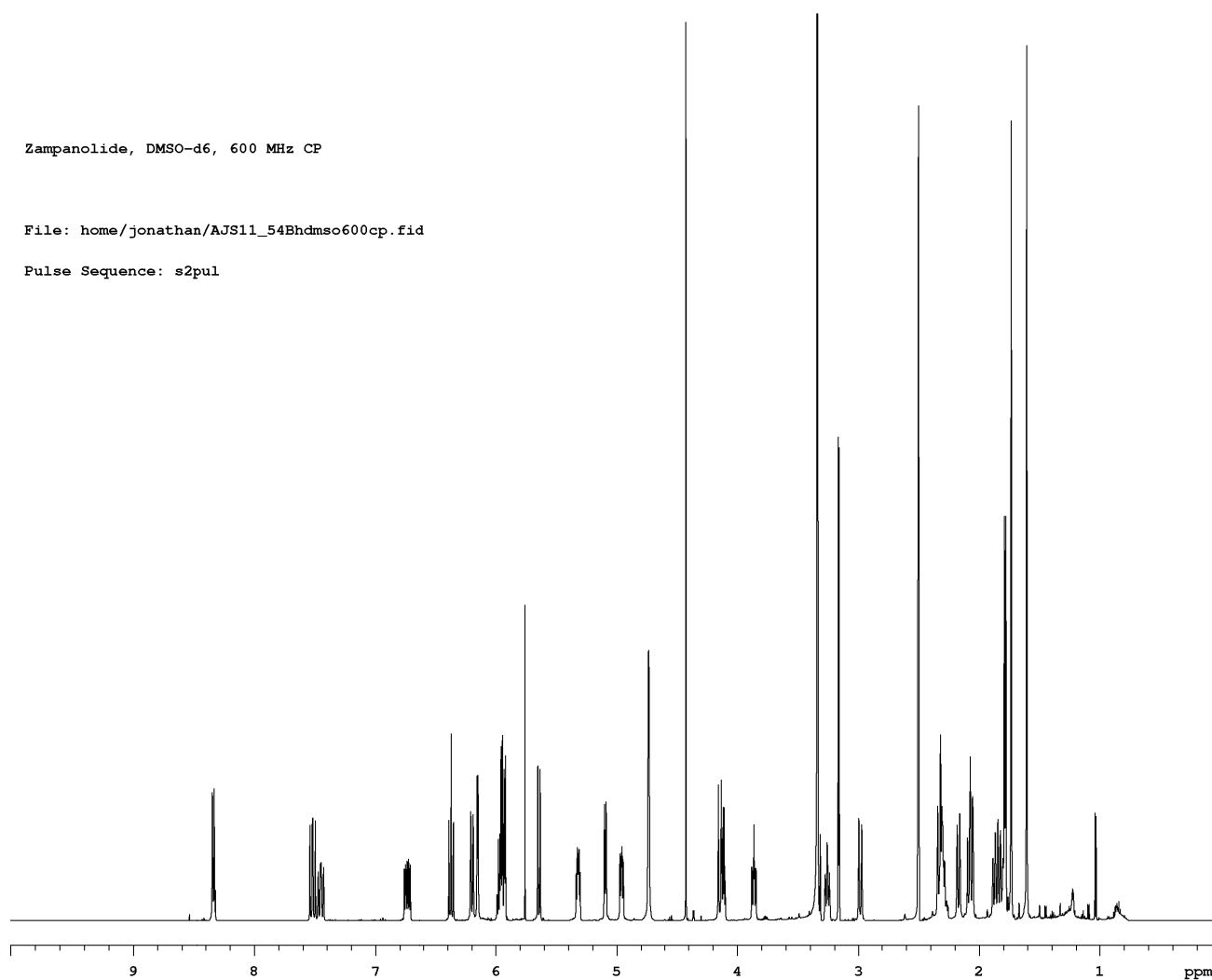
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Zampanolide, DMSO- $d_6$ , 600 MHz CP

File: home/jonathan/AJS11\_54Bhdms0600cp.fid

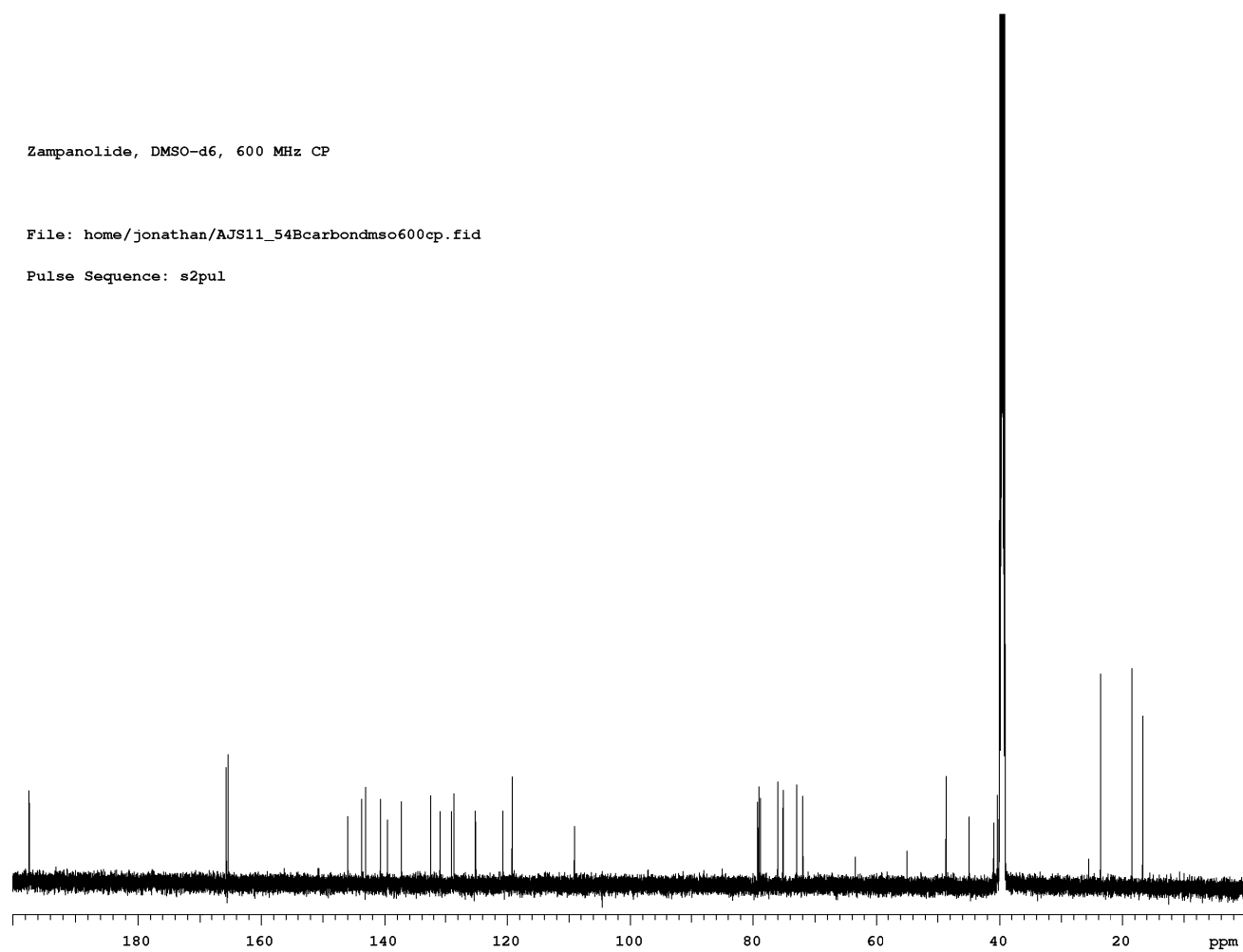
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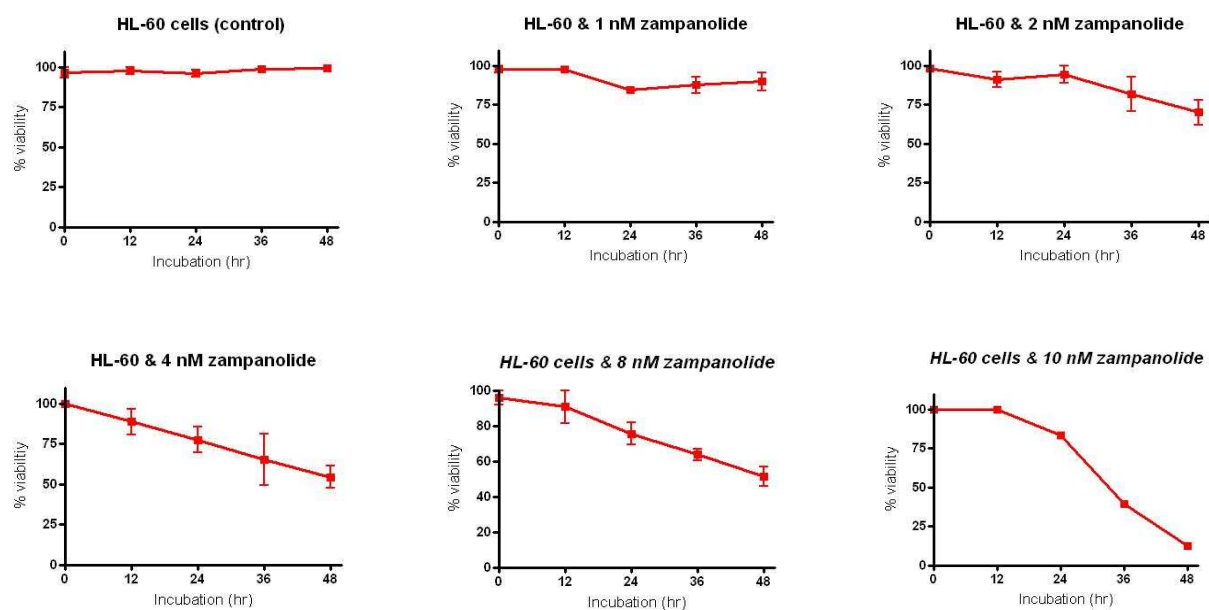


Note: resonance at  $\delta_H$  4.42 is nitromethane ( $\text{CH}_3\text{NO}_2$ ) added as an internal standard.

**Figure S1.**  $^1\text{H}$  NMR (600 MHz) spectrum of zampanolide (**1**) in  $\text{DMSO-}d_6$ .

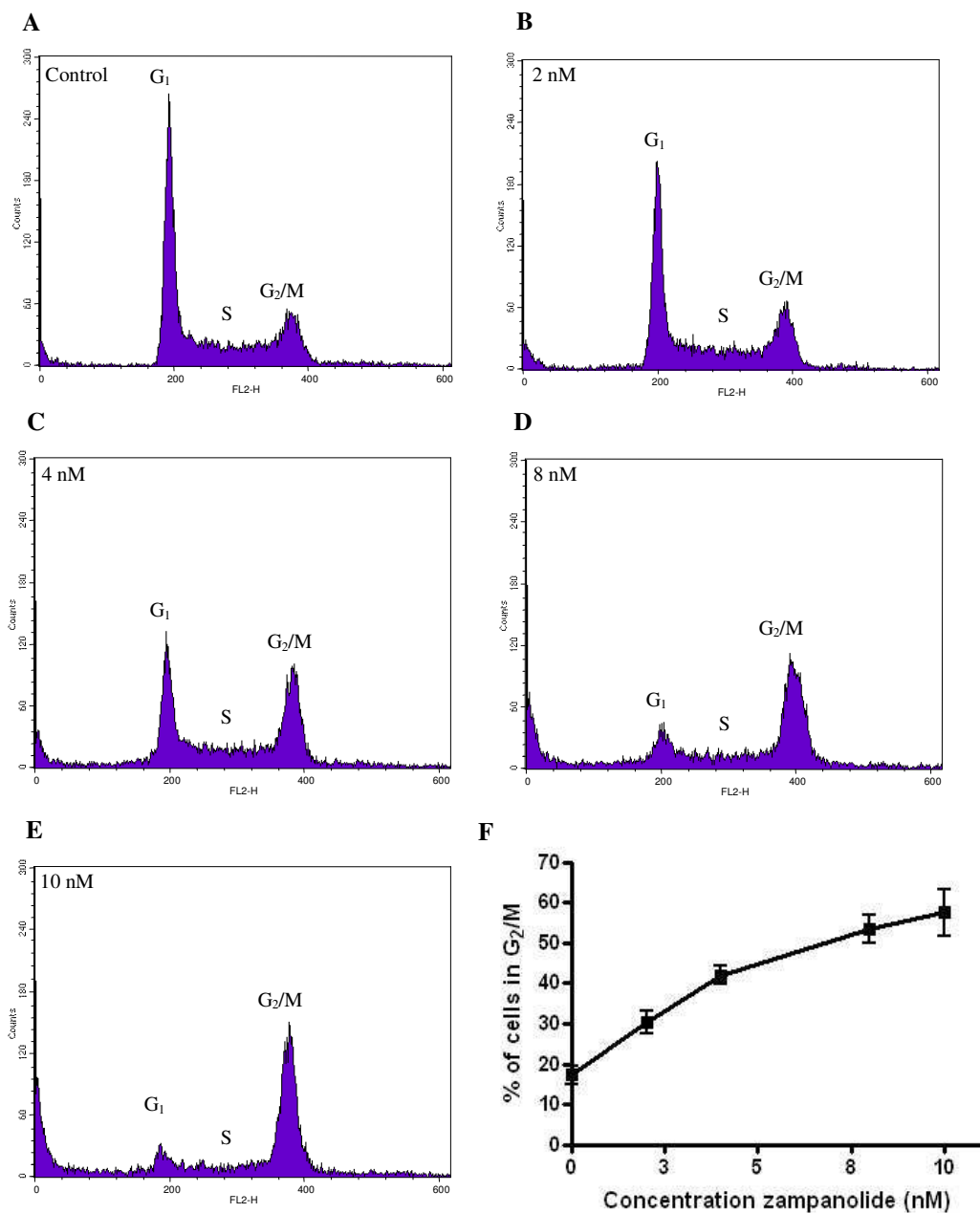
**Figure S2.**  $^{13}\text{C}$  NMR (150 MHz) Spectrum of zampanolide (**1**) in  $\text{DMSO-}d_6$ .



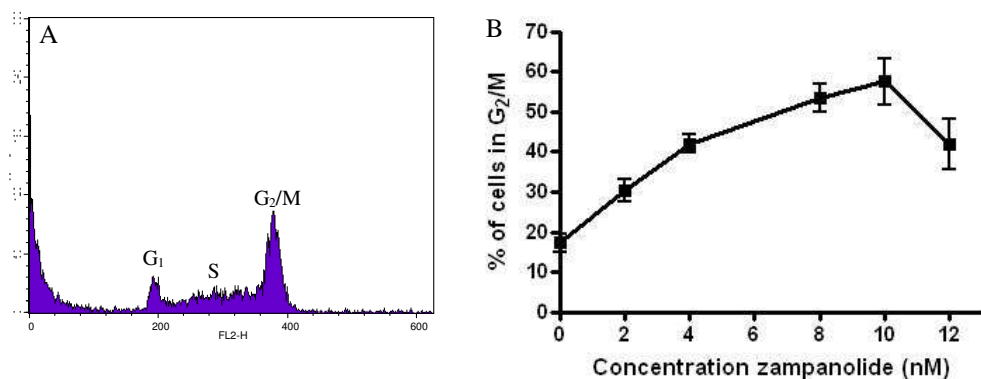


**Trypan blue dye exclusion assay for zampanolide.** Zampanolide effects on % viability of HL-60 cells. Cells were treated and aliquots taken every 12 hours from time 0 and assessed for cell viability using trypan blue dye exclusion. The results are presented as the mean  $\pm$  SEM ( $n = 2$  wells from two separate experiments).

**Figure S3.** Trypan blue dye exclusion assay for HL-60 cells.



**Flow cytometry results (representative results).** A–E. At 10 nM zampanolide, 58% of HL-60 cells are in the G<sub>2</sub>/M phase of the cell cycle compared to 17% in untreated cells. The number of cells in G<sub>2</sub>/M increases in a dose-dependent manner. F. Summary graph, values are represented as the means  $\pm$  SEM ( $n = 4$ ).



**Flow cytometry results for 12 nM zampanolide.** At 12 nM zampanolide there is a decrease in the number of cells arrested in G<sub>2</sub>/M and an increase in dead cells or cellular debris.

**Figure S4.** G<sub>2</sub>/M block series for HL-60 cells.