

# Supporting Information

## ***In silico and in vitro* study of Janus kinases inhibitors from naphthoquinones**

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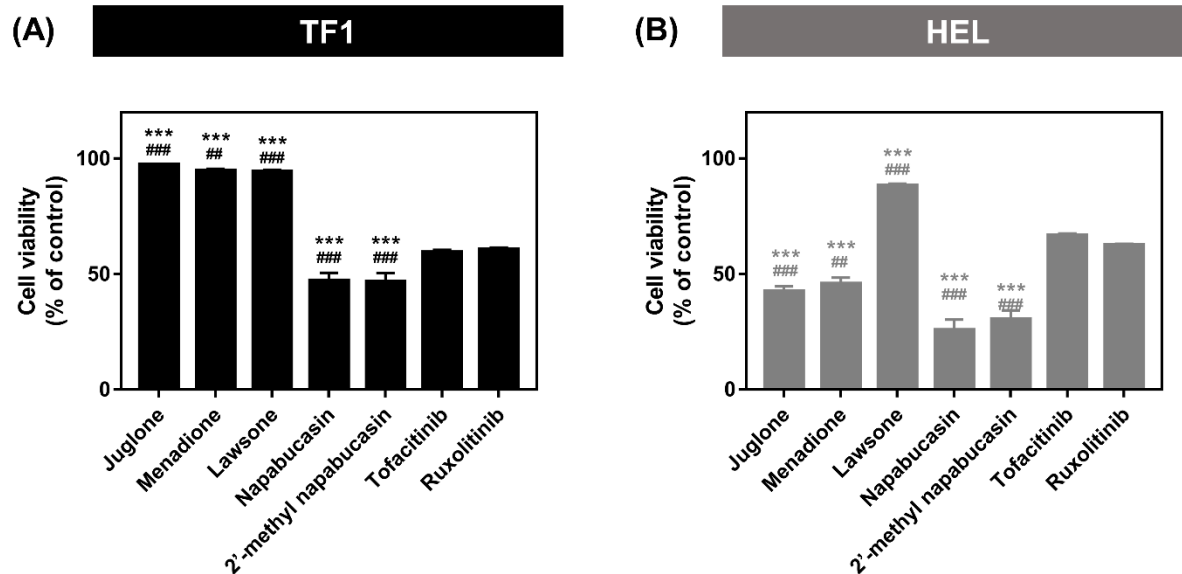
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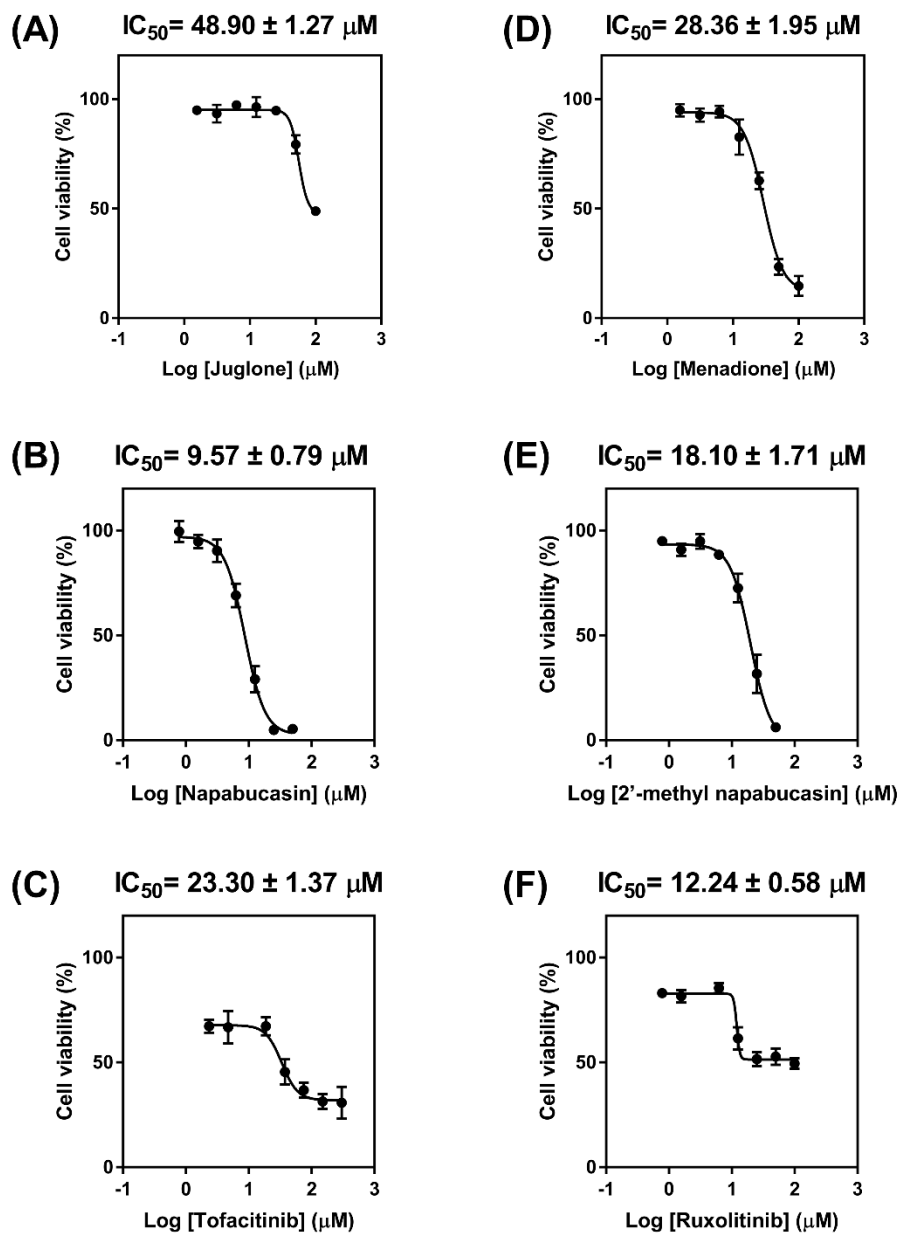
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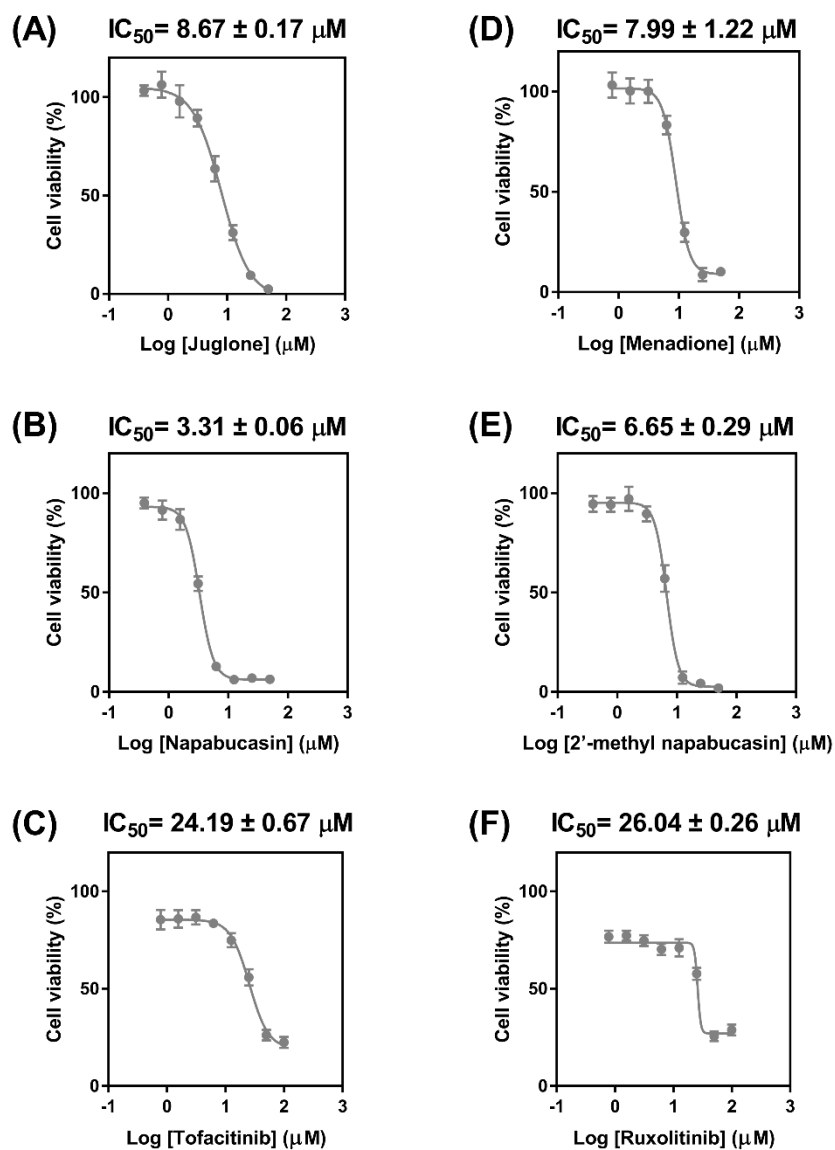
**Figure S1.** Cytotoxicity of naphthoquinones at 10  $\mu$ M against TF1 and HEL cells by Presto Blue assay.  
 \*\*\*  $p \leq 0.001$  vs. tofacitinib, #  $p \leq 0.01$  and ###  $p \leq 0.001$  vs. ruxolitinib.

# TF1

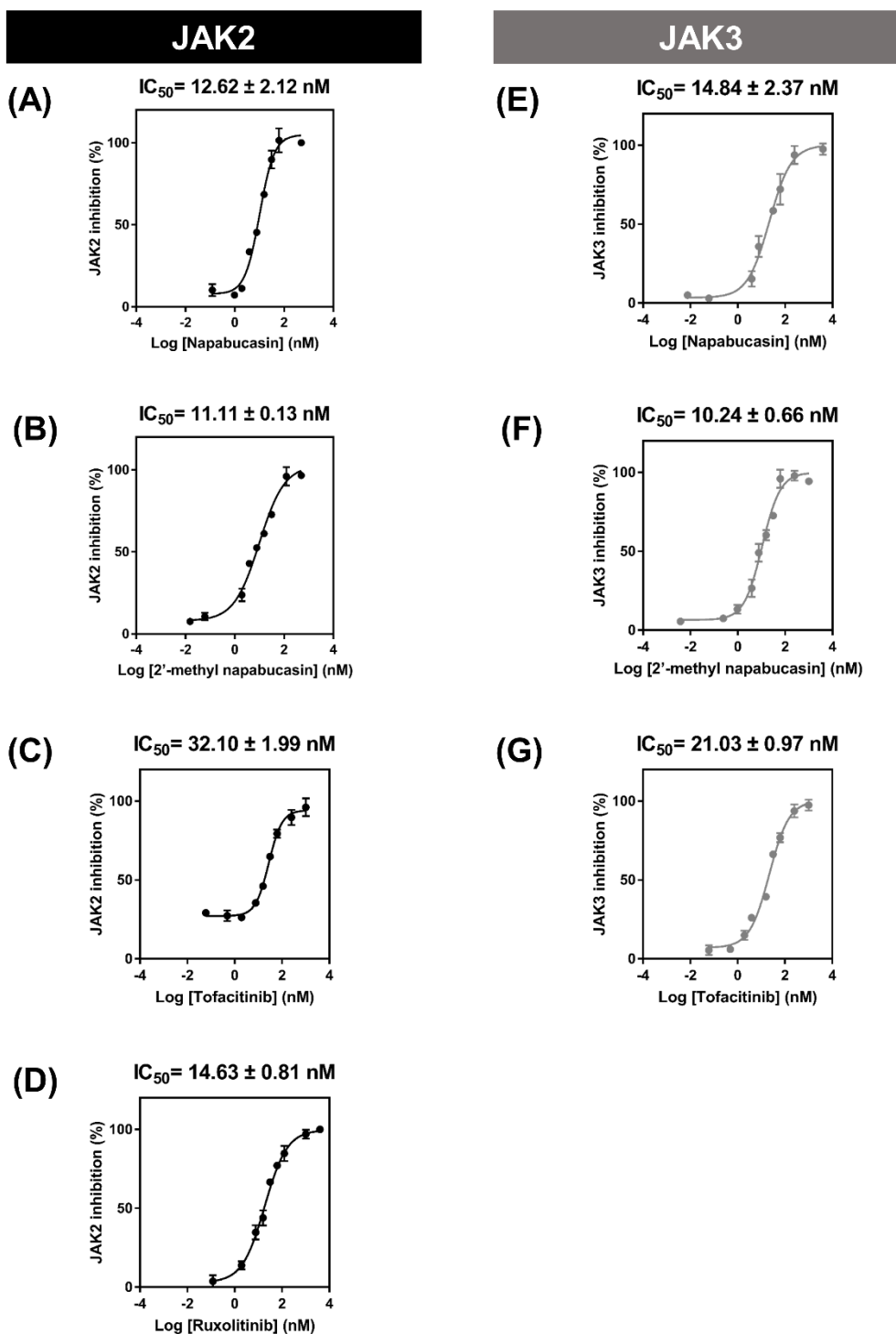


**Figure S2.**  $IC_{50}$  for cell viability inhibition of naphthoquinones and known drugs (tofacitinib and ruxolitinib) in TF1 cells by Presto Blue assay.

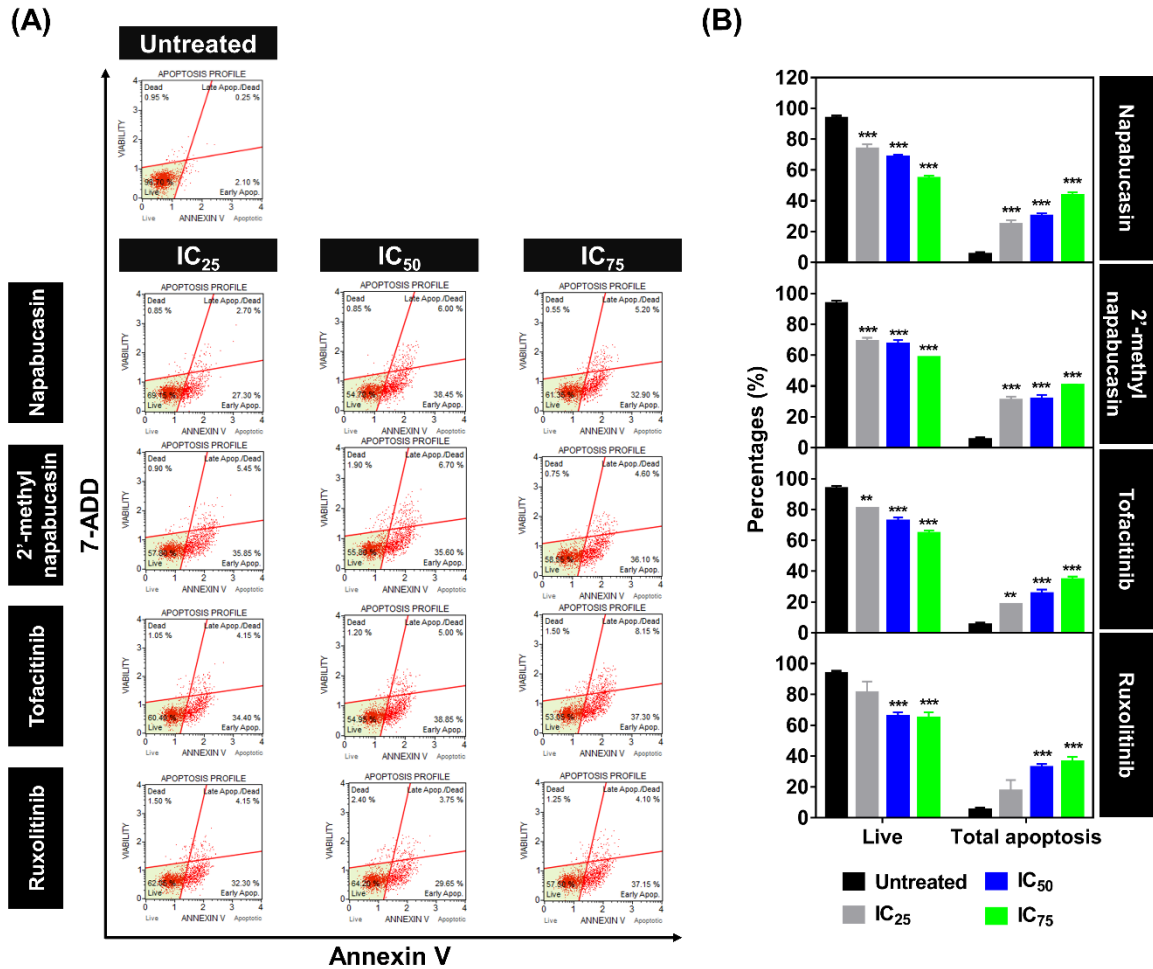
# HEL



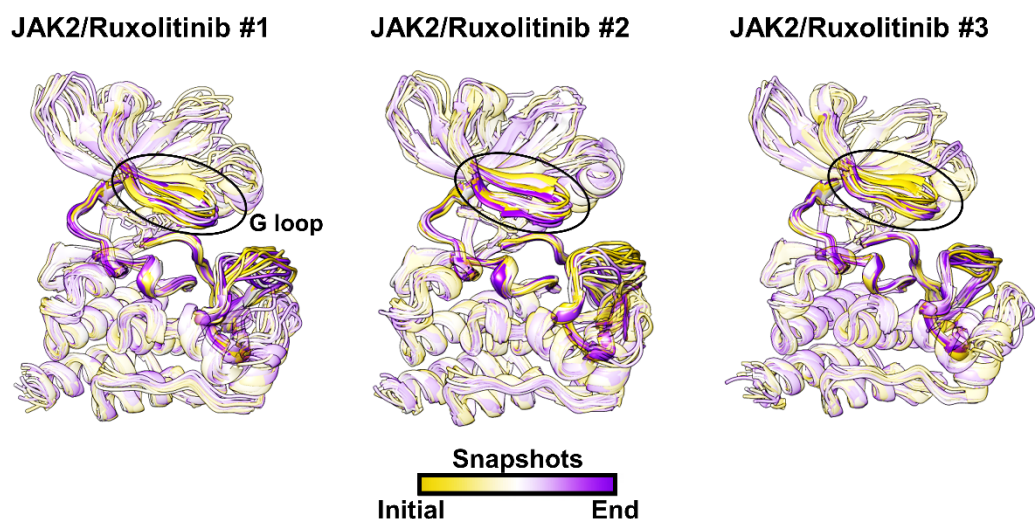
**Figure S3.**  $IC_{50}$  for cell viability inhibition of naphthoquinones and known drugs (tofacitinib and ruxolitinib) in HEL cells by Presto Blue assay.



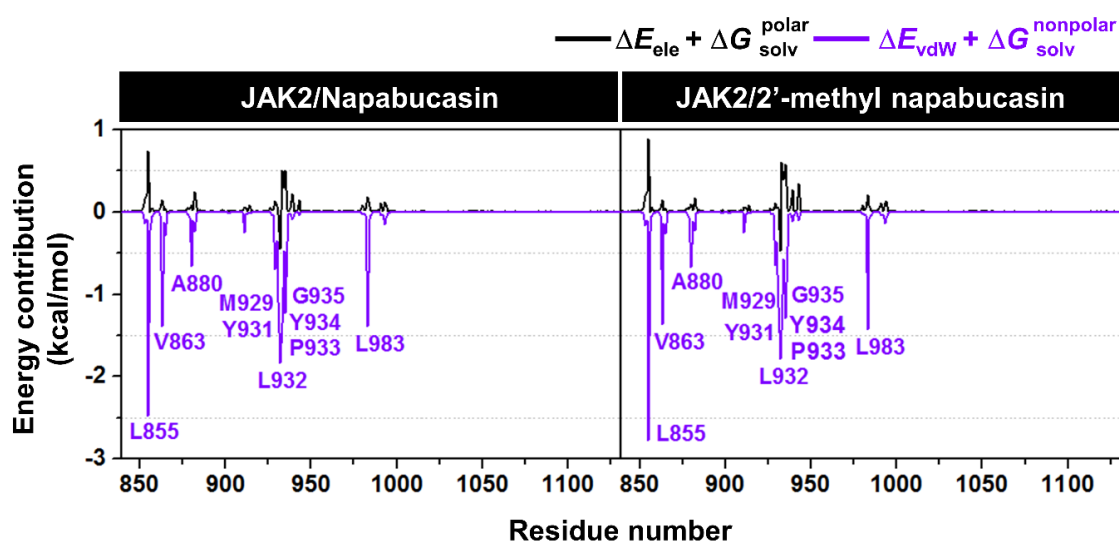
**Figure S4.**  $IC_{50}$  for JAK2/3 inhibitions of napabucasin and 2'-methyl napabucasin comparisons with known drugs, tofacitinib and ruxolitinib by ADP-Glo™ kinase assay.



**Figure S5.** Dose-dependent effect of napabucasin and 2'-methyl napabucasin comparison with drugs, tofacitinib and ruxolitinib at IC<sub>25</sub>, IC<sub>50</sub> and IC<sub>75</sub> values (nM) on the apoptosis of TF1 cells by annexin V for 24 h. (A) The flow cytometry dot plot of each group which divided into four quadrants: live cells, early apoptotic cells, late apoptotic cells, and necrotic cells. (B) The percentages of live cells and apoptotic cells. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$  compared with the untreated group.



**Figure S6.** Snapshot per time (25 frames) of complex between JAK2 and ruxolitinib along 500 ns simulations.



**Figure S7.** Energy contribution between napabucasin series within JAK2 in ATP binding pocket from the average three independent simulations (last 100 ns).

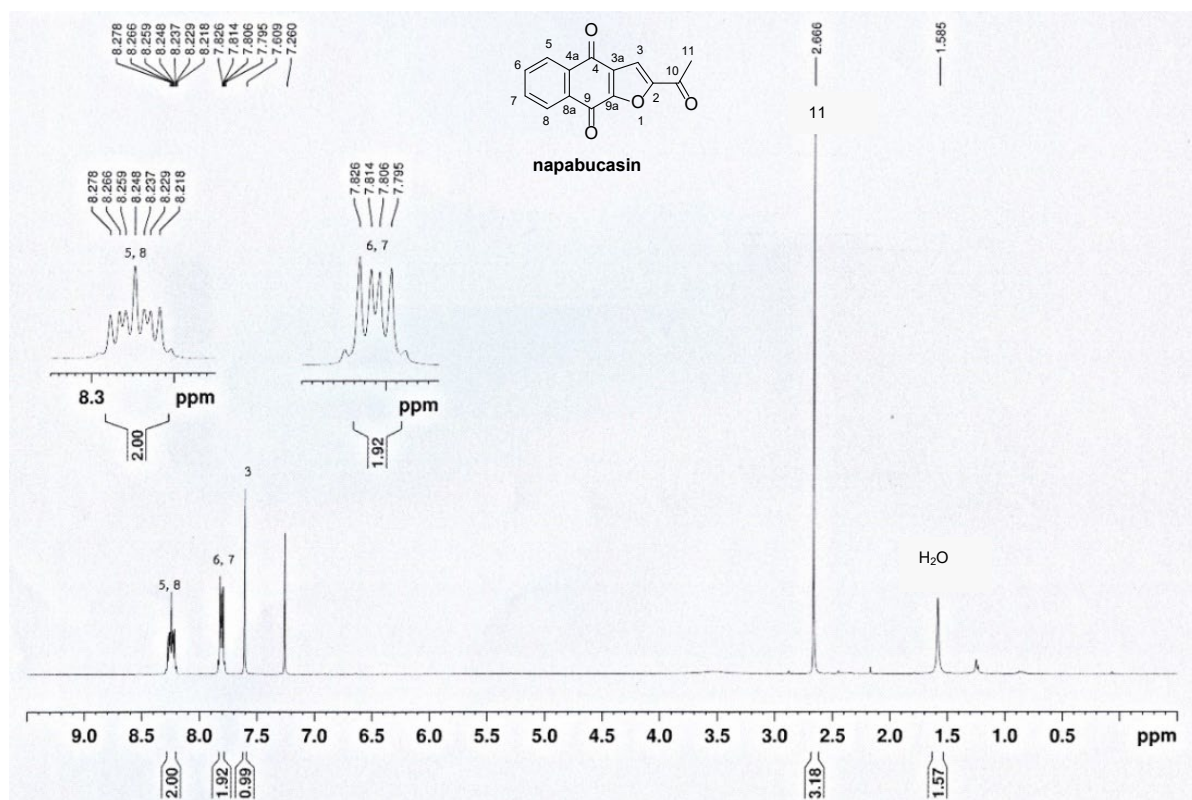


Figure S8.  $^1\text{H}$  NMR (300 MHz) spectrum of napabucasin in  $\text{CDCl}_3$ .

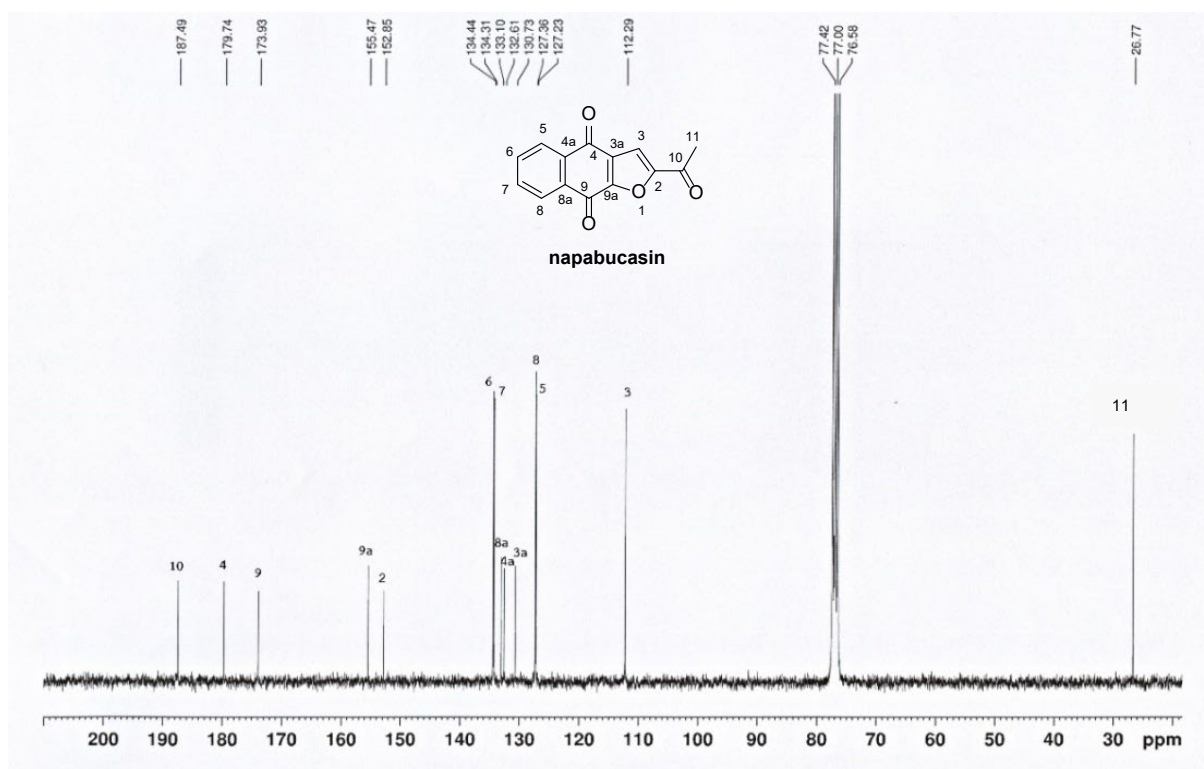
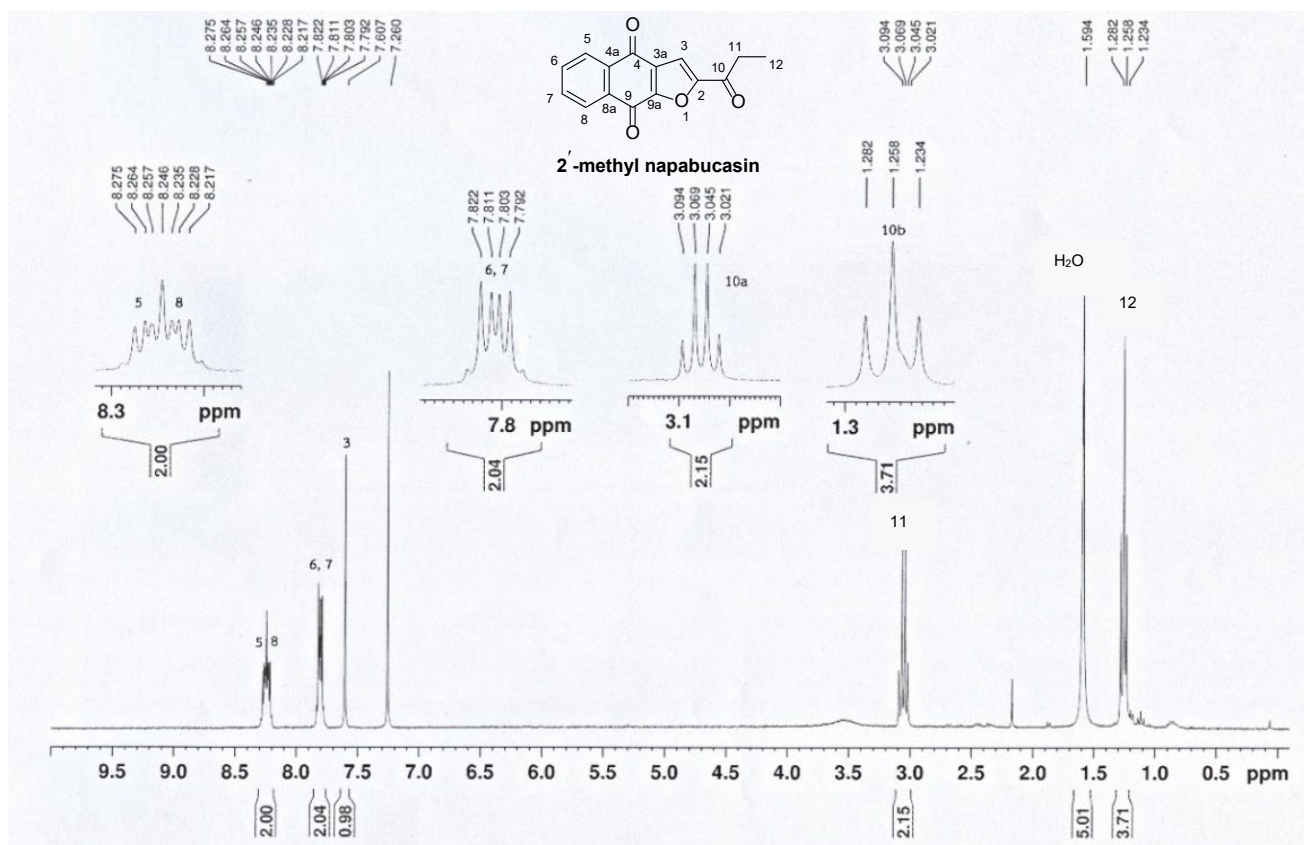
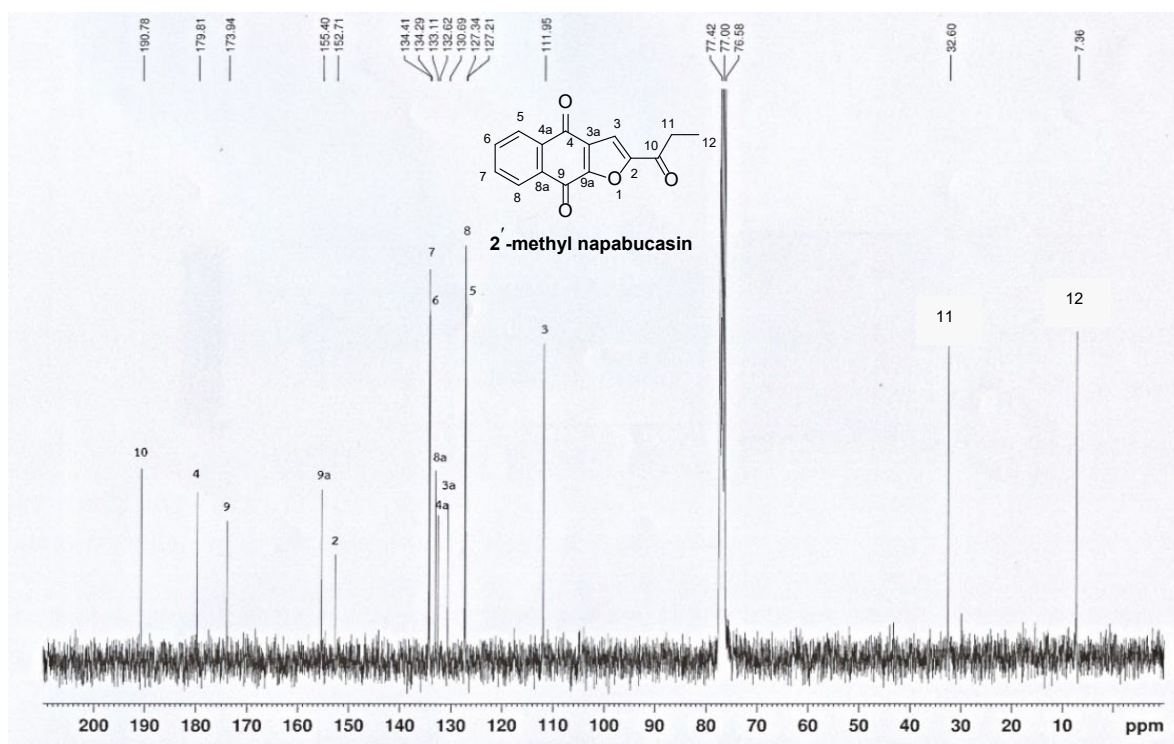


Figure S9.  $^{13}\text{C}$  NMR (100 MHz) spectrum of napabucasin in  $\text{CDCl}_3$ .





**Figure S10.** <sup>1</sup>H NMR (300 MHz) spectrum of 2'-methyl napabucasin in CDCl<sub>3</sub>.



**Figure S11.** <sup>13</sup>C NMR (100 MHz) spectrum of 2'-methyl napabucasin in CDCl<sub>3</sub>.