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Article

A Pyranoxanthone as a Potent Antimitotic and Sensitizer of Cancer Cells to Low Doses of Paclitaxel

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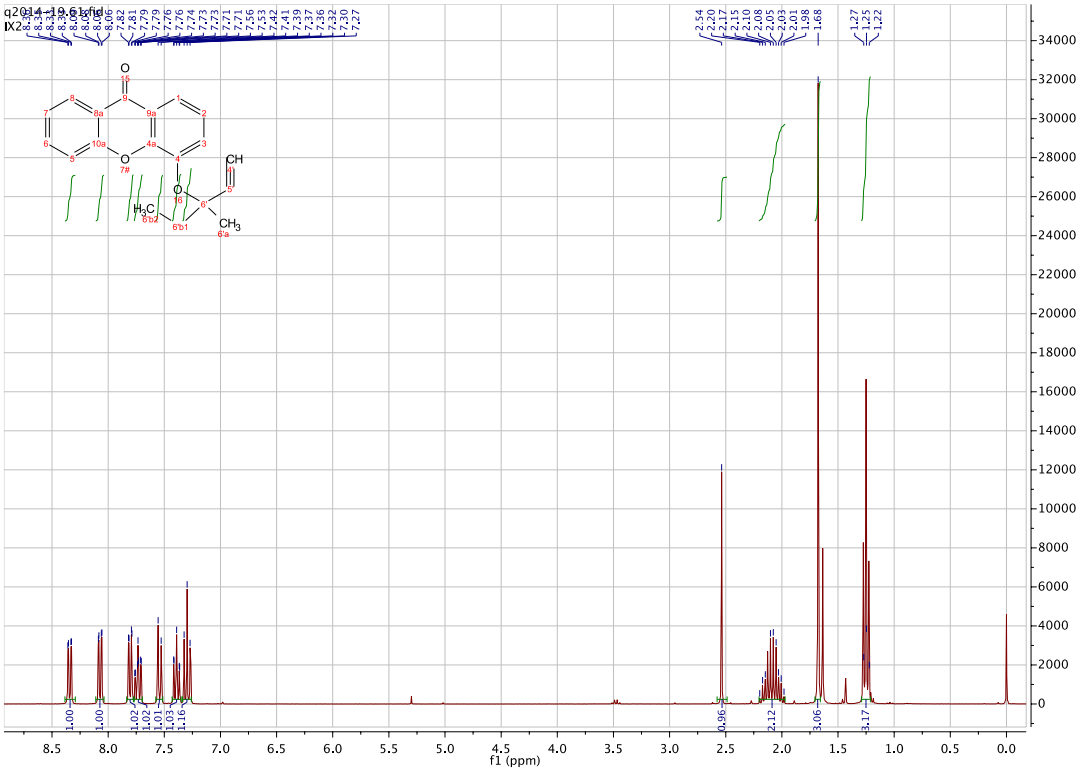

Figure 1. ^{1}H NMR spectrum of compound 8.

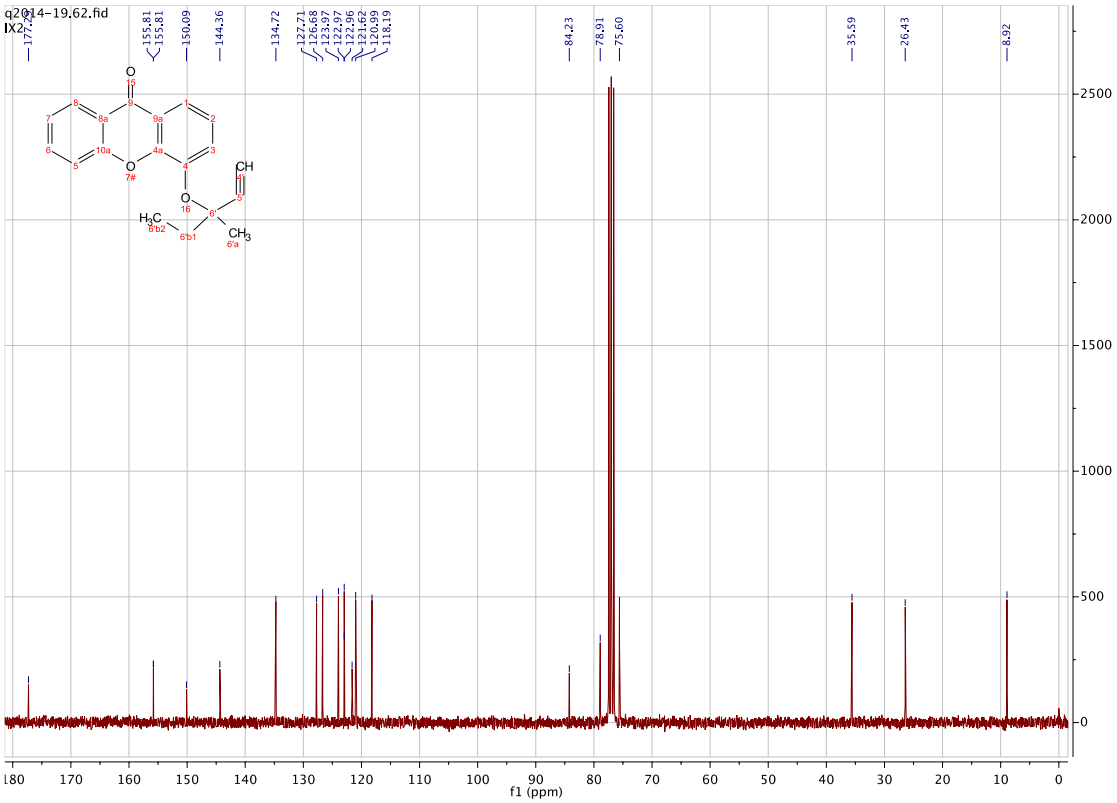

Figure 2. ^{13}C NMR spectrum of compound 8.

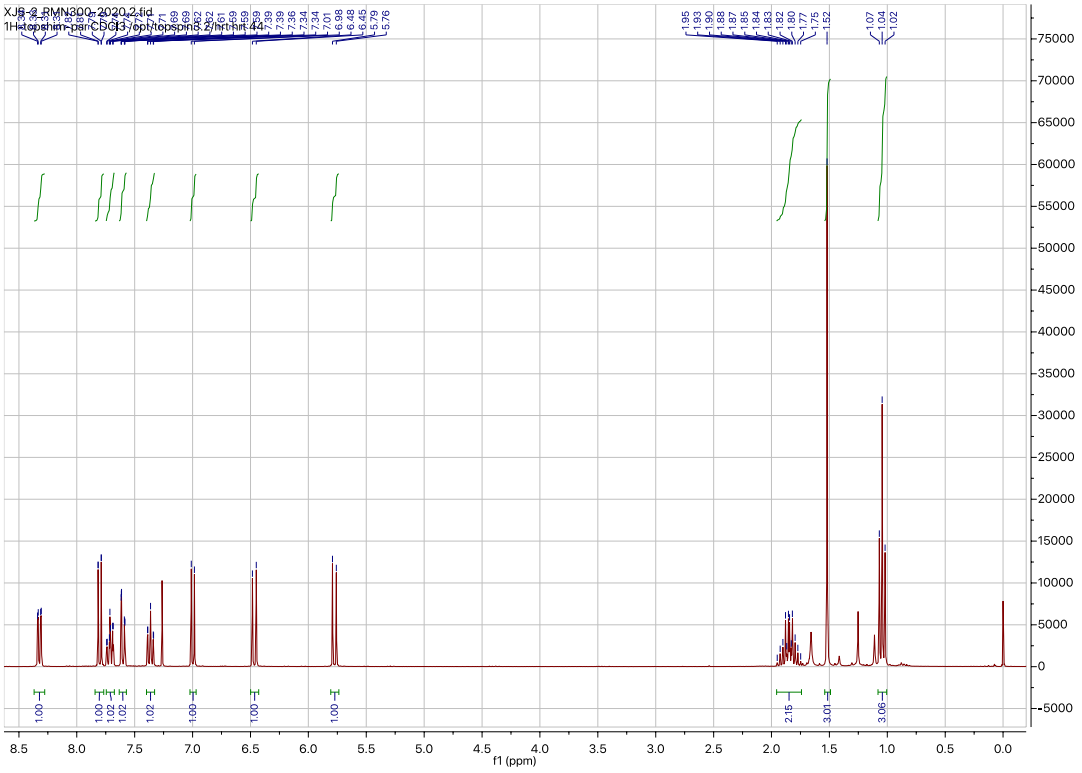

Figure 3. ^{1}H NMR spectrum of compound 2.

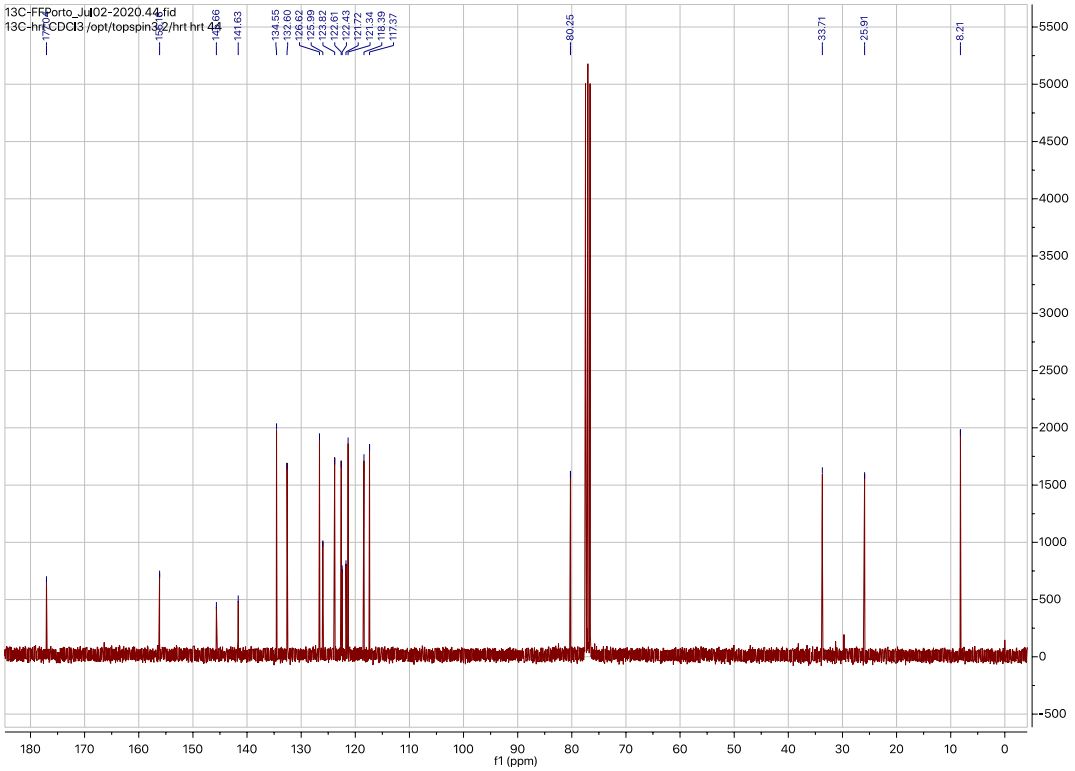

Figure 4. ^{13}C NMR spectrum of compound 2.

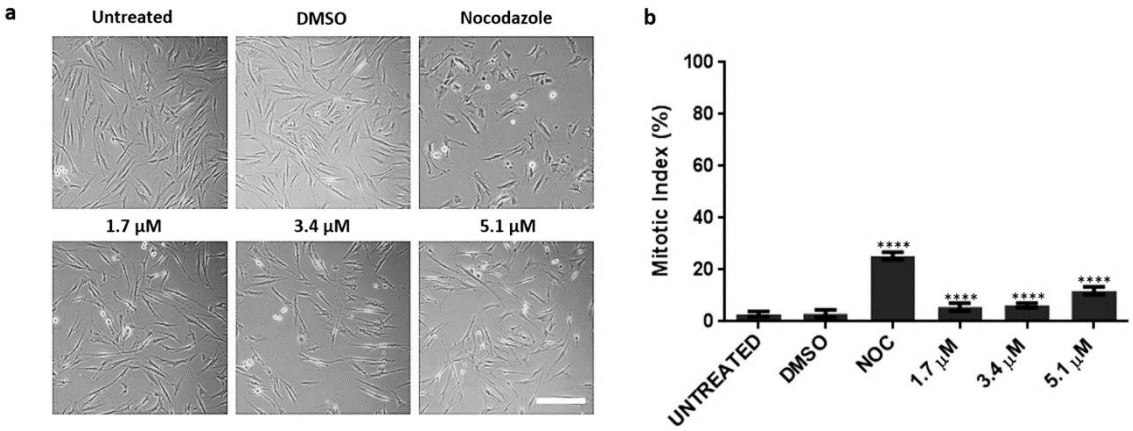

Figure S5: Lung non-tumor HPAEpiC cells are less sensitive to the antimitotic activity of pyranoxanthone **2** (PX2). (**a**) Representative phase contrast microscopy images, after 16 h treatment with pyranoxanthone 2 (PX2), at indicated concentrations. Nocodazole was used as a positive control. DMSO was used as a compound solvent control. Bar, 10 μm. (**b**) Mitotic index graph with statistical relevance of * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ by unpaired t-test.

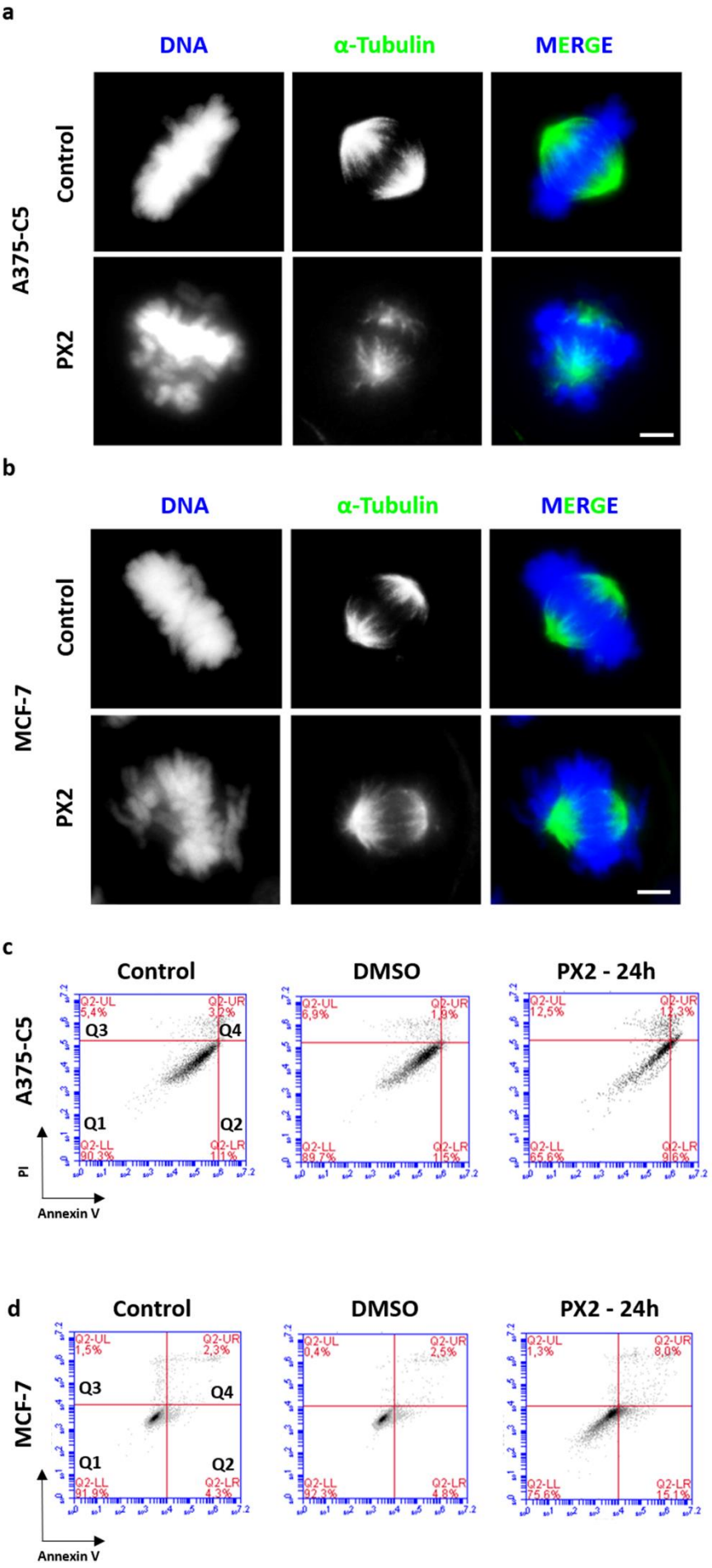
a
DNA
α-Tubulin
MERGE
A375-C5
Control
PX2
b
DNA
α-Tubulin
MERGE
MCF-7
Control
PX2
c
Control
DMSO
PX2 - 24h
A375-C5
Q1
Q2
Q3
Q4
PI
Annexin V
d
Control
DMSO
PX2 - 24h
MCF-7
Q1
Q2
Q3
Q4
Annexin V

Figure 6. Treatment with pyranoxanthone **2** (PX2) induces chromosome misalignment phenotype and leads to apoptotic cell death in MCF-7 (**a** and **c**) and A375-C5 (**b** and **d**) cancer cell lines. (**a** and **b**) Immunofluorescence images of untreated cell (Top), in metaphase, with all chromosomes aligned at equatorial zone, and PX2-treated cell (Bottom), arrested in prometaphase-like state, showing several misaligned chromosomes. Microtubules (green) were stained with anti-α-tubulin antibody and DNA (blue) with DAPI. Bar, 5 μm. (c and d) Flow cytometry analysis of apoptosis by Annexin V/PI co-staining, 24 hours after PX2 treatment. The quadrants Q were defined as Q1 = live (Annexin V- and PI-negative), Q2 = early stage of apoptosis (Annexin V-positive/PI-negative), Q3 = late stage of apoptosis (Annexin V- and PI-positive) and Q4 = necrosis (Annexin V-negative/PI-positive).