A novel microtubule disruptor exerts broad anticancer efficacy with a tolerable safety profile

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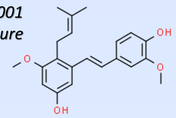
Background

Initial evaluation of the prenylated hydroxy-stilbene isolated from bee propolis, AUS_001, in the NCI 60 human cell line anti-cancer screen showed significant growth inhibitory effects with the most robust being observed in leukemia, central nervous system and breast cancer cell lines.

AUS_001 structure



Aim

The aim of the current study was to further assess the potency of AUS_001 *in vitro* and investigate its safety profile and mechanism of action.

Results

1. AUS_001 exerts broad *in vitro* anti-cancer activity

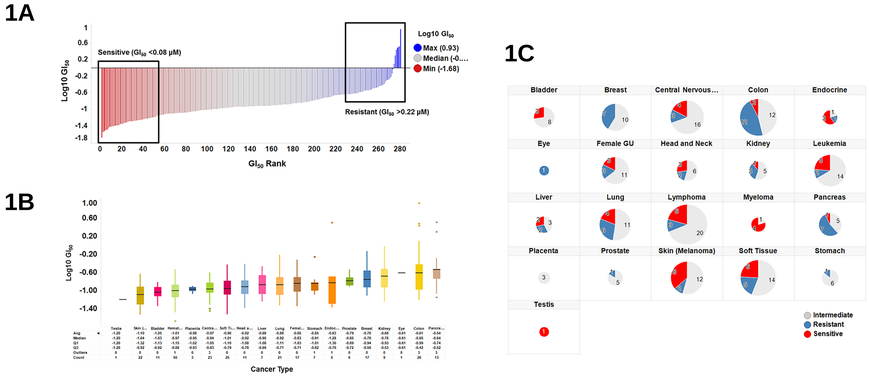


Figure 1. The proliferation response of cancer cell lines to AUS_001 treatments as assessed by high-content fluorescence imaging (Eurofins Panlabs). **(A)** All cell lines with cell count GI50 <0.08 uM were classified as sensitive to AUS_001, while those with GI50 >0.22 uM were classified as resistant. **(B)** Distribution of log10 concentration for 50% of maximal inhibition of cell growth (GI50) values from cell lines of the 19 most sensitive cancer types (site of primary tumor). **(C)** Breakdown of cell lines classified as sensitive, resistant, or intermediate within each cancer type represented in the OncoPanel™ Profiling Analysis. Pie sizes are relative to the total number of cell lines for each tissue/tumor type.

2. Orally administered AUS_001 is well-tolerated *in vivo*

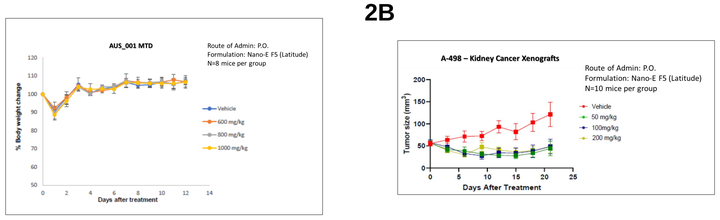
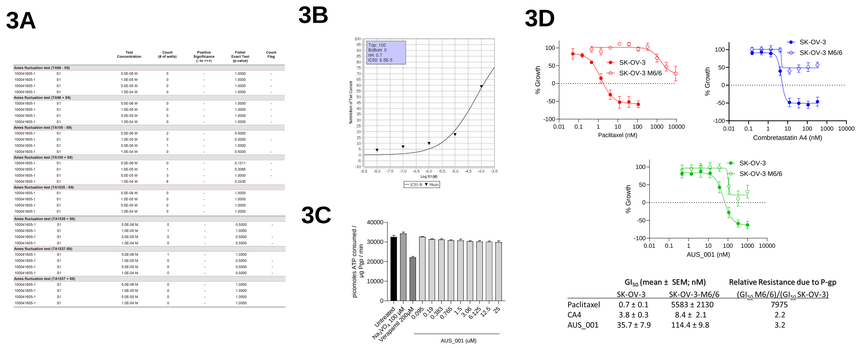


Figure 2. (A) BALB/c mice were treated once daily with 3 different AUS_001 doses (600, 800, 1000 mg/kg). Mice tolerated up to 1000 mg/kg of AUS_001 without significant changes in clinical outlook or weight for at least 14 days. **(B)** BALB/c nude mice were treated every 3 days with 3 different AUS_001 doses (50, 100, 200 mg/kg). AUS_001 presented significant tumor growth delay in all doses.

3. Predictive toxicology *in vitro* screening of AUS_001 supports a strong safety profile



	SK-OV-3	SK-OV-3-M6/6	Relative Resistance due to P-gp (GI50-M6/6/GI50-SK-OV-3)
Paclitaxel	0.7 ± 0.1	5583 ± 2130	7975
CA4	3.8 ± 0.3	8.4 ± 2.1	2.2
AUS_001	35.7 ± 7.9	114.4 ± 9.8	3.2

Figure 3. (A) Reversions of the histidine mutation as judged by the ratio of OD430/OD570 being greater than 1.0 are recorded as positive counts. **(B)** AUS_001 on hERG IC50 (hERG-CHO, automated patch-clamp). **(C)** AUS_001 is poor substrate of P-gp and does not inhibit drug-stimulated P-gp ATPase activity **(D)** P-gp overexpressing cellular models do not confer resistance to AUS_001.

4. Classification of AUS_001 as an aneugen

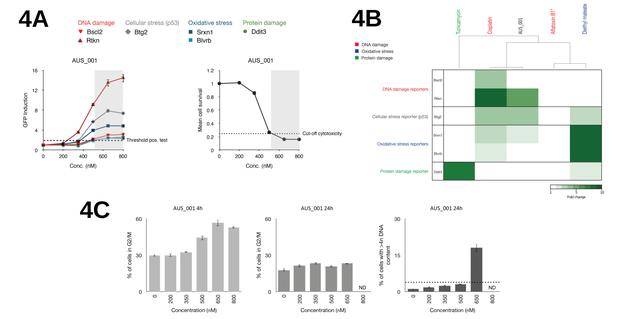


Figure 4. Genotoxicity evaluation of AUS_001 was performed using the ToxTracker ACE Assay. **(A)** Differential induction of GFP reporters was determined using flow cytometry. **(B)** Heatmap of reporter inductions at 50% cytotoxicity including clustering. **(C)** Accumulation of cells in G2/M after 4 h of exposure and the appearance of aneuploid cells after 24 h of exposure were observed.

5. AUS_001 acts as a tubulin destabilizing agent

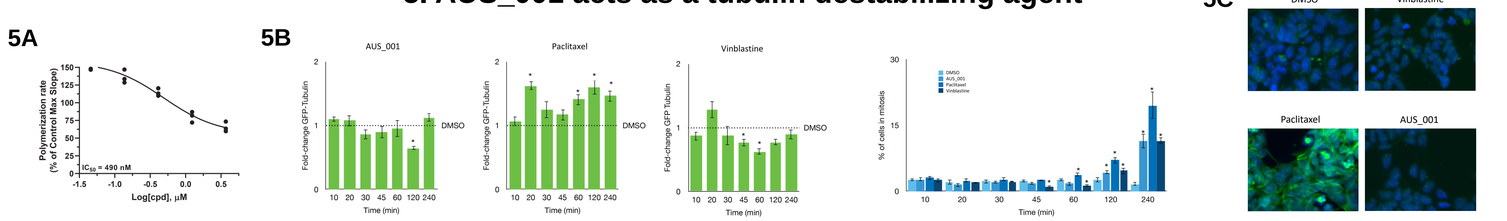


Figure 5. (A) Fluorescence-based assays were employed to assess blockage of neuronal microtubule polymerization by AUS_001. **(B)** Mouse embryonic stem cells (mES) containing a GFP-Tubulin reporter were used to visualize the microtubules after exposure to the test substance. AUS_001 resulted in a decrease in the GFP-tubulin signal and an increase in the percentage of mitotic cells. **(C)** Representative microscopy images of mES cells upon 120 min of compound treatments at 20x.

Conclusions

- ***Our studies have elaborated the mechanism of AUS_001 as an inhibitor of tubulin polymerization.***
- ***The favorable safety profile of AUS_001, along with its ability to circumvent Pgp-mediated multidrug resistance, provides potential for efficacy against multiple cancers.***

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