Supplementary information

Supplementary information contains 8 supplementary figures.

Supplementary Figure 1: The chemical structure of Paeonol derivatives. (A) The structure of Paeonol-2, (B) Paeonol-3, (C) Paeonol-4, (D) Paeonol-5, (E) Paeonol-6. (F) Crystal violet assay of cell viability of the indicated cell lines after treatment with Paeonol-2, Paeonol-3 (G), Paeonol-4 (H), Paeonol-5 (I) or Paeonol-6 (J) at the indicated concentrations. Panel F, G, H, I, and J represent mean ± SD of three independent experiments.

Supplementary Figure 2: Natural compounds induce apoptosis in colon cancer cell lines. (A) Pae (300 μM) and Pae1 (10 μM) were added to BjhTERT, HCT116, HT29, and SW48 cell lines. After 48 h, the cells were stained with DAPI to quantify dead cells by flow cytometry. (B) The cells were treated as in (A) and stained with Annexin V, and the apoptosis was quantified by flow cytometry. Panels A and B show single experiments from three independent replicates.

Supplementary Figure 3: Natural compounds induce apoptosis in a caspase-dependent manner in CRC lines. (A) CRC cell lines were treated with either aMan1 or Pae1 in the presence and absence of pan-caspase inhibitor z-vad-fmk for 72 h. After 72 h, the cells were stained with Annexin V and DAPI to quantify apoptosis and dead cells, respectively, by flow cytometry. Quantification of the apoptotic cells (in percentage) aMan1 (B) Pae1 (C) in the indicated different cell lines with the indicated treatments. Panels B and C represent mean ± SD of three independent experiments.

Supplementary Figure 4: aMan1 and Pae1 induce cellular metabolic stress in SW48 cell lines. (A and B) Principal components analysis (PCA) of BjhTERT and SW48 cell lines treated with either DMSO, aMan1 or Pae1. (C and D) Gene set enrichment analysis of the indicated canonical pathways (red highlighted pathways from figure 4C and D) in BjhTERT and SW48 cell lines treated with either DMSO, aMan1 or Pae1. The reported p-value is calculated using Wilcoxon signed-rank test (2-tail).

Supplementary Figure 5: aMan1 and Pae1 differentially induces expression of genes in SW48 cells. SW48 cells were treated with aMan1 (A) and Pae1 (B). Then, the expression of selected genes from RNA seq data were confirmed by qRT-PCR. The statistical significance was analysed by two-way ANOVA followed by Sidak’s multiple comparisons test. *adjP≤0.05, ** adjP≤0.01, *** adjP≤0.001, and ****adjP≤0.0001.

Supplementary Figure 6: aMan1 and Pae1 induce differentially metabolic stress in TP53 knockout HCT116 cell lines. (A) Protein level of p53 in HCT116 TP53_WT and TP53_KO was determined by Western blotting using p53 antibody (A upper panel), and beta-actin as a loading control (A lower panel). (B) Principal components analysis (PCA) of aMan1 or Pae1 treatment in HCT116 TP53_WT and TP53_KO cell lines. (C) Heat map of TP53, P21, and P15INK4b expression in aMan1, Pae1 or DMSO treated HCT116 TP53_WT and TP53_KO cell lines. (D) Gene set enrichment analysis of the indicated gene sets (form 4E) in HCT116_TP53_WT and TP53_KO cell lines treated with DMSO, aMan1 or Pae1. Panel A indicate single experiment from three independent replicates.

Supplementary Figure 7: aMan1 and Pae1 induce cellular metabolic stress largely in TP53 knockout HCT116 cell lines. (A and B) Gene set enrichment analysis of the indicated canonical pathways in HCT116_TP53_WT or TP53_KO treated with either DMSO, aMan1 or Pae1.
Supplementary Figure 8: Natural compounds induce cell death in organoids derived from cancer but not from healthy tissue. (A) Irinotecan (3, 6, and, 12 μM), aMan (25, 50, and 100 μM), aMan1 (25, 50, and 100 μM), Pae (100, 200, and 400 μM), or Pae1 (7.5, 15, and 30) was added to organoids derived from tumor or healthy colon epithelium of the same patient. After 48 h, the organoids were resuspended into single cells. Following staining with DAPI, the cell death was determined by flow cytometry. (B) Quantification of the dead cells (in percentage) in healthy and cancer organoid cells treated with aMan1 and Pae1. Panel A represents a single experiment from three independent replicates. Panel B shows the mean ± SD of three independent experiments. *P≤0.05, ** P≤0.01, and *** P≤0.001. The statistical significance between two groups were analysed by a two-tail unpaired T-test.
A
\[
\text{Paeonol-2} \quad (E)-1-((2\text{-hydroxy-4-methoxyphenyl})-3-(4-(\text{Piperidine-1-yl})\text{phenyl})\text{prop-2-en-1-one})
\]

B
\[
\text{Paeonol-3} \quad (E)-1-((2\text{-hydroxy-4-methoxyphenyl})-3-(4-\text{morpholinophenyl})\text{prop-2-en-1-one})
\]

C
\[
\text{Paeonol-4} \quad (E)-1-((2\text{-hydroxy-4-methoxyphenyl})-3-(4-(\text{pyrrolidin-1-yl})\text{phenyl})\text{prop-2-en-1-one})
\]

D
\[
\text{Paeonol-5} \quad (E)-1-((2\text{-hydroxy-4-methoxyphenyl})-3-(4-(\text{methylamino})\text{phenyl})\text{prop-2-en-1-one})
\]

E
\[
\text{Paeonol-6} \quad (E)-1-((2\text{-hydroxy-4-methoxyphenyl})-3\text{-phenylprop-2-en-1-one})
\]

F

G

H

I

J

Supplementary Figure 1
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
Supplementary Figure 5