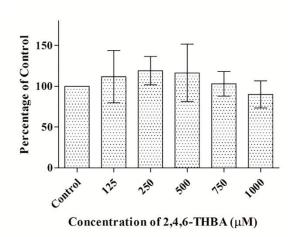




# The Flavonoid Metabolite 2,4,6-Trihydroxybenzoic Acid Is a CDK Inhibitor and an Anti-Proliferative Agent: A Potential Role in Cancer Prevention

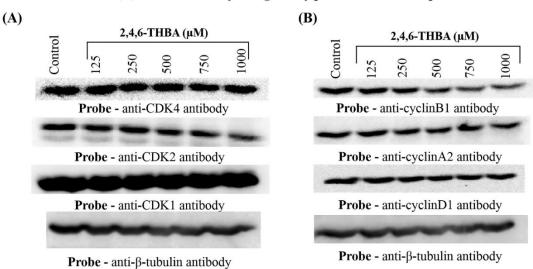
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### **Supplementary Material**



#### Effect of 2,4,6-THBA on HCT-116 cell proliferation

**Figure S1.** Effect of 2,4,6-THBA on HCT-116 cell proliferation. Cells were treated with 2,4,6-THBA for 72 h. Floating cells were collected, the adherent cells were washed, trypsinized and pooled with the floating cells and counted.



Effect of 2,4,6-THBA on cell cycle regulatory proteins in SLC5A8-pLVX cells

**Figure S2.** Western Blot analysis showing the levels of various CDKs and cyclins in SLC5A8-pLVX cells in response to 2,4,6-THBA.

# Expression of SLC5A8 in different cell lines



**Figure S3.** Western Blot demonstrating the expression levels of SLC5A8 protein in HCT-116, HT-29, MDA-MB-231 and SLC5A8-pLVX cell lines. Despite the expression of SLC5A8 in HCT-116 cells, uptake of 2,4,6-THBA was not observed (Figure 6A). In contrast, uptake was observed in SLC5A8-pLVX cells expressing the functional transporter. Consistent with the low expression of SLC5A8 in MDA-MB-231cells, low levels of 2,4,6-THBA was observed in these cells.

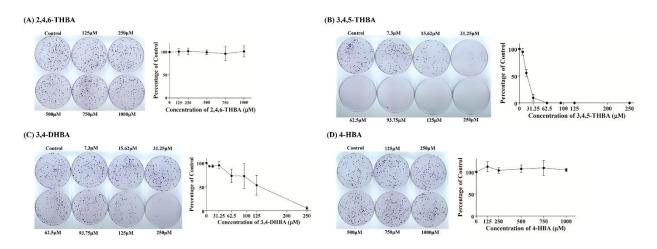
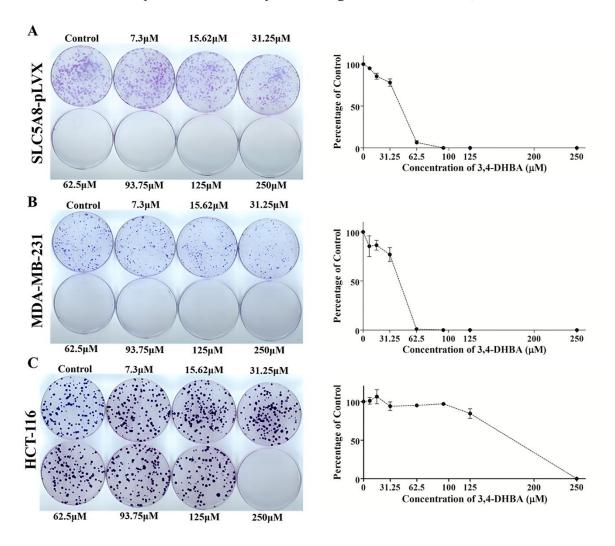
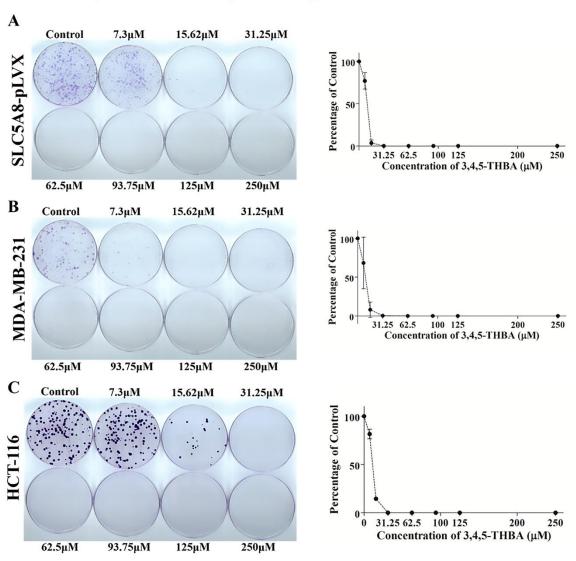


Figure S4. Effect of 2,4,6-THBA (A), 3,4,5-THBA (B), 3,4-DHBA (C) and 4-HBA (D) on colony formation in HT-29 cells.



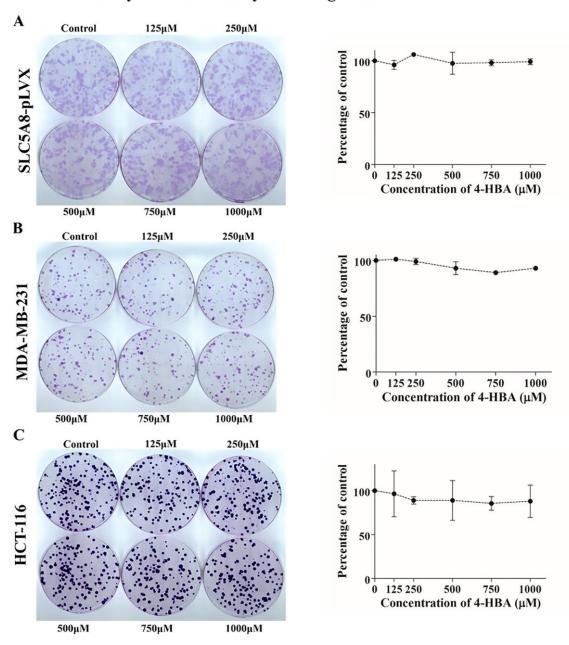
### Colony formation assay following treatment with 3,4-DHBA

**Figure S5.** Effect of different concentrations of 3,4-DHBA on colony formation in SLC5A8-PLVX (**A**), MDA-MB-231 (**B**), and HCT-116 (**C**) cells.



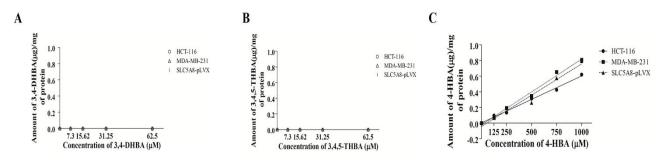
**Figure S6.** Effect of different concentrations of 3,4,5-THBA on colony formation in SLC5A8-PLVX (**A**), MDA-MB-231 (**B**), and HCT-116 (**C**) cells.

## Colony formation assay following treatment with 3,4,5-THBA



### Colony formation assay following treatment with 4-HBA

**Figure S7.** Effect of different concentrations of 4-HBA on colony formation in SLC5A8-PLVX (**A**), MDA-MB-231 (**B**), and HCT-116 (**C**) cells.



**Figure S8.** HPLC analysis showing the cellular uptake in SLC5A8-pLVX, MDA-MB-231 and HCT-116 cells following incubation with 3,4-DHBA (**A**), 3,4,5-THBA (**B**) and 4-HBA (**C**) in the cytosol.