Synthesis and Evaluation of Oleanolic Acid Carbamate Against Breast Cancer Cells, in vitro

Anjana Sinha[1], Subhasis Das[2], Saumya Roy[3], Vasudeva Naidu Sagi[3], Xianzhong Wu[4], Yue-Ming Li[4], Ajay Rana[2], Subhash C. Sinha[1,3,5]

[1]Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY, USA. [2]Department of Surgery, College of Medicine, The University of Illinois at Chicago, Chicago, IL. [3] Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA. [4]Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [5]Appel Alzheimer's Disease Research Institute, Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA
Email: arana@uic.edu or sus2044@med.cornell.edu

Abstract.
Complex natural products found abundantly in numerous edible and medicinal plants are rich source of bioactive compounds that can readily be obtained through simple chemical transformation and derivatization. Many of these compounds, such as Oleanolic acid (OA), **1** (**Fig. 1A**), a pentacyclic triterpene natural product (NP), are too complex to obtain through *de novo* synthesis for general usage. Instead, OA has been obtained from plant sources for commercial usages and converted to many modified OA analogs and derivatives that are known to possess anti-Cancer properties. These compounds inhibit tumor growth through multiple pathways, including inhibition of Notch signaling. The Herceptin-resistant breast cancer cells are known to overexpress Notch1 and to increase tumor growth through Notch1 signaling and Akt phosphorylation. Notch1 undergoes γ-secretase cleavage to produce Notch intracellular domain (NICD) as the signaling molecule. We synthesized and evaluated several OA carbamate derivatives *in vitro* using HeLa cell membranes that contain active γ-secretase enzyme and using BT474 cell model of Herceptin resistant breast cancer. Here, we report that all OA carbamates tested at 10 micromolar concentration using HeLa cell membranes inhibited Notch cleavage considerably (between 30 to 70% inhibition observed). Among six compounds tested with the BT474 cell model, two compounds, **2** and **3**, inhibited the growth of BT474 cells likely through Notch/Akt pathway, while the other four compounds (structure not shown) caused toxicity to both cancer and normal cells in a syngeneic cell model through an unknown mechanism.

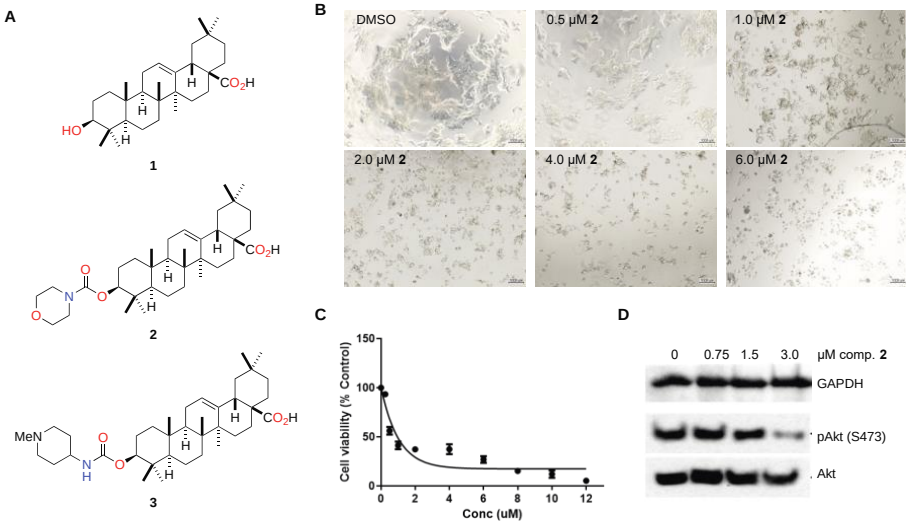


Fig. 1. Structure and in vitro activities. Shown are structure of OA and OA carbamates (A) and effects of compound **2** on cell viability (B-C) and on Akt phosphorylation (D) as determined using Herceptin resistant BT474 breast cancer cells.