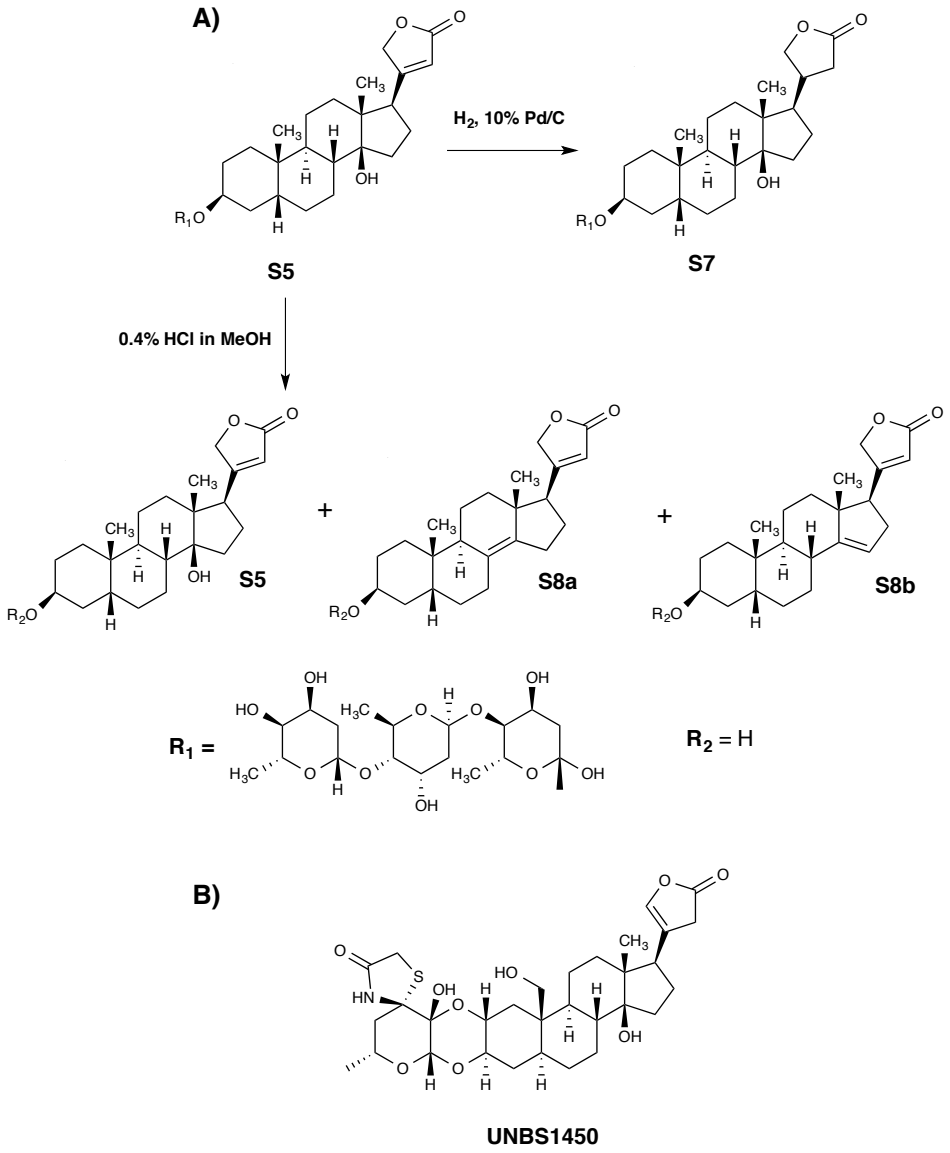
Cardiac glycoside activities link Na^+/K^+ ATPase ion-transport to breast cancer cell migration via correlarive SAR

Anniefer N. Magpusao, George Omolloh, Joshua Johnson, José Gascón, Mark W. Peczuh* and Gabriel Fenteany*

Department of Chemistry, University of Connecticut, 55 N. Eagleville Road, U3060, Storrs, CT 06269, USA

Supporting Information



Scheme S1. (A) Semi synthesis of digitoxin analogs S**5**-S**8**. (B) Structure of UNBS1450.

Inhibition of MDA-MB-231 breast cancer cell migration and the Na^+/K^+ ATPase by digitoxin analogs - To explore if the correlative SAR results for ouabain apply to other members of the cardiac glycoside family of natural products, analogs of hydrophobic digitalis cardiac glycoside digitoxin, were synthesized employing the same transformation as that used for **1, with the exemption of** the representative analog containing modification on steroid core (**S8a, S8b**). These analogs were found to be a

mixture of alkene isomers produced from dehydration of C14 hydroxyl obtained as a side product from the deglycosylation of digitoxin (Scheme S1).

The correlative SAR for the digitoxin derivatives (Table S1, Figure S1) was similar to that for the ouabain derivatives. The same SAR profile for inhibition of cell migration was observed for inhibition of the Na^+/K^+ ATPase activity (Figure S1a). These results imply that as with ouabain, the sugar is not very important and the steroid core is, in contrast, important for the inhibition of both cell migration and Na^+/K^+ ATPase activity. However, it appears that modifying the structure of digitoxin has more pronounced effects in its ability to inhibit Na^+/K^+ ATPase than in its ability to inhibit cell migration as shown by the decrease in potency of digitoxin analogs for the two assays (Figure S1a). The IC_{50} values of digitoxin for antimigratory and Na^+/K^+ ATPase inhibitory activities correlate very well. The strong correlation between the SAR profiles of digitoxin in both cell migration and Na^+/K^+ ATPase inhibition assays corroborates the conclusion from the correlative SAR results for the ouabain analogs that the observed antimigratoy effect of cardiac glycosides in MDA-MB-231 breast cancer cells is directly related to the inhibition of Na^+/K^+ ATPase activity.

Table S1. IC_{50} values for the inhibitory activity of digitoxin and analogs in the Na^+/K^+ ATPase assay and the cell migration assay.

Compound	Na^+/K^+ ATPase	Cell migration
S5	0.005	0.046
S6	0.097	0.260
S7	0.097	0.260
S8a and S8b	3.2	10.8

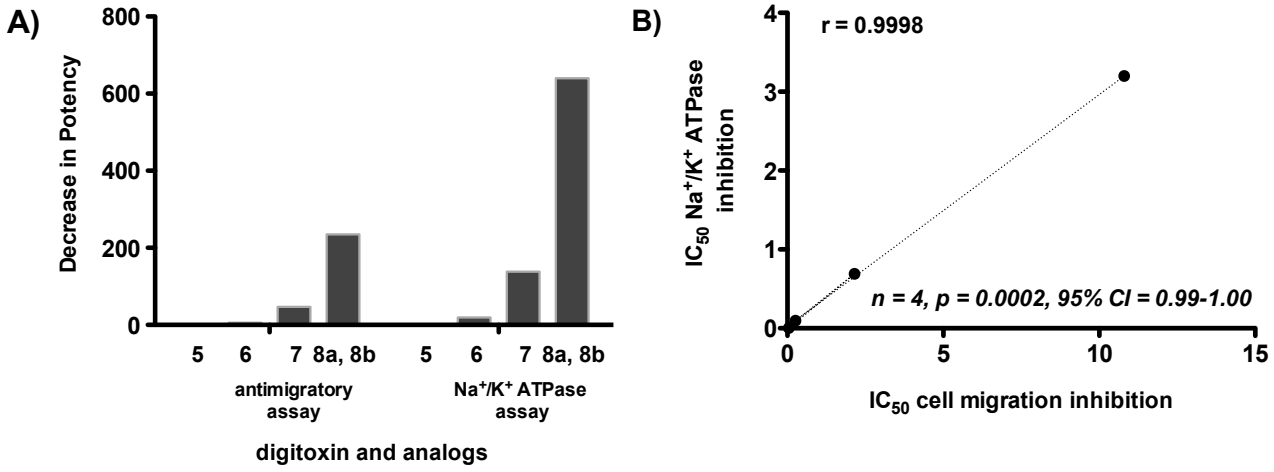


Figure S1. The (A) SAR profiles (decrease in potency of digitoxin analogs in the antimigratory assay and the Na^+/K^+ ATPase assays are very similar. (B) Pearson correlation plot of IC_{50} values (Correlative SAR) of digitoxin and analogs in the antimigratory assay and the Na^+/K^+ ATPase assay showed a very strong correlation implying that the inhibition of breast cancer cell migration is directly related to inhibition of Na^+/K^+ ATPase ion transport function.

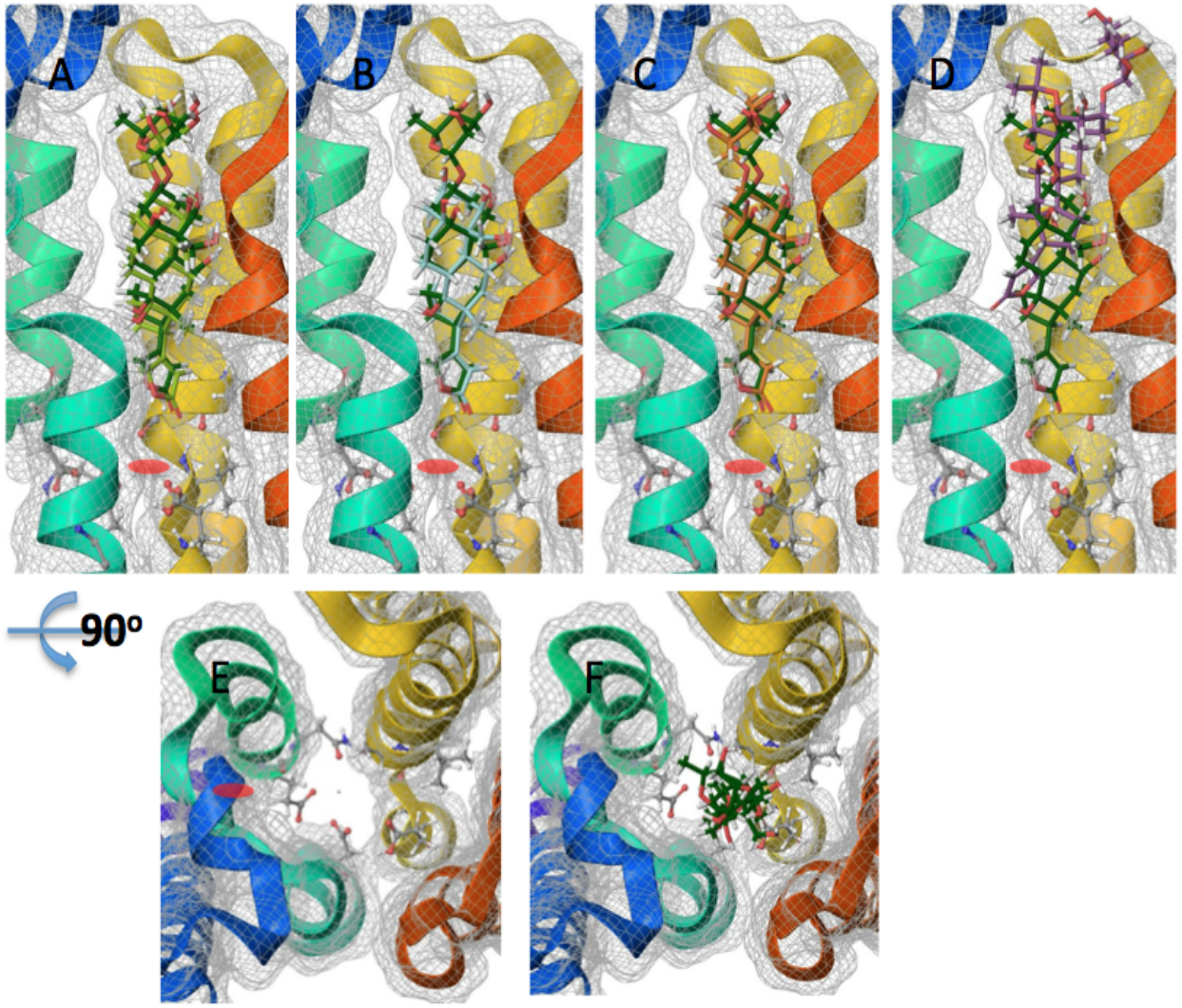


Figure S2. (**A**) Overlayed Crystal structure of ouabain with docked ouabain. (**B**) Overlayed Crystal structure of ouabain with docked analog **2** (**C**) Overlayed Crystal structure of ouabain with docked analog **3**. (**D**) Overlayed crystal structure of ouabain with docked analog **4**. (**E**) extracellular view of cation permeation path and residues involved in K^+ binding site (**F**) extracellular view of cation permeation path with ouabain.

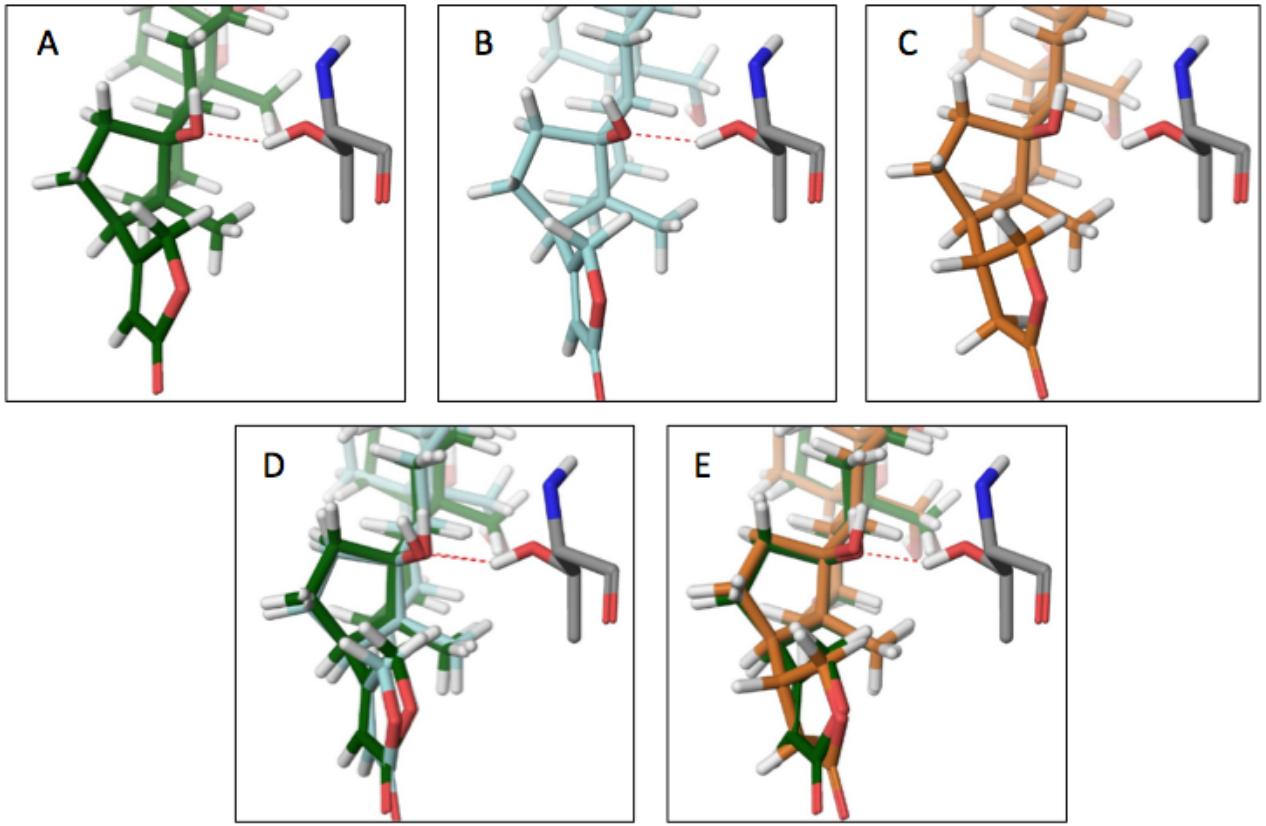


Figure S3. One of the critical interactions for inhibition of Na^+/K^+ ATPase by cardiac glycoside is the interaction between the well conserved T797 and C14-OH of oubain (**A**). This interaction is similarly accessible in (**B**) analog **2** but is not accessible in (**C**) analog **3,** in which the C14-OH was shifted in an orientation that might have resulted to a less ideal H-bonding angle with T797. This shift in orientation in analog **3** could be due to the hydrogenation of the lactone ring olefin of ouabian. The effect of modifications (deglycosylation (analog **2**) and hydrogenation (analog **3**)) on cardiac glycoside to this critical hydrogen-bonding interaction might explain the 5x and 25x decrease in potency for analogs **2** and **3** respectively.

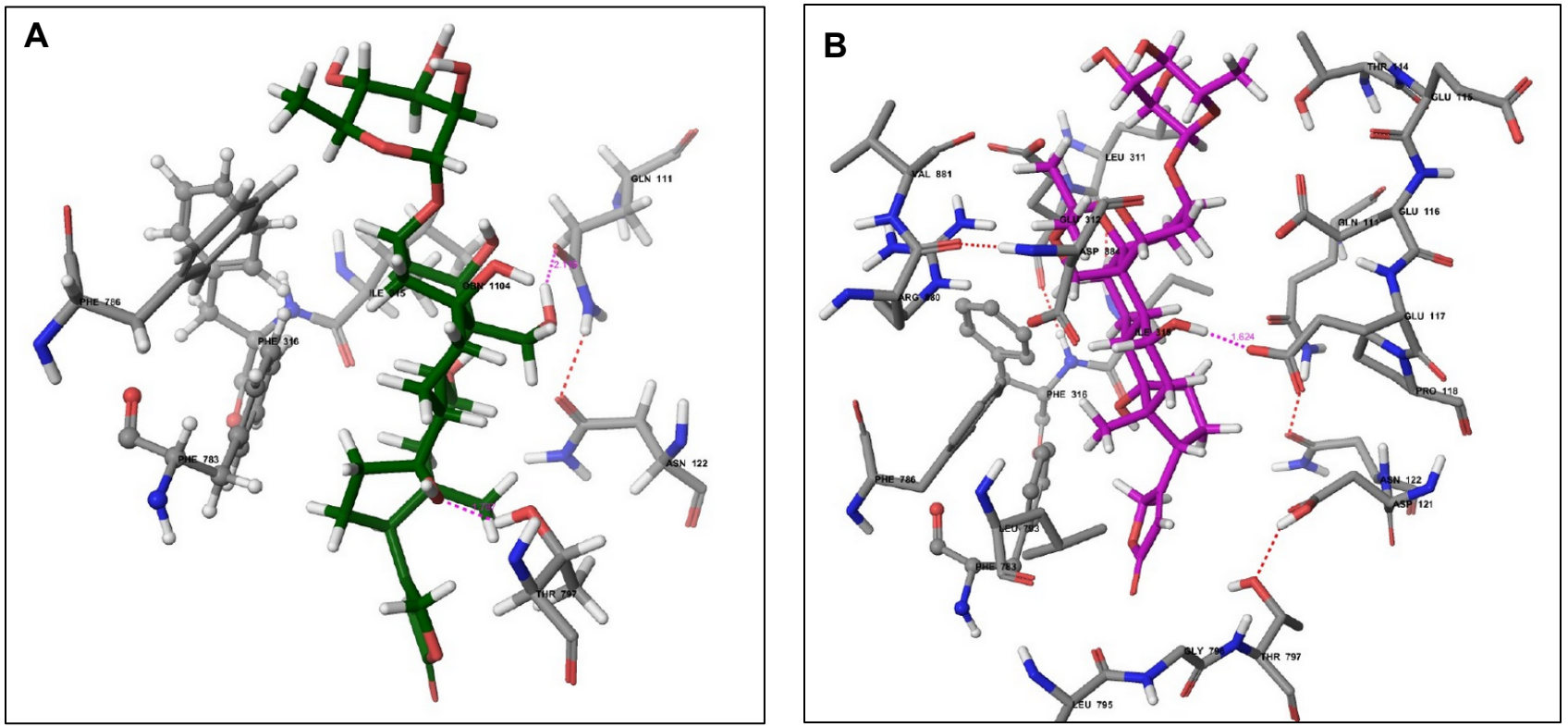


Figure S4. Ouabain (**A**) and analog **4** (**B**) hydrophobic and H-bonding interactions with amino acid residues in Na^+/K^+ ATPAse. The orientation of analog 4 does not allow for H-bonding interactions with Q111 and T797, residues identified by mutagenesis studies to be critical for ouabain's inhibition of Na^+/K^+ ATPAse. The high binding energy of analog 4 is however possibly stabilized by hydrophobic interactions of I315, F316, F783 with the steroid rings C and D, and H-bonding interaction of C19-hydroxyl with E117 instead of Q111.

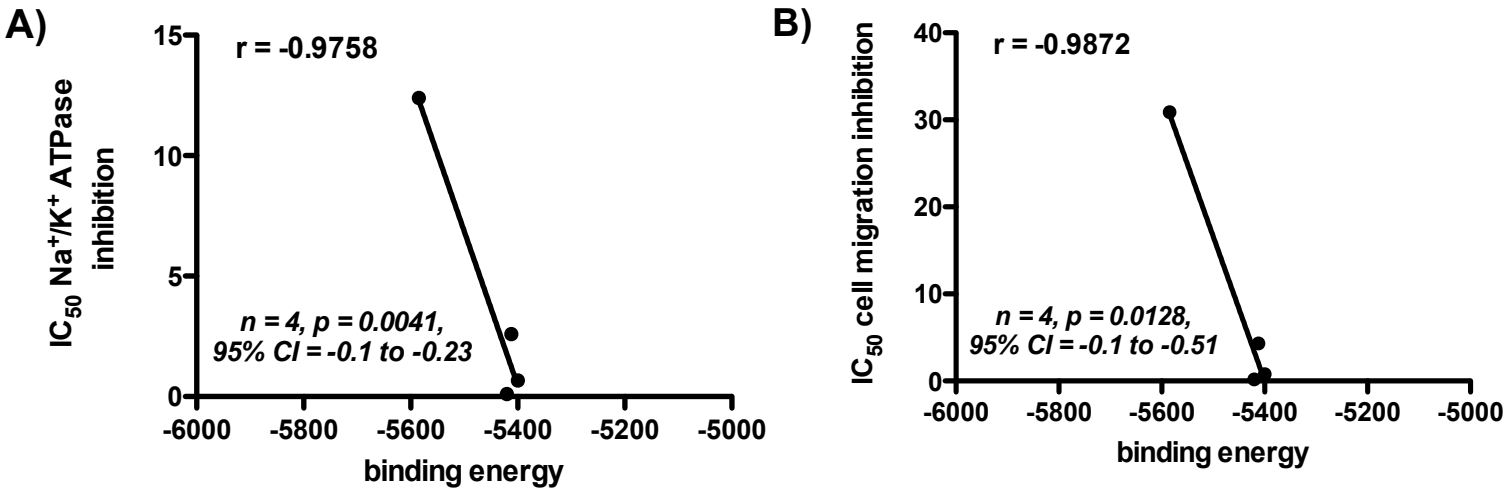


Figure S5. (**A**) Pearson correlation plot for IC_{50} values of ouabain and analogs in the Na^+/K^+ ATPase assay and the binding energy. (**B**) Pearson correlation plot for IC_{50} values of ouabain and analogs in the cell migration assay and the binding energy.

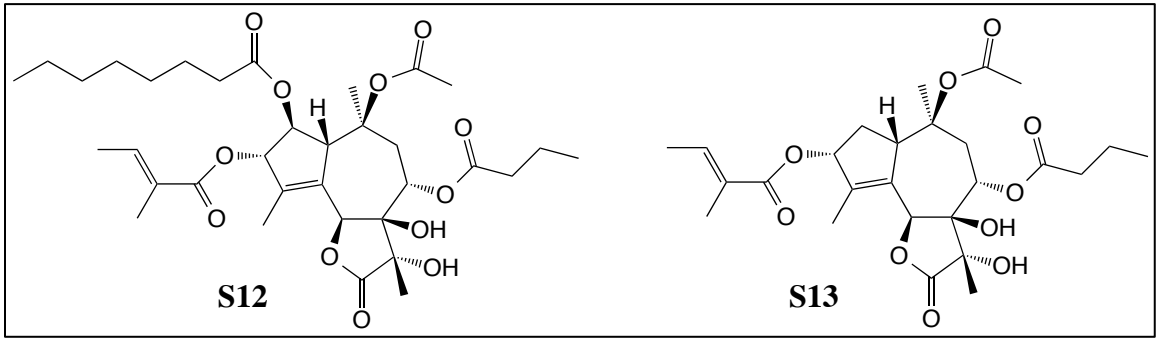


Figure S6. Thapsigargin (**S12**) and analog (**S13**) were found to inhibit the migration of MDA-MB-231 breast cancer cell from chemical genetic screening. Both **S12** and **S13** were toxic above 10 μM (Maximum Lethal Concentration) in MDA-MD-231 and have antimigratory activity above 400 nM (Minimum Inhibitory Concentration).