**High-Throughput Screening in Drug Discovery:**

**Identification and Characterization of Novel Inhibitors of ANO1**

Wan Namkung

College of Pharmacy and Yonsei, Institute of Pharmaceutical Sciences, Yonsei University, 85 Songdogwahak-ro, Yeonsu-gu, Incheon 21983, Republic of Korea.

**ABSTRACT**

High-Throughput Screening (HTS) offers an innovative approach that can accelerate the identification of new drug candidates, unlock the secrets of biological pathways, and revolutionize materials design. This approach involves the rapid and systematic testing of large libraries of compounds against specific targets or biological systems, utilizing advanced automation, robotics, and data analysis tools. Through this systematic evaluation, researchers can identify leads for further investigation and ultimately speed up the development of new drugs. Anoctamin 1 (ANO1)/transmembrane protein 16A (TMEM16A), a calcium-activated chloride channel (CaCC), is widely expressed in various cell types including airway and intestinal epithelial cells, smooth muscle cells, intestinal pacemaker cells, sensory neurons, and several tumors, and known to be involved in many physiological functions such as fluid secretion, smooth muscle contraction, nociception and cancer progression. We have performed a cell-based HTS to identify highly potent and specific small-molecule modulators of ANO1. Screening of ~160,000 synthetic small molecules revealed several novel ANO1 inhibitors and activators that fully blocked or activated ANO1 channel activity with an IC50 < 3 μM. Recently, we developed the first nanomolar small-molecule inhibitors of human ANO1. Ani9 completely inhibited ANO1 chloride current with IC50 approximately 77 nM, without interfering with intracellular calcium signaling. Notably, Ani9-5f, a novel Ani9 derivatives, is very potent (IC50 = 22nM) and >1000-fold more selective for ANO1 compared to ANO2, which shares a high amino acid homology to ANO1. In addition, Ani9-5f did not affect CFTR chloride channel and epithelial Na+ channel (ENaC) function. The ANO1 inhibitors permit pharmacological dissection of ANO1/CaCC function and may be potential development candidates for drug therapy of cancer, hypertension, pain, diarrhea and asthma.