MLSCN Probe Summary

Screening Center: Vanderbilt Screening Center for GPCRs, Ion Channels, and Transporters

Grant Number: 1R03MH076402-01

Principal Investigator (PI) Name: Ming Zhou

CRISP Project Title: Molecule Modulators: Voltage-Dependent Potassium (RMI)

Specific Aim: To identify small molecule modulators of Kvβ that are cell permeable and have a Km sufficient for in vitro cellular physiology assays.

Significance: Probes developed from these efforts will aid in the understanding of the influence of Kvβ in Kv channel-controlled physiological processes such as the rhythmic beating of the heart, communication between neurons and secretion of hormones.

Rationale: Voltage-dependent potassium channels (Kv) are integral membrane proteins that, in response to membrane voltage changes, catalyze potassium ions to diffuse across the cell membrane. In a cell, Kv channels are always assembled with other proteins, and channel function is regulated by cellular environment through these associated proteins. The beta subunit (Kvβ), a potential aldo-keto reductase (AKR), attaches to the intracellular side of the Kv1 family channels. They are ubiquitous in the brain and abundantly expressed in heart, and have been implicated in control of cell excitability and in sensing cellular redox state. The long-term goal of this research is to understand the role of Kvβ in cell biology. The PI, Ming Zhou, identified two Kvβ substrates and for the first time demonstrated that Kvβ is a functional AKR. Zhou also found that the substrates, when perfused to the intracellular side of the channel, modulate channel functions only when Kvβ is co-expressed. These exciting new results led to the hypothesis that Kv1 channel functions are coupled to Kvβ enzymatic activities and thus a modulator of Kvβ would modulate the physiology of Kv1 channels.

PubChem Bioassay Identifier (AID): 634, 623, 622

Puchem Bioassay Name: Voltage-Dependent Potassium Channel Beta Subunit (KvBeta) Substrate Screen

PubChem Assay Description: Recombinantly expresssed Kvβ was combined with 10uM test compound. Substrate turnover was measured using the intrinsic fluorescence of the enzyme-bound cofactor nicotinamide adenine dinucleotide phosphate (NADPH). If Kvβ catalyzed the reduction of the test compound, NADPH was oxidized, and consequently, the fluorescence signal from NADPH decreased over time. 4-

cyanobenzaldehyde (4CY) was used as the positive control, and the compound vehicle, dimethyl sulfoxide (DMSO), was used as the negative control. The Hamamatsu FDSS kinetic plate reader equipped with 340nm excitation and 460nm emission filters was used for data collection.

Center Summary of Screen: 12,364 compounds from the 3K and 10K libraries were screened using the primary assay protocol described in the PubChem Bioassay Summary. 20 compounds were designated as primary hits based on statistical analysis of the dataset as described in PubChem. The 20 compounds were cherrypicked in house and retested. 2 compounds had confirmed activity at 30μM. The two compounds, SID 857311 and SID 856002, were tested in dose response. SID 856002, commonly known as ebselen (Figure1, Panel A), showed dose-dependent activity with an EC50 value of 25.84 to 115.7μM at 95% confidence compared to the EC50 of >250mM for the control compound 4CY. In secondary assays, ebselen was unable to oxidize NADPH independent of Kvβ (Figure 1, Panel B). Ebselen was reordered as a powder for testing in cell based electrophysiological assays (Figure 1, Panel C) that demonstrated that Kvβ is required for modulation of the potassium channel by ebselen. Based on the structure of ebselen, Darren Orton, Ph.D. hypothesized a potential mechanism by which ebselen could modulate Kvβ via covalent linkage. Dr. Orton synthesized a selenium free analog (Figure 2, Panel A) that shows dose-dependency in the cell-based system (Figure 2, Panel B). Dr. Ming is currently co-crystallizing ebselen and the analog with Kvβ to determine the atomic details of the interactions.

Center Comments: In summary, the Vanderbilt Screening Center for GPCRs, Ion Channels and Transporters in collaboration with Dr. Ming Zhou have identified a new small molecule modulator of the novel target Kvβ that influences potassium channel activity. Previous to our work, no modulators of Kvβ were known making ebselen's discovery a significant advancement in the current art. Furthermore, we have synthesized a selenium-free-ebselen-analog that noncovalently and reversibly modulates Kvβ which is allowing Dr. Zhou to hypothesize and test the mechanism of regulation. With these two tools in hand, Dr. Zhou is poised to explore the physiological role of Kvβ in Kv channel-controlled physiological processes such as the rhythmic beating of the heart, communication between neurons and secretion of hormones.

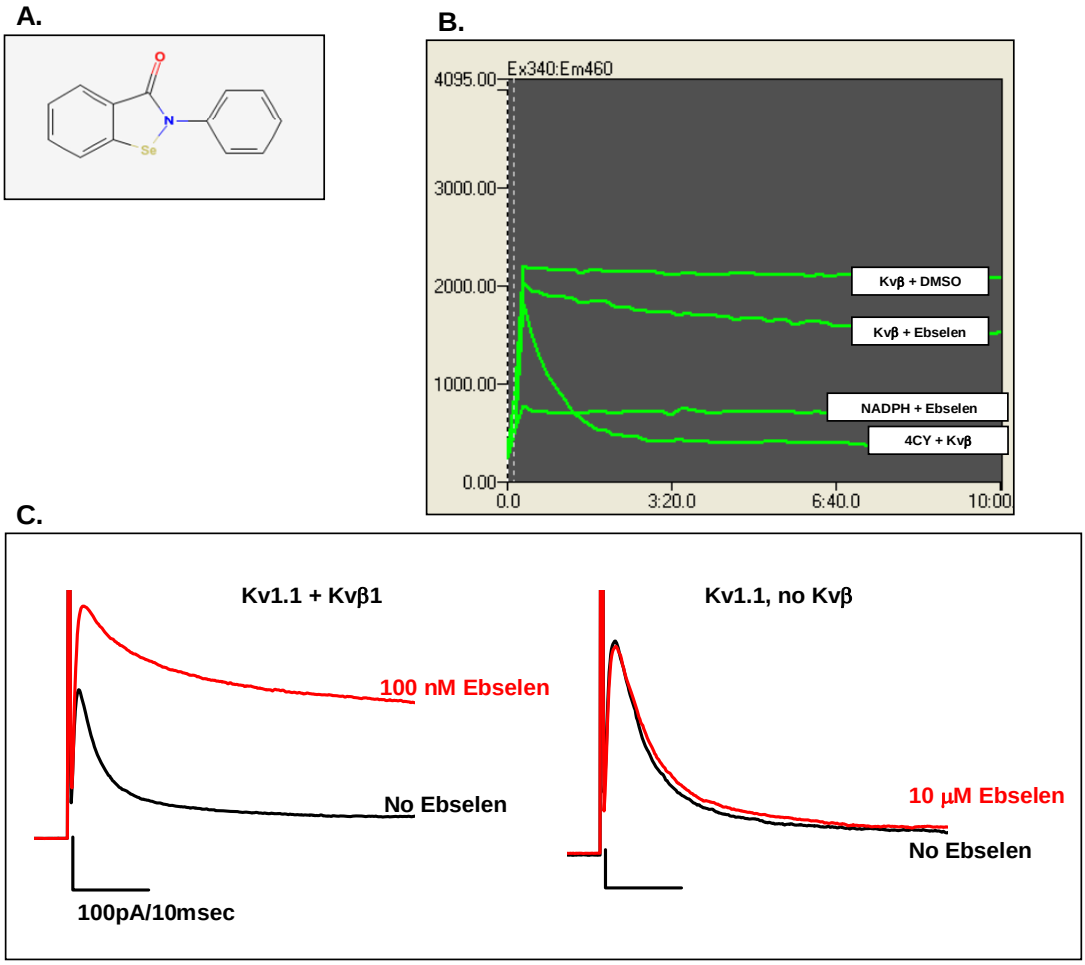
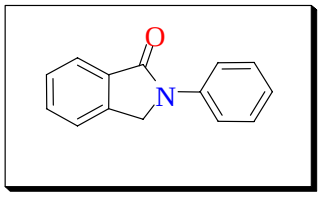


Figure 1. A. Structure of SID 856002, commonly referred to as ebselen.; **B.** Activity of Kvβ with DMSO (vehicle control for the screen), Kvβ with 30μM Ebselen, 30μM ebselen with 50μM NADPH, and Kvβ with 135μM 4CY (known substrate); **C.** Modulation of channel current in cells with (left panel) and without (right panel) Kvβ before (the black trace) and after (the red trace) bath perfusion of ebselen. Potassium current was elicited by a 60mV depolarization from a holding potential of -100mV on an inside-out patch.

A.



B.

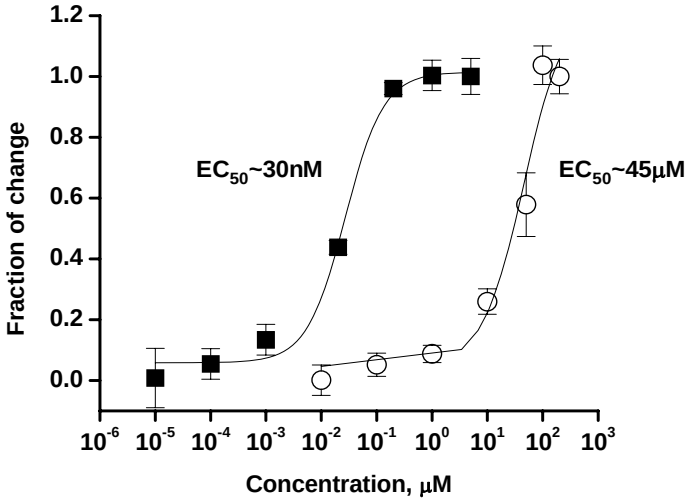


Figure 2. A. Structure of ebselen analog that was synthesized by Darren Orton, Ph.D.to aid Dr. Ming Zhou in elucidating the mechanism of ebselen's modulation of Kvβ.; **B.** Dose-dependent activity of ebselen (black square) and the analog (open circle) in cell-based assays measuring potassium channel modulation.