Case Study

Development of a High-Throughput Screening Platform for Kv2.2 Drug Discovery

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Introduction

Voltage-gated potassium (Kv) channels are transmembrane proteins that mediate the selective passage of K^+ ions across cellular membranes, playing critical roles in the regulation of neuronal and cardiac excitability, muscle contraction and hormone secretion. The Kv channel family is categorized into twelve subfamilies—Kv1 to Kv12- each consisting of multiple α-subunits that assemble into functional tetrameric channels. These channels exhibit distinct biophysical and pharmacological properties and play a critical role in modulating cellular excitability.

Kv2.2 Therapeutic Potential

A recent study identified Kv2.2 as a critical maintenance driver in SHH medulloblastoma, where it regulates potassium homeostasis, plasma membrane tension and EGFR signalling (Fan *et al.*, 2025). Loss of Kv2.2 reduces tumour cell proliferation and enhances the efficacy of SHH pathway inhibitors, highlighting its potential as a therapeutic target. Importantly, Kv2.2 is largely dispensable for normal development, offering a favourable safety profile for targeted therapies aimed at disrupting medulloblastoma growth while minimizing off-target toxicity.

Fan *et al.*, 2025, Development Cell *60*, 1-18.

Supporting Your Research

At SB Drug Discovery, we offer unparalleled expertise in ion channel drug discovery research, equipping researchers with cutting-edge tools to explore the therapeutic potential of targeting ion channels. Our extensive suite of ion channel assays and advanced electrophysiology technologies is strategically designed to cater to diverse research objectives precisely and efficiently. Providing a wide array of assay solutions, including fluorescence-based and electrophysiology assays, and leveraging a variety of platforms to accommodate different experimental formats, our innovative technologies and seasoned team of experts deliver comprehensive support for ion channel drug discovery.

Methods

HEK cells stably expressing Kv2.2 were produced by Sygnature Discovery.

Fluorescence-Based Screening Assay

Kv2.2 activity was assessed using fluorescence-based thallium assay. Following overnight incubation, media was replaced with loading buffer and plates were incubated at room temperature for 30 minutes in the dark. After pre-incubation with test compounds, 10 µL stimulus buffer was added and fluorescence was measured on a FLIPR platform (488 nm excitation/510-570 nm emission) over 5 minutes.

Electrophysiology

Automated patch-clamp recordings were conducted on the SyncroPatch 384i (Nanion Technologies) using HEK cells over-expressing Kv2.2. Cells were held at a membrane potential of -80 mV depolarized to +40 mV for 200 ms and then returned to -80 mV every 15 seconds. The maximum outward current at +40 mV was analyzed. Data analysis was performed using DataControl V3.2.1 (Nanion Technologies) and Prism V10.1 (GraphPad).

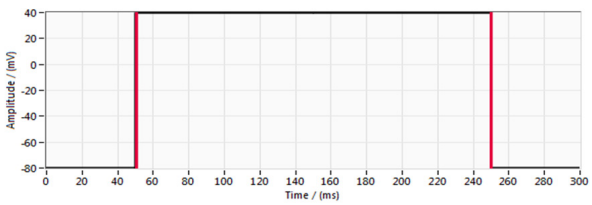


Figure 1. Voltage protocol showing Kv2.2 current elicited by using repeated peak-pulse steps, holding cells at -80 mV, stepping to +40 mV for 200 ms, then returning to -80 mV, repeated every 15 seconds.

Electrophysiological Characterization

Automated electrophysiology was used to confirm the presence of Kv2.2 channels. Under control conditions, HEK cells overexpressing Kv2.2 channels exhibited voltage-dependent activation resulting in robust outward potassium currents (Figure 2A).
Application of 30 µM Quinine, a known Kv2.2 channel blocker, resulted in a marked inhibition of Kv2.2 activity (Figure 2B). The corresponding I-V relationship further demonstrated the inhibition of Kv2.2 currents by Quinine in a voltage-dependent manner (Figure 2C). To further characterize the Kv2.2-expressing cell line, concentration-response curves were generated for the selective Kv2 antagonist RY796 and the non-selective antagonist, Quinine (Figure 2D). Due to its increased potency and specificity, RY796 was used as the reference inhibitor for subsequent high-throughput screening studies.

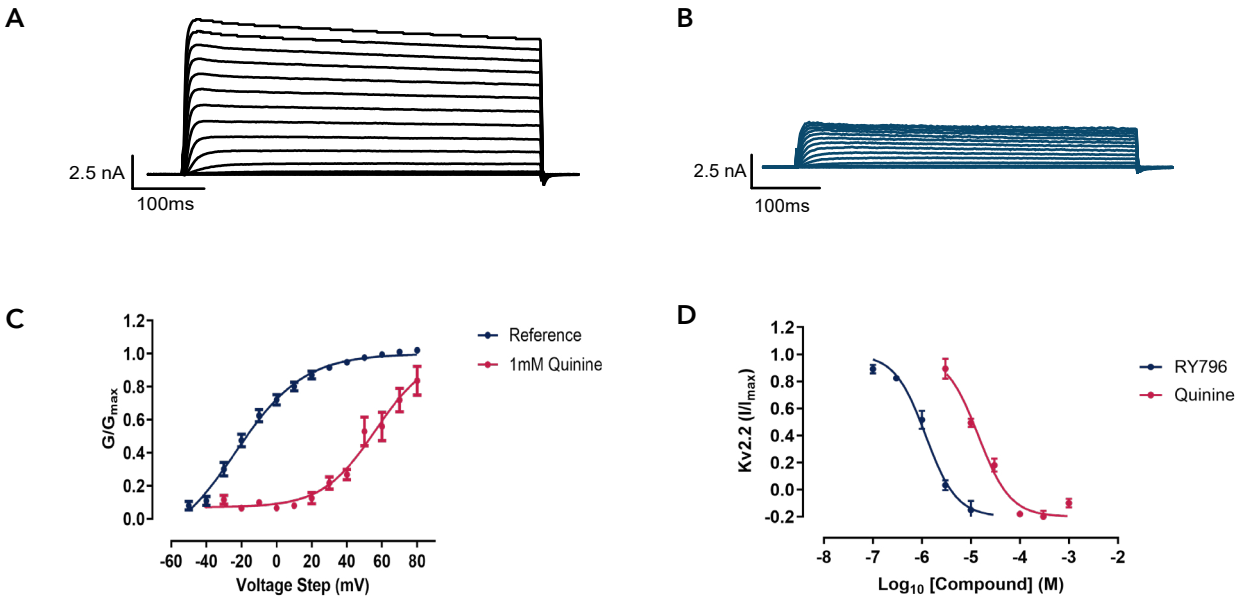


Figure 2. Kv2.2 currents elicited by 10 mV voltage steps from -80 mV to +80 mV under control conditions (A) and in the presence of 30 µM Quinine (B). Kv2.2 conductance-voltage relationship under control conditions and in the presence of 1 mM Quinine (C). Concentration-response curves comparing the inhibitory effect of two reference antagonists, RY796 and Quinine (D).

Fluorescence-Based High-Throughput Screening

To identify novel small molecule inhibitors of Kv2.2, a high-throughput, 384-well, fluorescence-based thallium assay was developed. The assay exhibited a strong signal in transfected Kv2.2 cells (Figure 3) which was inhibited in a concentration-dependent manner by the reference inhibitor RY796 (Figure 4). No measurable response was detected in untransfected cells.

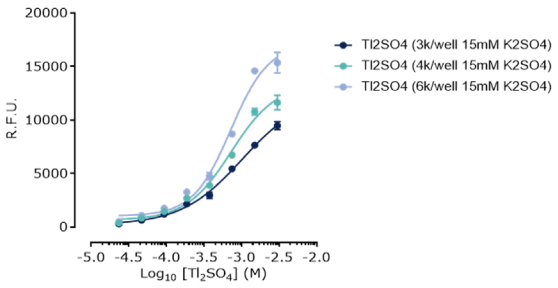


Figure 3. Concentration response curve for Tl_2SO_4 across different seeding densities.

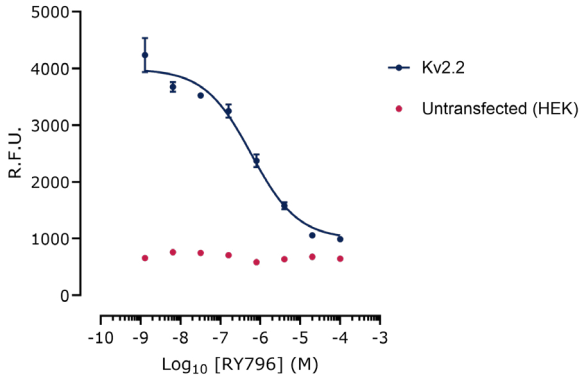


Figure 4. Concentration response curve showing the inhibition of Kv2.2 activity by RY796. The IC_{50} was 0.6 µM.

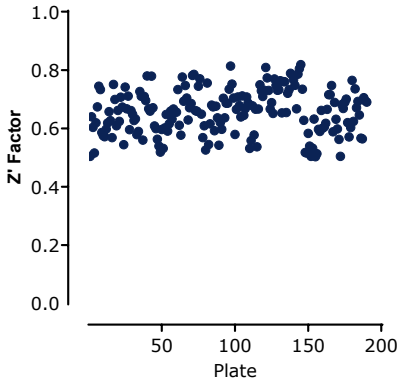


Figure 5. Scatter plot showing Z′ factor values for the full HTS campaign, measured across 190 independent screening plates using the reference inhibitor RY796. The assay maintained high reproducibility, with Z′ factors exceeding 0.5 across all plates.

Following assay optimization, high-throughput screening was conducted using a 30,000-compound library, with each compound tested at 10 µM in duplicate. Z′ factor values were monitored throughout the screening campaign, with scatter plot analysis confirming excellent reproducibility across all plates (Figure 5). Compounds exhibiting >40% inhibition in the primary screen were designated as hits, leading to the identification of 529 preliminary hit compounds.

To confirm the hit compounds identified from the primary screen, preliminary hit compounds were subject to secondary screening at two concentrations (10 µM and 1 µM) against Kv2.2-expressing HEK cells and against untransfected cells. This hit confirmation study confirmed 370 active hits that inhibited Kv2.2 while showing no significant activity in untransfected cells.

Electrophysiological Assessment

The top 65 compounds identified from the screening campaign were further characterized using automated electrophysiology. An application protocol was employed, consisting of three additions of physiological extracellular solution for a minimum of 3 minutes to obtain a control period, followed by application of a single test compound concentration (minimum 2 minutes) and finally a saturating concentration (30 µM) of the inhibitor RY796 for a minimum of 1 minute.

The results confirmed that all 65 compounds selected for electrophysiological assessment demonstrated inhibitory effects on Kv2.2 activity, confirming the initial findings from the fluorescence-based screen. All test compounds were shown to inhibit Kv2.2 currents in a concentration-dependent manner, with IC_{50} values ranging from 3 µM to 88 µM.

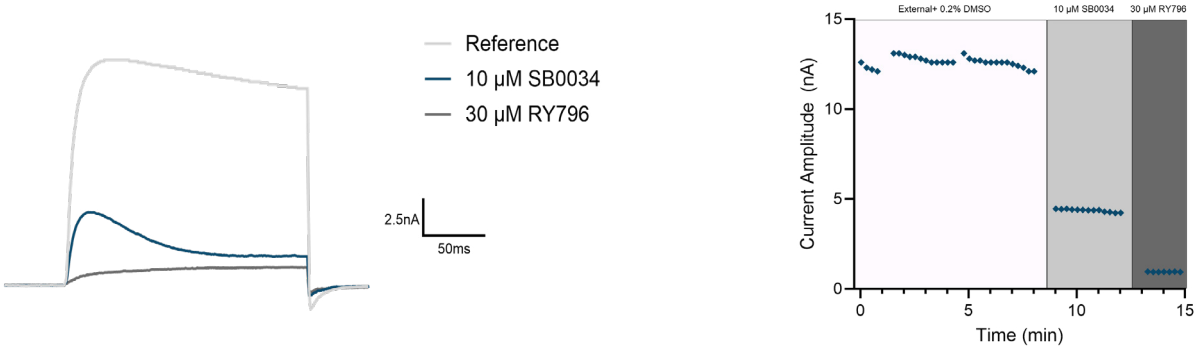


Figure 7. Representative current trace and corresponding time course of Kv2.2 under control conditions, following application of the hit compound SB0034 and subsequence full inhibition by the reference antagonist RY796 (30 µM).

Advancing Kv2.2 Drug Discovery

SB Drug Discovery's ion channel screening platform provides reliable, high-quality data to support the search of novel starting points for Kv2.2 drug discovery. By integrating well-characterized cellular tools with high-throughput fluorescence-based screening and automated electrophysiology, we offer a robust and scalable workflow designed to efficiently progress hit compounds through to candidate selection.

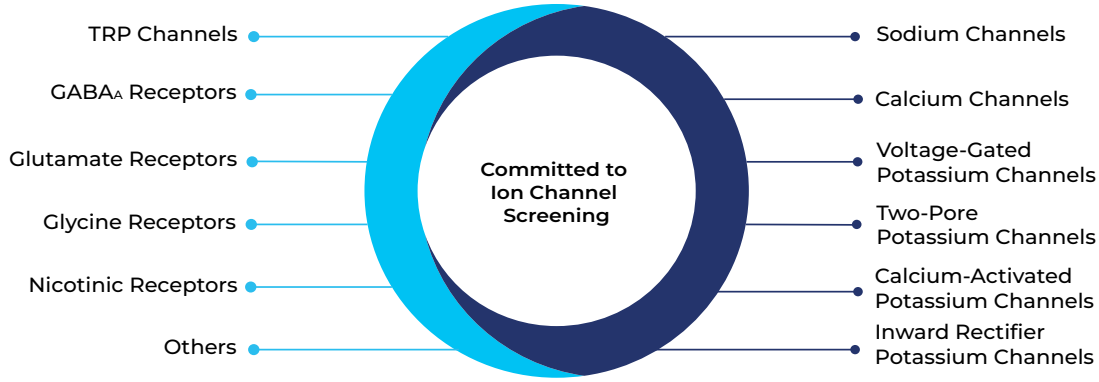
With extensive expertise in ion channel pharmacology, we tailor each screening cascade to meet the specific needs of the project, ensuring both primary screening and follow-up electrophysiology profiling generate data that supports informed decision-making.

SB Drug Discovery's Potassium Channel Expertise

SB Drug Discovery offers extensive expertise across a wide range of potassium channel families, including:

- Voltage-gated (Kv) channels
- Two-pore domain (K_2P) channels
- Inward rectifier (Kir) channels
- Calcium activated channels

Our capabilities span custom cell-based assay development, high-throughput fluorescence screening and automated electrophysiology, allowing us to design flexible, data-driven workflows to address the unique biology and pharmacology of each target. This expertise enables us to support your research across diverse therapeutic areas, from neuroscience and cardiovascular disease to oncology and metabolic disease.



Advance your research with SB Drug Discovery's ion channel screening platform. Offering end-to-end hit identification and validation, we deliver the results you need to progress your ion channel drug discovery research.

Contact us today to explore how our screening and electrophysiology services can accelerate your research and drive meaningful advancements in therapeutics.

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