FluxOR™ Differentially Identifies K+ Channel Antagonists Using FDSS6000

Introduction

Current assays for recording ion flux in cell based assays use No Wash Membrane Potential kits. These kits measure transmembrane voltage without regard to fluxing of specific ions. By contrast ion specific dyes like those measuring calcium channels (Fluo-4, Fura-2) are better probes as the specific ions fluxing across the membrane are identified.

Invitrogen (IVGN) offers a fluorescent dye kit[1)] for measuring potassium channel activity using the surrogate ion Thallium. In this assay cells are loaded with the FluxOR dye.

Cells are agonized through ligands or K^+ in the presence of Thallium. Open K^+ channels allow an influx of Thallium ions which binds to the dye; fluorescence increases. For K^+ specific models IVGN offers BacMam vectors with one of a panel of K^+ channels such as hERG[2)].

Materials and Methods

Three K^+ Channels, hERG, Kir2.1, and Kv7.2, were differentially expressed in U-2 OS cells using BacMam technology. Following an overnight incubation cells were dye loaded using FluxOR then incubated with compounds or controls. Each of the four compound plates (Tocris) was tested against the three cell lines hERG, Kir2.1, and Kv7.2. Following a thirty minute incubation with compound K^+ and Thallium ions were added to the cells using the FDSS6000 and data collected. The data was analyzed using CeuticalSoft (Hudson, NY).

The results using 10 μM Cisapride against hERG channel were analyzed for optimal z' score based on reaction time. The results in Figure 1 show a 16 sec reaction time gives a z' score over 0.8. Longer reaction times did not improve the z' score.

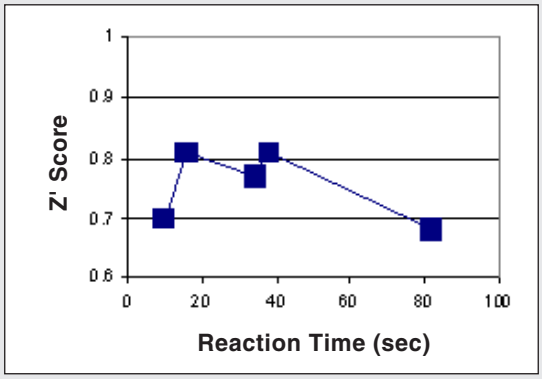

Fig. 1: Effect of reaction time on z' scores using hERG cells and Cisapride, a known hERG blocker. The minimal reaction time for a z' score over 0.8 is 16 sec.

In fact at an 80 sec reaction time the z' decreases; this is due to the decrease in the signal window as signal from Cisapride treated cells increase with time (Figure 2).

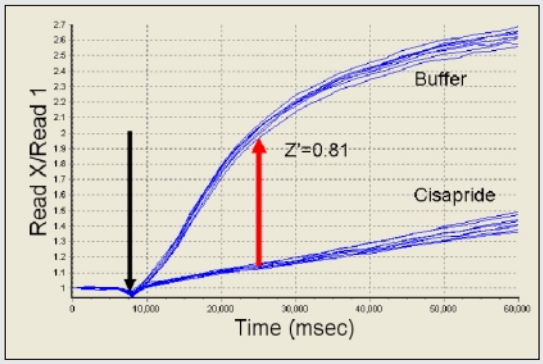

Fig. 2: Effect of 10 μM Cisapride on Thallium ion uptake against hERG cells. Black arrow, injection of Thallium, Potassium; Red Arrow, window for z' score used for analyzing Tocris compounds. N=8 per group. Note Cisapride inhibition is less than 100 % across the assay.

For all three targets a 16 sec reaction window was used. Hits were identified as having activity less than half of the buffer controls. The results are expressed as the percentage of hits common and unique to the three K^+ channels (Figure 3).

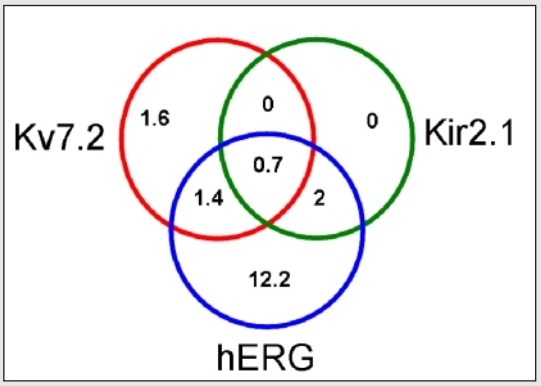

Fig. 3: Venn diagram of percentage of compounds with greater than 50 % inhibition against cells expressing indicated gene using BacMam technology.

The results show how a panel of cell lines differentially identifies K^+ channel specific antagonists. Besides antagonists specific to a given channel (hERG, Kv7.2) there are some hits less specific, i.e. antagonist against two K^+ channels. As expected there were no antagonists specific to Kir2.1, a K^+ channel with no such identified antagonists. Pan-antagonists (0.7 %) need to be carefully scrutinized for artifacts, i.e. fluorescence compounds, acute toxins, etc.

References

1. FluxOR™ Thallium Detection Kits. Invitrogen Product Insert.
2. BacMam-hERG Potassium Channel. Invitrogen Product Insert.

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