Primary screening of a 1564 compound focused diversity set against the human cardiac sodium channel $Na_v1.5$ using the IonWorks Quattro system

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Abstract

Population Patch Clamp™ (PPC) technology was recently introduced by Molecular Devices in the IonWorks™ Quattro system. PPC records from up to 64 cells at a time, and greatly reduces biological variability, achieving nearly 100% success rates and highly consistent data quality (Z'-Factors between 0.6 – 0.8). We conducted a feasibility study for using the IonWorks Quattro system in a primary screen of a focused library (1564 compounds) for modulators of the cardiac sodium channel $Na_v1.5$ (hH1)-—an important target for anti-arrhythmia therapeutics. The screen was carried out in duplicate at a single concentration (10 uM) in less than two working days. There were 37 actives with 50% or more inhibition identified (2.4% hit rate), which were all followed-up for potency (10-point dose-response run in duplicate) and selectivity (counter screened against $K_v1.3$). A number of compounds show use-dependence and selectivity. This rapid assay campaign demonstrates that the IonWorks Quattro system can be used for directed primary screens of voltage-gated ion channel modulators. The daily throughput of the IonWorks Quattro is >2000 data points per day. It is estimated that a 10,000 compound single-point screen with follow-up pharmacology could be completed in approximately 2-4 working weeks.

Original PatchPlate **PatchPlate PPC**

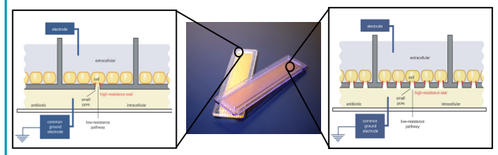

Figure 1. **Planar Patch Clamp and Population Patch Clamp (PPC) technique.** Top left: Schematic of planar patch clamp technology employed in the original IonWorks HT system, whereby a single cell is drawn by vacuum into a single hole at the bottom of the PatchPlate well. Top right: Schematic of the PPC technique, whereby multiple cells seal onto an array of holes at the bottom of the PatchPlate well. Top center: photograph of the single-hole (yellow) and multi-hole (blue) PatchPlate substrates.

Bottom, a magnified view of PatchPlate PPC wells. 64 recording sites in each well can be seen under microscope.

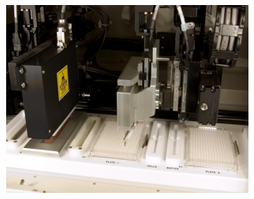

Figure 2. **The IonWorks Quattro instrument.** Photographs of the instrument (left) and the experimental deck inside the instrument (right) showing the electronic and fluidic heads, compound plates, electronic and fluidic head wash stations and PatchPlate™PPC substrate. The systems supports both 96- and 384-well compound plates.

Material and Methods

Cells: Chinese hamster lung (CHL) cells expressing the $Na_v1.5$ sodium channel

Internal Buffer (in mM): 140 KCl, 2 $MgCl_2$ 5 EGTA, 10 Hepes pH to 7.2 with KOH (Sigma Cat. #'s P-9333, M-1028, E-0396, H-7523, P-5958);

External Buffer (in mM): 137 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 Hepes, 10 Glucose, pH to 7.4 with NaOH (Sigma Cat #'s S-7653, P-9333, M-1028, Fluka Cat. # 21115, Sigma Cat. #'s H-7523, G-7528, Fisher Cat. # SS266-1)

PPC PatchPlate™ consumables (Molecular Devices Cat. # 9000-0902)

Compound plate layout

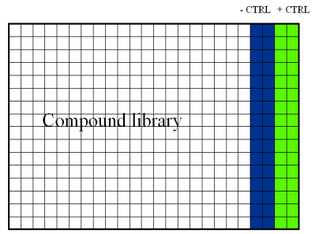

Figure 3. **Plate layout of compounds and controls.** Compound library consists of a set of 5 microtiter plates in 384 format. Each plate contains up to 320 compounds from the chemical library. Columns 21 and 22 contain negative controls and columns 23 and 24 are positive controls (TTX).

Na channel currents and metrics

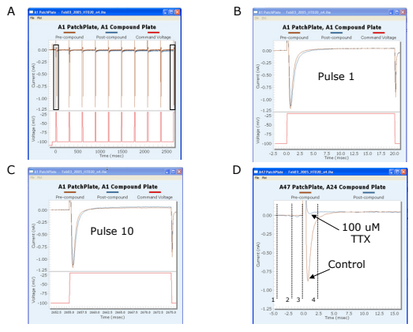

Figure 4. **$Na_v1.5$ ionic current measurements.** The voltage protocol used to elicit currents is shown (A), a train of 10 depolarizing steps to −20 mV from a holding potential of −100 mV was delivered. The estimation of the resistive leak is derived from the current acquired during the voltage step to −110 mV delivered just before the pulse train. Negative control current recordings are shown for pulse 1 (B) and pulse 10 (C), the currents before (brown) and after (blue) the addition of the negative control is shown. Typical positive control recordings are shown for the first pulse in the train (D), note full block of the current after the addition of 100 uM TTX. The regions of the sweep used to calculate the current magnitude and the percent change in peak currents is shown. The magnitude of the peak negative current between the dotted lines shown at (3) and (4) was used. An average of 3 ms holding current between (1) and (2) was calculated and subtracted from the peak currents to yield the measured current.

Data consistency and stability

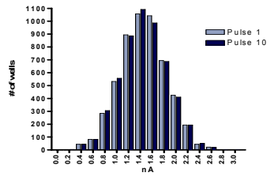

Figure 5. **Nav1.5 current amplitudes.** Pulse 1 and pulse 10 current amplitudes are similarly distributed. Also, note that PPC technology eliminated the non-expressing assay wells (with current near zero).

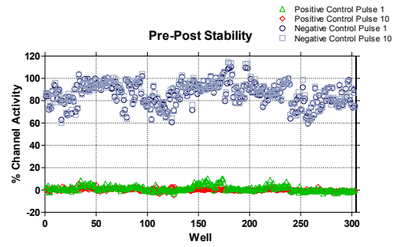

Figure 6. **The stability of Nav1.5 current at pulse 1 and pulse 10.** The data points for negative controls are shown on the top of the figure; positive controls are shown at the bottom.

Screening of 1564 compounds

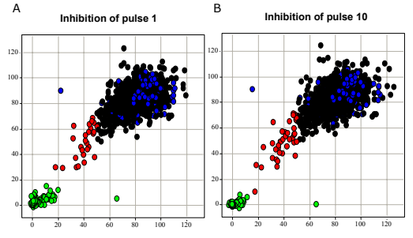

Figure 7. **Results of the compound screen run in duplicate.** Compounds from the chemical library were tested in duplicate and their channel activity graphed. All 1564 test compounds are shown as well as the negative and positive controls. Duplicate compound plates were freshly prepared from DMSO stocks and run on the IonWorks Quattro system. The results from duplicate assays are shown with the first assay on the x-axis and the second assay on the y-axis. Graphed in this manner the potent positive controls (green) cluster in the lower left part of the plot and the negative controls cluster in the upper right (blue). The results from pulse 1 (figure A) and pulse 10 (figure B) are graphed separately.

Use-dependency and selectivity

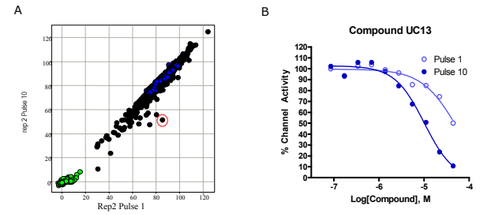

Figure 8. **Screening for use-dependent compounds.** A) Graph of the channel activity measured from the first pulse (x-axis) versus the tenth pulse (y-axis) for all compounds and controls (A). Compounds that demonstrate use-dependence show higher channel activity in the tenth pulse and are below the diagonal line formed between the positive and negative controls. An example of a use-dependent compound is shown (compound UC13). B). Dose response curves for UC13 calculated from pulse 1 and pulse 10.

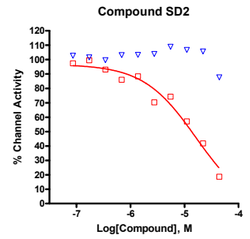

Figure 9: **Counter screening**. A counter screen of the 38 compounds inhibiting Nav1.5 was performed against the Kv1.3 channel. Figure shows an example compound (SD2) exhibiting no activity against the Kv1.3 channel.

Summary and conclusions:

- 1600 compounds wells screened in duplicates
  - → 10 PPC runs for the screen
- 38 hits with activity > 50% (hit rate 2.4%)
- All hits were followed up with 10-points IC50s
  - → 4 PPC runs for IC50 follow up (32 IC50s/plate)
- Use-dependent compounds easily picked up
- Hit compounds counter-screened against Kv1.3

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