Increasing the Throughput of a High Content Imaging-Based Developmental Neurotoxicity Proliferation Assay

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Background

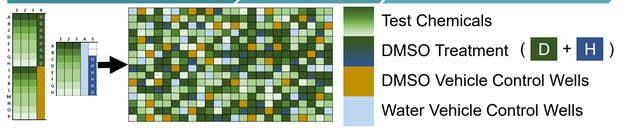
- The majority of chemicals in our environment have yet to be assessed for developmental neurotoxicity (DNT) hazard.
- To close this gap, a number of assays covering a variety of key neurodevelopmental processes have been developed to form the DNT *In Vitro* Battery (DNT-IVB).
- One such DNT-IVB assay is a fluorescence microscopy-based 5-bromo-2'-deoxyuridine (BrdU) proliferation assay performed in human neural progenitor (hNP1) cells in 96-well format.
- In keeping with the goals underlying the DNT-IVB, the aim of this study is to increase the throughput of the existing BrdU proliferation assay by optimizing it to 384-well format.

The DNT-IVB BrdU proliferation assay is optimized to 384-well format using enhanced laboratory automation and high-content imaging capabilities.

This optimization represents a first step towards increasing the overall throughput of the EPA DNT New Approach Methodologies battery.

Experimental Design

Coat Plates with PLO **24h** Plate Cells **24h** Dose Cells **20h**



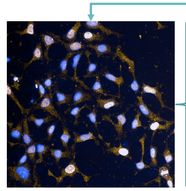
Cultures	Replicate Plates / Culture	Technical Replicates / Plate	Vehicle Controls/ Biological Replicate
3	3	4	36 DMSO + 32 Water

Chemicals

Known Antiproliferatives	Negatives	Known Apoptotic
Aphidicolin 5-fluorouracil (5-FU) Cytosine arabinoside (Cytarabine)	Hydroxyurea‡ Caffeine‡ Ketamine‡ Saccharin Sorbitol	Staurosporine + DMSO in 7-pt Concentration-Response

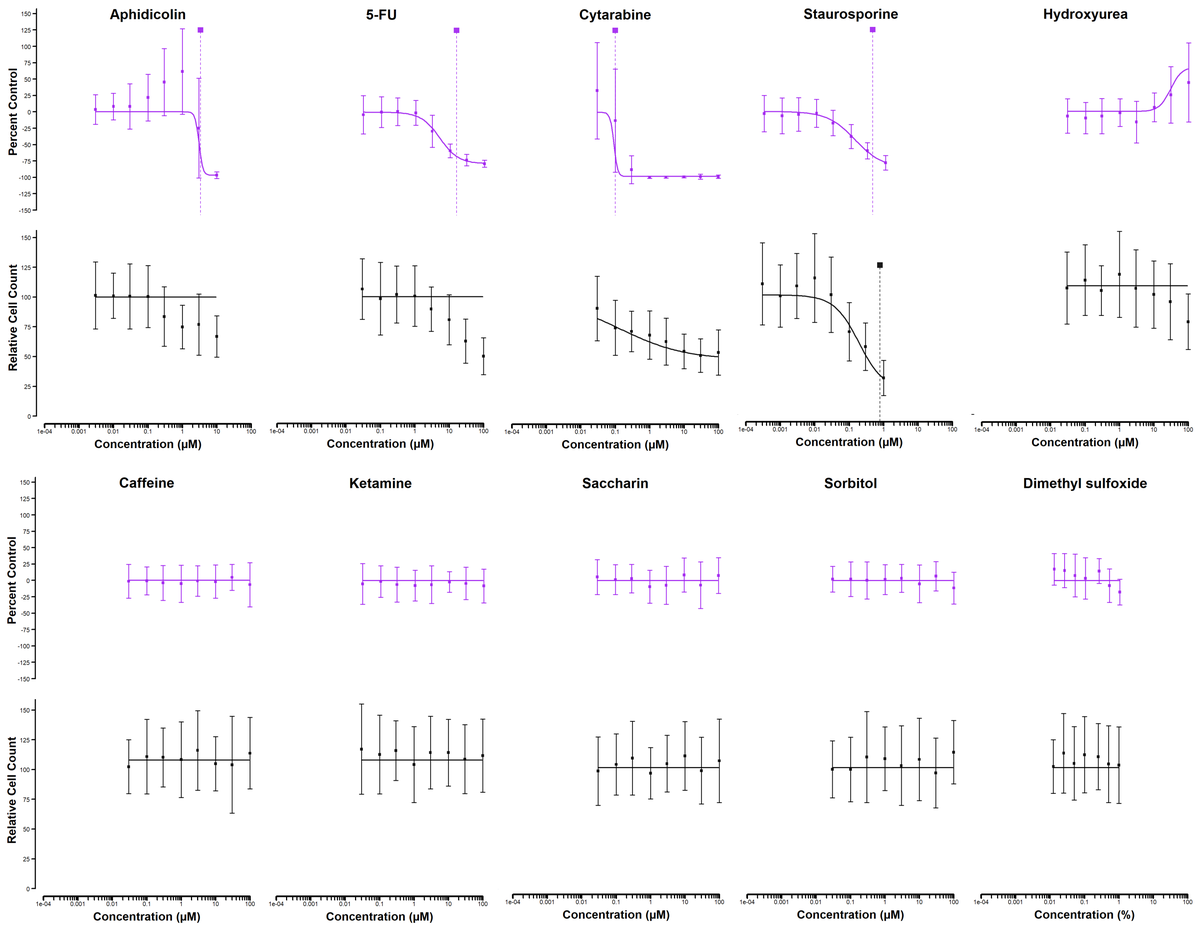
‡Water-solubilized chemicals

Add BrdU **4h** Fix **10m** Blocking/ Permeabilization **56h** Antibody Labeling **24h** Image Analyze



Channel Name	Stain / Label	Analysis
DNA	Hoechst-33342	*Harmony Software:* collect intensity, morphology, and texture measurements *R:* Data normalization and visualization
BrdU	Alexa Fluor™ 546 goat anti-mouse	

Successful Scaling of BrdU Proliferation Assay to 384-well Format in Human Neural Progenitor Cells



The results above are *tcplfit2* curve fitting of percent control (purple) and relative cell count (black) data. Curves were fit if the median response exceeded either 3 (percent control) or 2 times (relative cell count) the normalized median absolute deviation of vehicle control data. The benchmark dose was identified where a 50% deviation from vehicle control occurred, providing an EC50 value. Large square symbols with vertical dotted lines trailing down towards the x-axis represent the determined EC50 values where applicable. Well-level cell count was normalized to vehicle control to determine relative cell count. Subtracting 100 from well-level percent BrdU response values normalized to vehicle control provided percent control values.

Concordance with Previous Data

Chemical	384-Well EC50 (μM)	384-Well AC50 (μM)	96-Well AC50 (μM)
Aphidicolin	3.24	2.91	10[a, f]
5-fluorouracil	14.47	4.65	4.62[b]
Cytosine arabinoside	0.14	0.11	0.08[b]
Staurosporine	0.46	0.15	*No Data*
Hydroxyurea	--	26.22	--[b]
Caffeine	--	--	--[b]
Ketamine	--	--	--[b]
Saccharin	--	--	--[c]
Sorbitol	--	--	--[c]
Dimethyl sulfoxide	--	--	*No Data*

[a]Culbreth et al, 2012
[b]CompTox Chemicals Dashboard (htpps://comptox.epa.gov/dashboard/)
[c]Harrill et al, 2018
[f]ReNcell® CX cells, no data available in hNP1 cells

Conclusions and Future Directions

- The EC50 and AC50 values derived for 5-fluorouracil and cytosine arabinoside were consistent with previous observations.
- The EC50 and AC50 values derived for aphidicolin were relatively consistent with previous observations in ReNCell CX® cells.
- All except one negative chemical had no measurable effect on proliferation, including DMSO.
- Hydroxyurea resulted in an AC50 for increased BrdU incorporation.

With the DNT-IVB BrdU proliferation assay successfully optimized to 384-well format, we will pursue multiplexing this approach with caspase activation to simultaneously measure proliferation and apoptosis.

References

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