The Development and Validation of a High throughput Cell-Based Acetylcholinesterase Assay Using Mouse N2A

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Abstract

Acetylcholinesterase (AChE) resides in chemical synapses where it can hydrolyze acetylcholine: a neurotransmitter associated with muscle movement, cognition, and other neurobiological processes. While AChE inhibitors have been used to treat Alzheimer's disease, glaucoma, and myasthenia gravis, excess amounts of the inhibitor can lead to toxicological effects such as gastrointestinal upset, vomiting, and muscular paralysis. Nevertheless, many compounds have unknown AChE inhibitory effects.

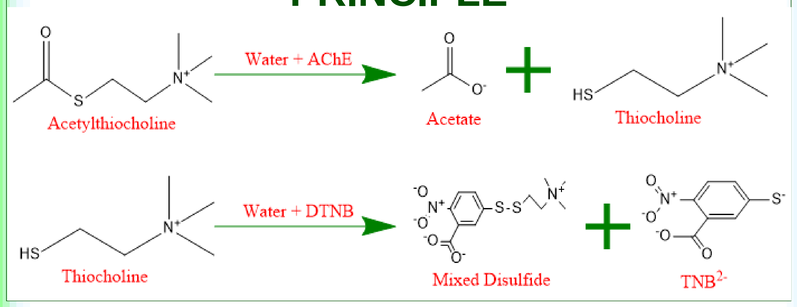
In order to counteract this problem, our study developed a cell-based assay using mouse neuro2a cells to screen for AChE inhibitors in a high-throughput screening fashion. Our cell-based assay was optimized and miniaturized into a 1536-well format using the Ellman method, in which AChE activity was determined by measuring the absorption intensity of the DTNB-thiocholine adduct that results when AChE hydrolyzes acetylthiocholine.

The assay was then validated against a cherry-picked 1247 compounds that were identified from the 10 k library using human SH-SY5Y cells. Many compounds show inhibitory effects for AChE in both cell lines, such as Acid Red 337. And there are also some compounds that only show effects in one of the cell lines, such as Arbutin. Overall, this assay will aid in the identification of chemical compounds that inhibit the activity of AChE in certain cell lines.

The Amplite™ Colorimetric Acetylcholinesterase Assay Kit (11400) was purchased from AAT Bioquest. Compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

Principle and Method

PRINCIPLE

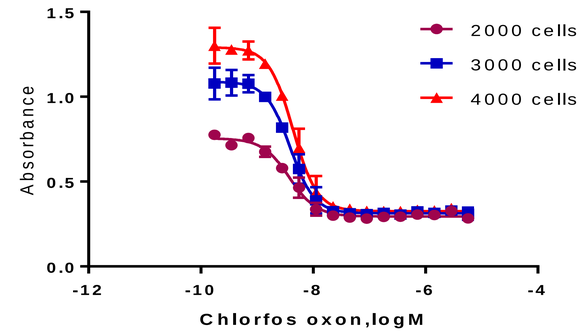


METHOD

Step	Parameter	Value	Description
1	Plate Cells	4 µL	3000 cells/well using a MultiDrop
2	Incubation	18 hr	Incubation at 37 °C
3	Compound	23 nL	Pintool transfer of control + compounds
4	Incubation	60 min	Incubation at 37 °C
5	Reagent	4 µL	Addition with BioRAPTR
6	Incubation	40 min	Incubation at room temperature
7	Readout	Envision	Ex: 405 Absorbance

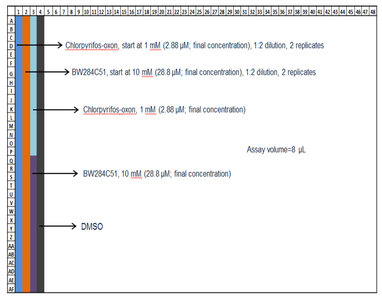
Optimization and Results

1A)



	2000 cells	3000 cells	4000 cells
HillSlope	-1.808	-2.025	-2.026
IC50	3.767e-009	3.903e-009	4.354e-009

1B)



1C)

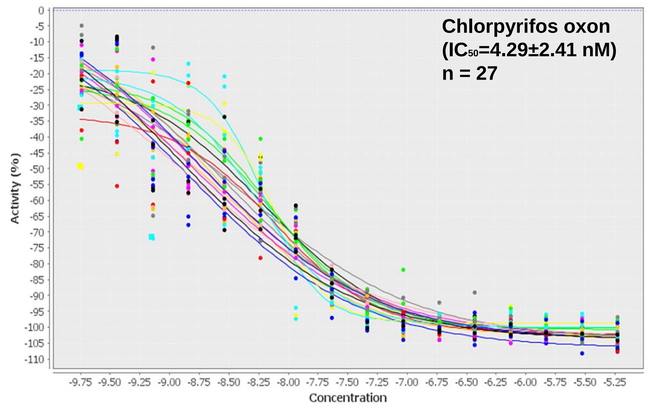
Chlorpyrifos	cv	S/B	Z' factor
2000 cells	0.070742	2.810946	0.557939
3000 cells	0.036339	3.674934	0.78805
4000 cells	0.045609	4.056431	0.78365

BW284c51	cv	S/B	Z' factor
2000 cells	0.070742	2.620679	0.554892
3000 cells	0.036339	3.532044	0.801868
4000 cells	0.045609	3.856086	0.763435

Figure 1:
1A: Dose response curves for the positive control compounds
1B: Assay control plate
1C: CV, Z' factor, and S/B values for 2000, 3000, and 4000 cells/well are listed in Table 1C. The CV values shown are the average values for the plates, excluding the top concentration points.

Positive Controls

2A)



2B)

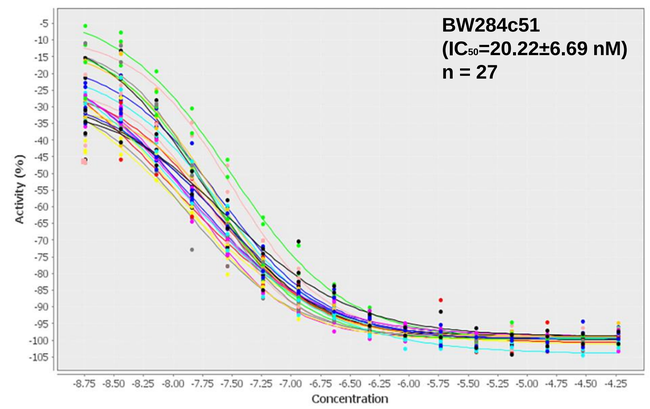
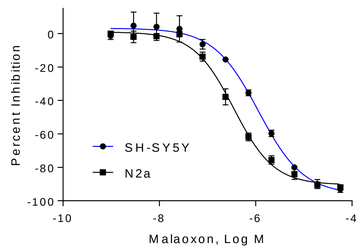


Figure 2:
2A: Dose response curves for Chlorpyrifos oxon (positive control).
2B: Dose response curves for BW284c51 (positive control).

Comparison Between Cell Lines

3A)



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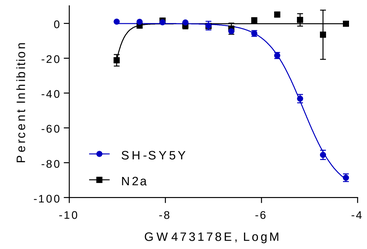
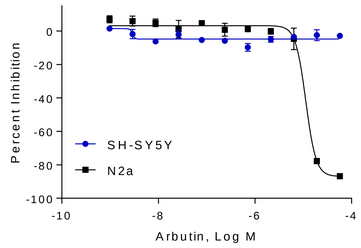


3C)



3D)

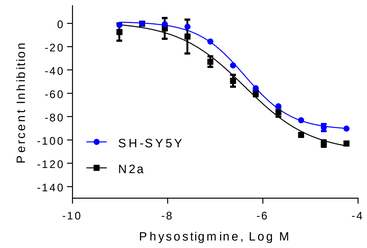


Figure 3:
3A: Dose response curves for Malaoxon in SH-SY5Y and N2a cells
3B: Dose response curves for GW473178E in SH-SY5Y and N2a cells
3C: Dose response curves for Arbutin in SH-SY5Y and N2a cells
3D: Dose response curves for Physostigmine in SH-SY5Y and N2a cells

Conclusion

1) **This AChE assay using N2a cells showed good performance in screening AChE inhibitors.**

References

Li, S., Huang, R., Solomon, S., Liu, Y., Zhao, B., Santillo, M. F. and Xia, M. Identification of acetylcholinesterase inhibitors using homogenous cell-based assays in quantitative high-throughput screening platforms. Biotechnol. J., 2017.

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