The sigma-2 receptor modulator and investigational therapeutic CT1812 is neuroprotective against 4-HNE-induced cell death in a disease-relevant neuronal model

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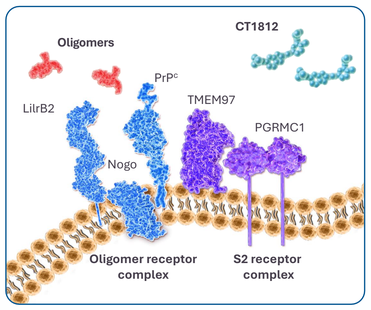
COGNITION™ Therapeutics

Poster # 2550

Introduction

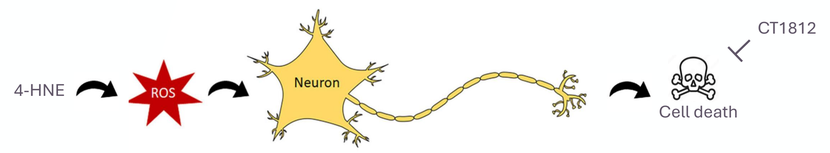
The sigma-2 receptor (S2R/TMEM97) modulator CT1812 (zervimesine) is an allosteric amyloid-beta (Aβ) oligomer antagonist currently in clinical development for Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). Preclinical studies have shown that CT1812 can displace Aβ and alpha-synuclein oligomers from synapses, preventing synaptotoxicity and restoring neuronal function[1], and can rescue oxidative stress-mediated deficits in retinal pigment epithelium (RPE) cells[2].

In the Phase 2 clinical trial SHINE (COG0201/NCT03507790), conducted in patients with mild to moderate AD, we have also demonstrated an impact of CT1812 on neurodegeneration such as reducing CSF NfL level with CT1812 treatment. Since neuronal damage and cell death in AD, and other neurodegenerative diseases like DLB, are driven by oxidative stressors as well as by toxic oligomers, we aim to further elucidate the neuroprotective mechanisms of CT1812 in preventing oxidative stress-induced cell death in a human neuronal cell model.



Schema 1.

Methods



- SH-SY5Y cells were differentiated using retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) for 7 days.
- Differentiation markers (MAP2, SYP, ID1, TH, DDC and ChAT) were characterized at the mRNA level via RT-qPCR and cell morphology was assessed via immunocytochemistry (ICC).
- Differentiated cells were treated with 4-Hydroxynonenal (4-HNE, oxidative stressor) to induce neuronal cell death.
- CellTiter-Glo and LDH-Glo assays were performed to measure cell viability.
- Z-VAD (apoptosis inhibitor) was utilized to determine whether apoptotic signaling is involved in the 4-HNE-induced cell death pathway.

Results

Characterization of differentiated SH-SY5Y cells as an in vitro model of oxidative damage in neurodegeneration

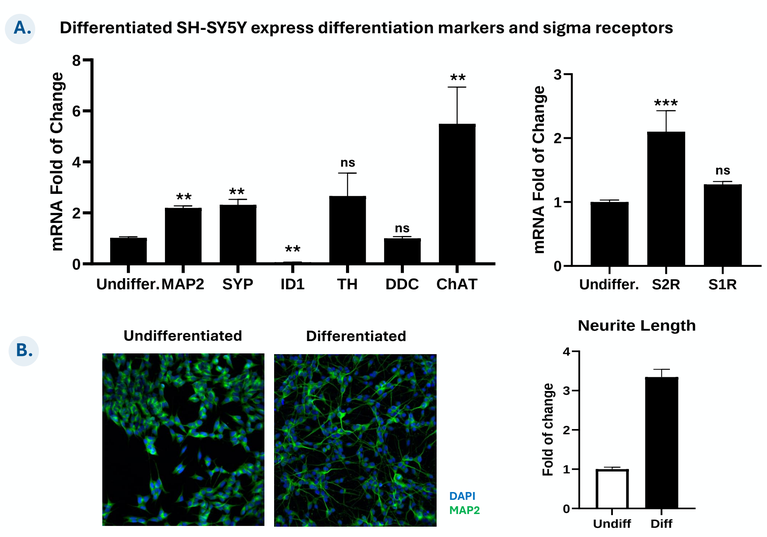


Figure 1. (A) mRNA level of differentiation-neuron markers [MAP2, SYP and inhibitor of differentiation (ID1)]; dopaminergic markers [tyrosine hydroxylase (TH) and DOPA decarboxylase (DDC)]; cholinergic neuron marker [choline acetyltransferase (ChAT)]; and sigma receptors (S1R and S2R/TMEM97). Normalized by undifferentiated cells. **(B)** Cell images of nuclear (DAPI, blue) and neuron (MAP2, green). Neurite length of differentiated cells was induced 3.35-fold compared to undifferentiated cells' one. Significance of p<0.05 determined by unpaired t test (differentiation marker vs undifferentiated). ns: not significant

The S2R modulator CT1812 protects neuronal cells from oxidative stress, but not PRE-084, a S1R-selective modulator

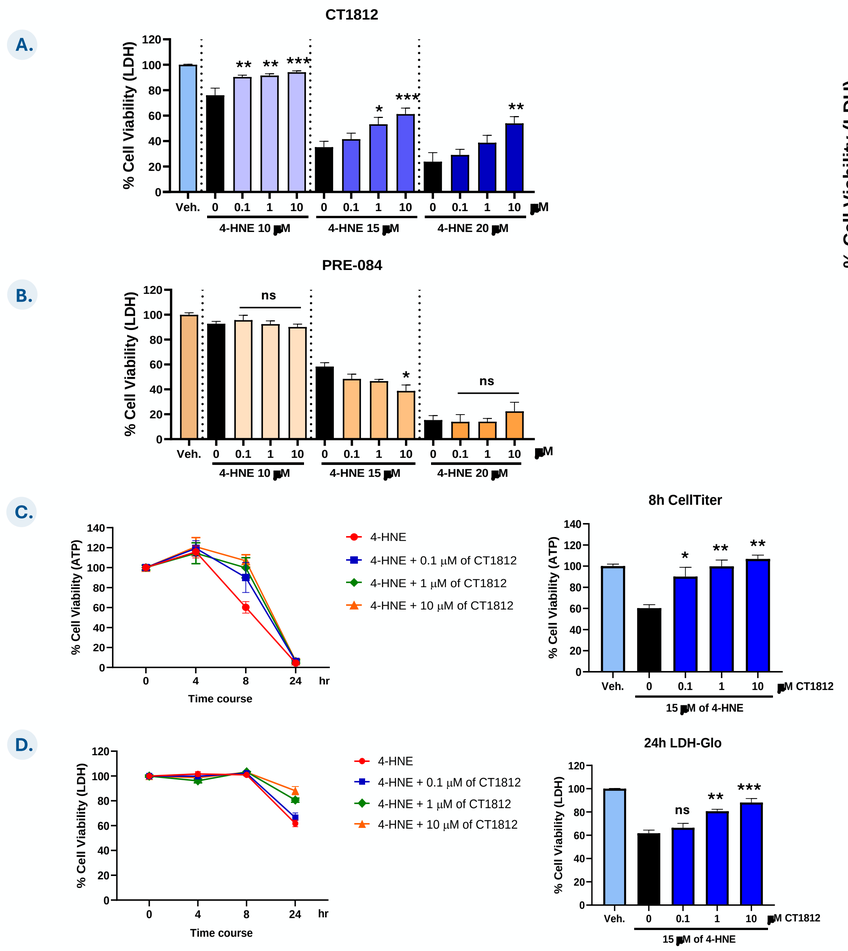


Figure 3. LDH-Glo assay for cell death detection with 10 μM 4-HNE in the presence of vehicle (DMSO) or **(A)** CT1812 or **(B)** PRE-084 for 24 h. **(C)** CellTiter-Glo assay or **(D)** LDH-Glo assay performed following treatment of cells with 4-HNE -/+ CT1812 over time with 8 h **(C)** or 24 h **(D)** graphed separately in bar-graph form for visualization (Significance of p<0.05 determined by one-way ANOVA (4-HNE + CT1812 vs 4-HNE). ns: not significant

S2R/TMEM97 is required for CT1812-mediated rescue of cell viability under oxidative stress conditions

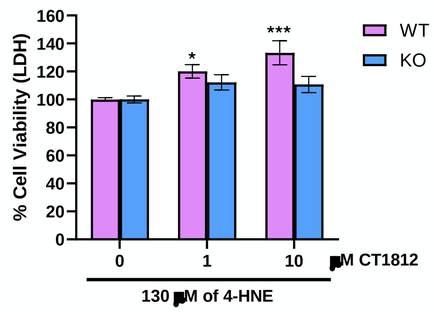


Figure 4. LDH-Glo assay for cell death detection in presence of 4-HNE and CT1812 was performed in a wild type or TMEM97 knockout HEK293T cells to confirm target engagement (Significance of p<0.05 determined by one-way ANOVA (4-HNE + CT1812 vs 4-HNE treatment).

Cell death induced by 4-HNE treatment might involve activation of apoptotic signaling pathways

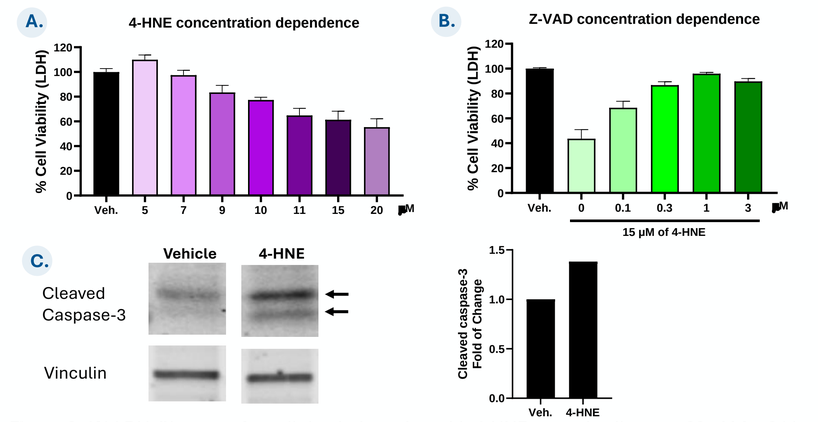
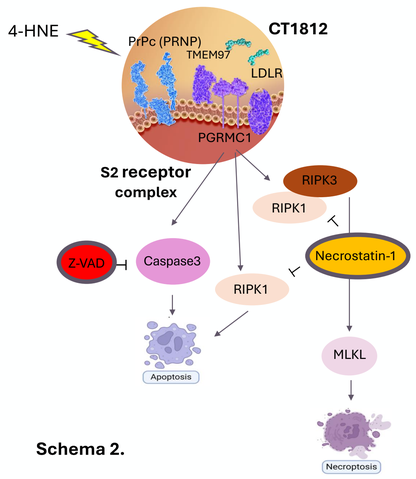


Figure 2. (A) LDH-Glo assay for cell death detection with 4-HNE treated cells up to 20 μM for 24 h. **(B)** LDH-Glo assay performed following treatment of cells with 15 μM of 4-HNE -/+ apoptosis inhibitor (Z-VAD) for 24 h showed rescue of cell viability. **(C)** 4-HNE treatment at 15 μM induced caspase-3 activation as demonstrated by Western Blot analysis of Cleaved caspase-3 after 8h of treatment. Vinculin was used for loading control. Densitometry was performed using ImageJ.

Conclusions

- Preliminary data suggests that cell death induced by 4-HNE treatment might involve activation of apoptotic signaling pathways.
- CT1812 can restore cell viability under oxidative stress conditions induced by 4-HNE treatment.
- S2R/TMEM97 is required for the role of CT1812 in neuroprotection with 4-HNE in HEK293T cells.
- These findings are consistent with an impact of CT1812 on cell survival and neuroprotection through a S2R/TMEM97 mediated mechanism of action.



Schema 2.

- Next studies will focus on investigating which key proteins in the apoptotic signaling pathway are impacted by treatment with CT1812 and if other cell death processes like necroptosis are involved.

Other Posters on CT1812 by Cognition Therapeutics

Poster # 1525: CT1812 preserves neurons and decreases levels of neurodegeneration biomarker NfL in disease-relevant neuronal model
Jill K Thiel, Eunah Cho, PhD, Anthony O Caggiano, MD, PhD, Valentina Di Caro, PhD, and Mary E Hamby, PhD

Poster # 1507: Sigma-2 receptor modulators including CT1812 modulate low-density lipoprotein uptake in primary rat neurons
Nicole Knezovich, Jill K Thiel, Britney N Lizama, PhD, Aidan reaver, Anthony O Caggiano, MD, PhD, Valentina Di Caro, PhD· Mary E Hamby, PhD

References: [1] Izzo NJ et al., Alzheimer's Dement. 2021 Aug 17(8):1365-1382.
[2] Lizama et al., Sci Rep. 2025 Feb 10;15(1):4256.

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