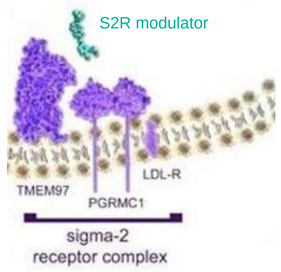
Sigma-2 Receptor Modulators Alter Low-density Lipoprotein Receptor-mediated Lipid Trafficking in Retinal Pigmented Epithelial Cells

Britney N. Lizama, PhD[1], Aidan Reaver, B.S.[1], Eunah Cho, PhD[1], Valentina Di Caro, PhD[1], Anthony O. Caggiano, MD PhD[2], and Mary E. Hamby, PhD[1].

(1) Cognition Therapeutics, Inc., Pittsburgh, PA, USA, (2) Cognition Therapeutics, Inc., Purchase, NY, USA

INTRODUCTION

The sigma-2 receptor (S2R, TMEM97) has been linked to dry age-related macular degeneration (AMD) in genome-wide association studies. We demonstrated that small molecule modulators of S2R rescue RPE photoreceptor outer segment (POS) trafficking deficits[1]. Mechanisms underlying compound-mediated rescue are under investigation. S2R interacts with proteins involved in lipid trafficking, such as low-density lipoprotein receptor (LDLR) and Niemann-Pick C Protein 1 (NPC1)[2]. Lipid homeostasis is necessary for effective POS trafficking and clearance[3], and loss of LDL trafficking contributes to RPE degeneration *in vitro*[4] and *in vivo*[4,5]. In this work, we develop tools to assess effects of chemically-distinct S2R modulators and hypothesize that S2R modulators alter LDLR-mediated lipid trafficking in RPE cells.

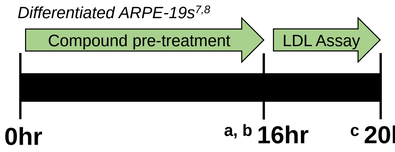


Schema 1. The sigma-2 receptor is comprised of TMEM97 and PGRMC1 (*purple*) and interacts with the LDL receptor (LDLR). We have shown that S2R modulators prevent toxic amyloid-β oligomer binding in neurons[6] and oligomer-induced POS trafficking deficits in RPE. The effects of S2R modulators on LDLR-mediated signaling remain to be elucidated.

METHODS

Aim 1: Characterize S2R modulator effects on LDL levels

Differentiated ARPE-19s[7,8]



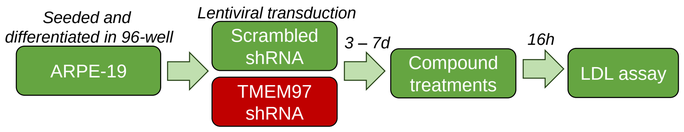
Endpoints
a. qRT-PCR
b. Western Blot
c. Quantification of LDL-DyLight550 fluorescence

Compound	µM	Description	S2R K_i	Specificity
CT1812	0.1 – 10	S2R modulator	8.5 nM	7.4-fold S2>S1R; >100-fold S2R, screened against 118 proteins
CT2074	0.1 – 10	S2R modulator	21 nM	6.6-fold S2R<S1R; >100-fold S2R, screened against 117 proteins 67-fold M2; 62-fold M3
CT2168	0.1 – 10	S2R modulator	1.4 nM	1.3-fold S2R>S1R; >100-fold S2R, screened against 116 proteins
Z2877893998	0.1 – 10	S2R modulator	3.4 nM	318-fold S2R>S1R; >100-fold S2R, screened against 320 proteins[9]
U18666A[10]	5	Inhibits LDL-mediated cholesterol transport		

Aim 2: Target validation of S2R modulators

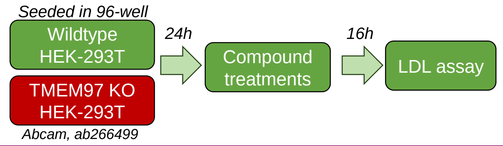
a) TMEM97 knockdown in RPE cells

Test the hypothesis that TMEM97 deficiency reduces effects of S2R modulators on LDL uptake in mature RPE cells



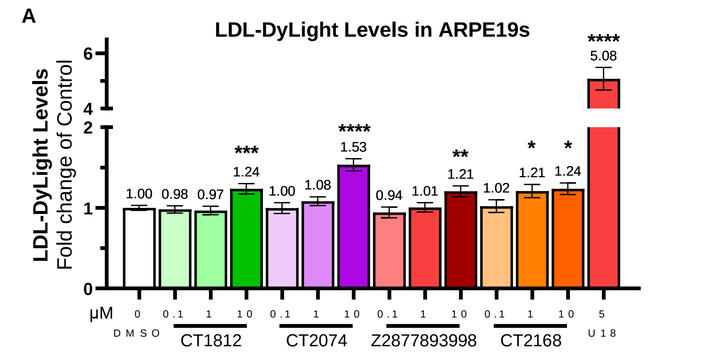
b) Genetic TMEM97 knockout in independent cell line

Confirm that S2R modulator-mediated LDL uptake is TMEM97 dependent



RESULTS

S2R modulators elevate internalized LDL



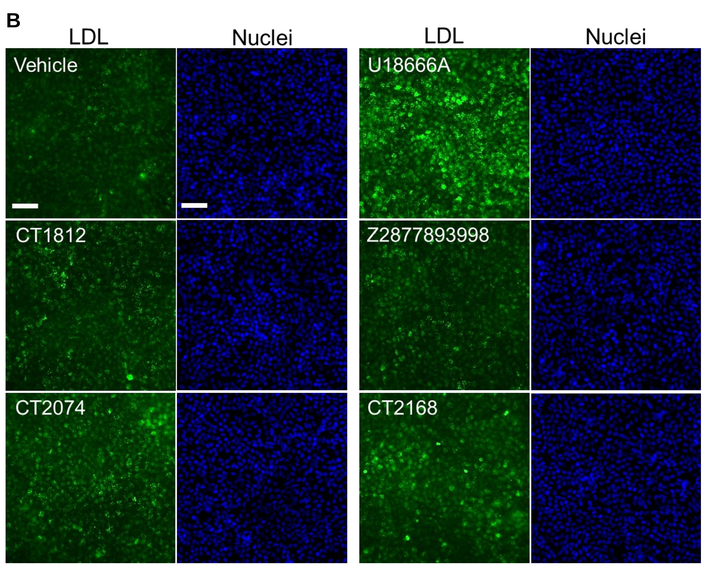


Figure 1. A. Quantification of LDL-Dylight550 fluorescence (spot total intensity per cell) assessed by CX7 Spot Detector after treatment with increasing concentrations of CT1812, CT2074, CT2168, and Z2877893998. U18 5 µM treatment was used as positive control. N=5 independent experiments, normalized to vehicle (DMSO); mean +/- SEM, ordinary one-way ANOVA. *p<0.05; **p<0.01; ***p<0.001; **** p<0.0001. B. Representative images of Hoechst-positive nuclei and LDL-DyLight550 fluorescence in vehicle-treated, compound-treated (10 µM), or U18-treated (5 µM) cultures. Scale bar = 50 µm.

S2R modulators do not significantly impact TMEM97 or LDLR protein levels in RPE cells

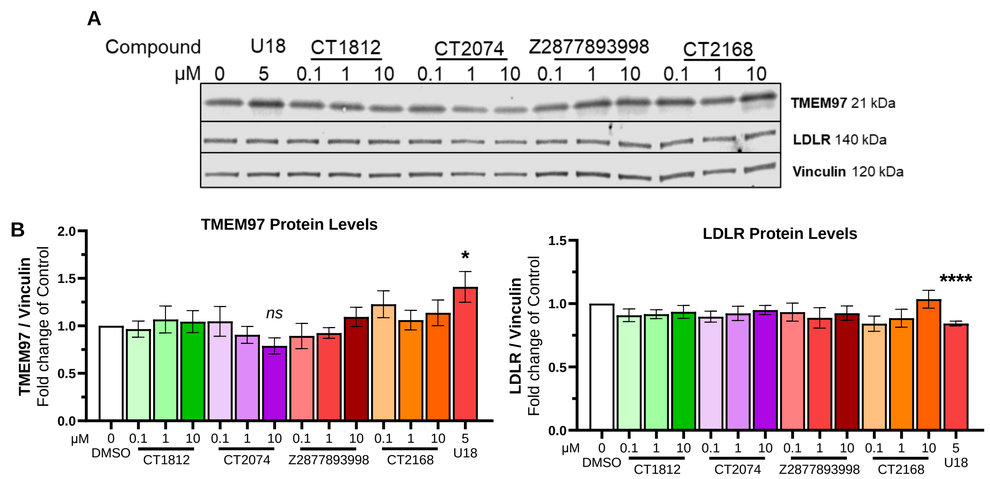


Figure 2. A. Protein levels of TMEM97 and LDLR were assessed by western blotting. Membrane-associated protein vinculin was assessed as loading control. B. Densitometry of western blots was performed, with TMEM97 or LDLR normalized to vinculin, normalized within experiment as fold change of DMSO control (0 µM). N=5, mean +/- SEM, significance assessed by one-way ANOVA of compound vs vehicle or two-tailed t-test U18 vs vehicle. *p<0.05; ****p<0.0001.

S2R modulators do not strongly impact TMEM97 or LDLR transcript levels in RPE cells

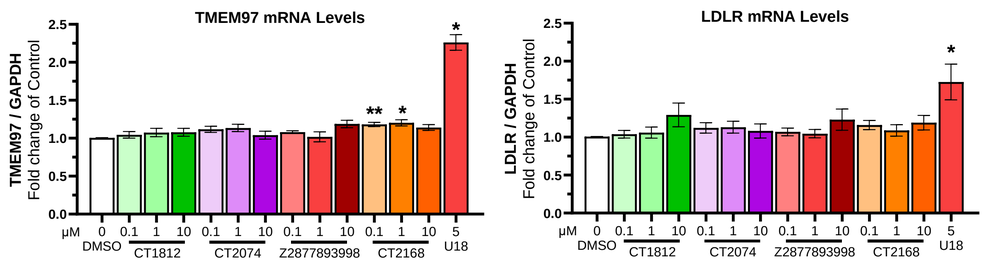


Figure 3. Transcript levels of TMEM97 and LDLR were assessed by qRT-PCR after treatment with ascending concentrations of compounds. GAPDH was assessed as housekeeping gene control. Graphs depict TMEM97 or LDLR normalized to GAPDH, fold change of DMSO control (0 µM). N=5, mean +/- SEM, significance assessed by one-way ANOVA of compound vs vehicle or two-tailed t-test U18 vs vehicle. *p<0.05; ****p<0.0001.

LDL is elevated in a TMEM97-dependent manner in RPE cells

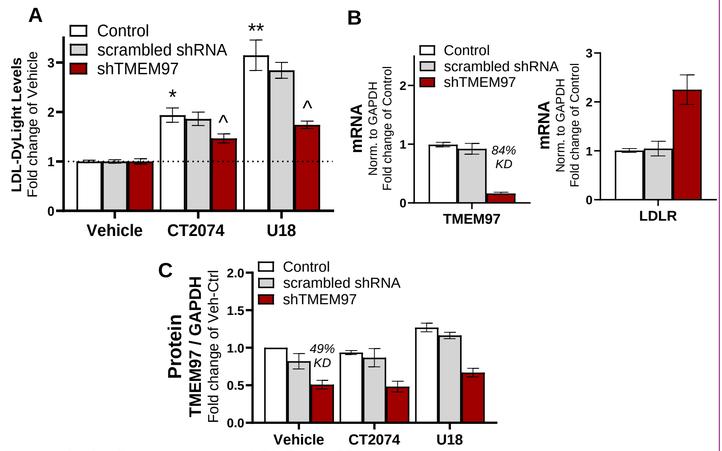
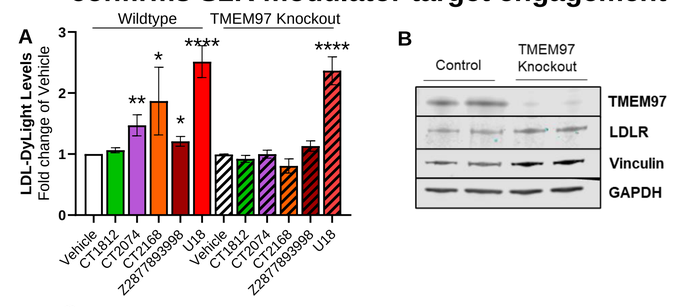


Figure 4. A. Quantification of LDL-Dylight550 fluorescence (spot total intensity per cell) assessed by CX7 Spot Detector after treatment with vehicle (DMSO), CT2074 (10 µM) or U18 (5 µM). N=3 independent experiments, normalized to vehicle; mean +/- SEM, two-way ANOVA. *p<0.05, **p<0.01 compound vs vehicle; ^p<0.05, shTMEM97 vs control. B. qRT-PCR of TMEM97 or LDLR normalized to GAPDH in vehicle-treated cells. C. Densitometry of western blots was performed, with TMEM97 normalized to GAPDH, normalized within experiment as fold change of control; N=5, mean +/- SEM, percent TMEM97 knockdown (KD) indicated.

TMEM97 knockout HEK-293T cell line confirms S2R modulator target engagement



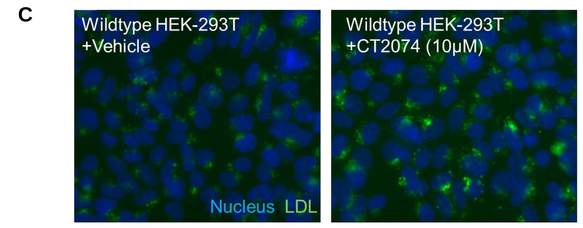


Figure 7. A. Quantification of LDL-Dylight550 fluorescence (spot total intensity per cell) assessed by CX7 Spot Detector after treatment with vehicle (DMSO), CT1812 (10 µM), CT2074 (10 µM), CT2168 (5 µM), Z2877893998 (10 µM), or U18 (1 µM). N=3-6 independent experiments, normalized to vehicle; mean +/- SEM, one-way ANOVA. *p<0.05, **p<0.01, compound vs vehicle. B. Western blots were performed to confirm absence of TMEM97 in TMEM97 knockout HEK-293T cells, and LDLR expression in wildtype and TMEM97 knockout cells. GAPDH and vinculin were assessed as loading controls. C. Representative images of cells treated with vehicle or CT2074, labeled with LDL-DyLight (*green*) and Hoechst (*blue*).

CONCLUSIONS

- Chemically distinct S2R modulators exhibit differential capacity to increase LDL uptake in RPE cells.
- S2R modulators induce LDL uptake in a TMEM97-dependent manner.
- Given lipid homeostasis is important for vesicular and POS trafficking, these data support the potential of S2R modulators to alter critical RPE functions through LDL/LDLR.
- Ongoing work aims to further develop robust tools to study S2R signaling to identify mechanisms underlying S2R modulator effects on RPE trafficking functions.

References:
1. Malagise E. et al. Sigma-2 receptor modulators rescue POS trafficking deficits in RPE-based models of dry AMD. Poster presented at ARVO 2022; Denver, CO.
2. Ebrahimi-Fakhari D. et al. Reduction of TMEM97 increases NPC1 protein levels and restores cholesterol trafficking in Niemann-pick type C1 disease cells. *Hum. Mol. Gen.* 2016; 25(16):3588-3599.
3. Rao, S.R. et al. Compromised phagosome maturation underlies RPE pathology in cell culture and whole animal models of Smith-Lemli-Opitz Syndrome. *Autophagy.* 2018; 14(10):1796-1817.
4. Sreekumar, P.G. et al. Oxidative Stress and Lipid Accumulation Augments Cell Death in LDLR-Deficient RPE Cells and Ldlr−/− Mice. *Cells.* 2022; 12(1): 43.
5. Claudepierre, T. et al. Lack of Niemann–Pick type C1 induces age-related degeneration in the mouse retina. *Mol. Cell. Neurosci.* 2010; 43(1): 164-176.
6. Izzo, N.J. et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. *Alzheimers. Dement.* 2021; 17(8); 1365-1382.
7. Hazim, R.A. et al. Rapid differentiation of the human RPE cell line, ARPE-19, induced by nicotinamide. *Exp. Eye Res.* 2019; 179: 18-24.
8. Reaver, A. et al. Differentiated retinal pigment epithelial cells as a model for uncovering sigma-2 receptor functions and novel therapeutics for dry AMD. Poster presented at ARVO 2024; Seattle, WA.
9. Alon, A., Lyu, J., Braz, J.M. et al. Structures of the σ2 receptor enable docking for bioactive ligand discovery. *Nature.* 2021; 600: 759–764.
10. Shen H. et al. BRD2 regulation of sigma-2 receptor upon cholesterol deprivation. *Life Science Alliance.* 2020; 4(1).

Contacts:
blizama@cogrx.com
mhamby@cogrx.com

COGNITION™ Therapeutics