Testing The Ability of Novel Drugs to Inhibit TNFα-Induced Inflammation via NFκB Activation Pathway Using Luciferase Assay in HEK293 cells

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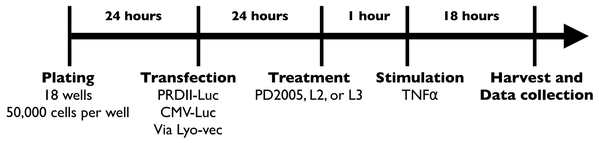
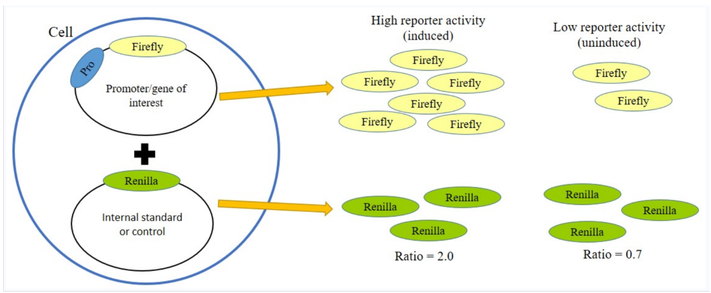
Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and chronic neuroinflammation. Inflammatory signaling pathways such as nuclear factor-κB (NF-κB) play a critical role in the progression of neurodegeneration by regulating the expression of pro-inflammatory cytokines such as TNF-α and IL-1β. Targeting NF-κB signaling therefore represents a promising therapeutic strategy for reducing inflammation associated with AD. This study evaluated the effects of several novel anti-inflammatory compounds provided by P2D Biosciences and Dr. Geen's research lab on TNF-α–induced NF-κB activation. HEK293 cells were transfected with an NF-κB responsive PRDII luciferase reporter and a CMV luciferase control, followed by treatment with novel compounds and stimulation with TNF-α. Luciferase activity was measured to quantify the effect of our molecules on TNF-α-induced NF-κB transcriptional activation. Results demonstrated dose-dependent reductions in NF-κB activation for several compounds, suggesting potential anti-inflammatory activity. These findings contribute to ongoing efforts to identify novel small molecules capable of modulating NF-κB signaling and may support future therapeutic development targeting neuroinflammation in Alzheimer's disease.

Methods



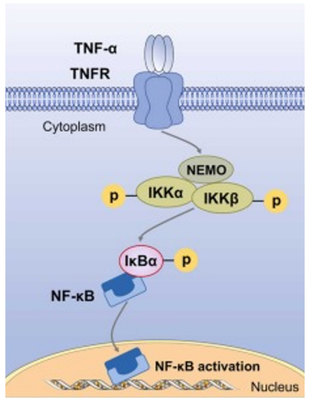


UEBio. (n.d.). Dual-luciferase reporter assay diagram [Figure]. UEBio. http://www.uebio.com/newsDe_2.html

Hypothesis

PD2005, L2, and L3 reduce inflammation by inhibiting NF-κB activation.

NF-κB Signaling Pathway



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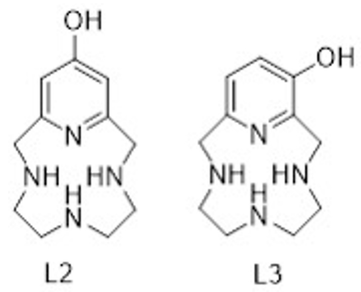
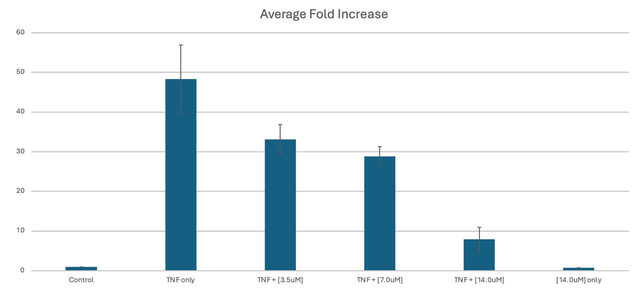
Compounds

PD2005



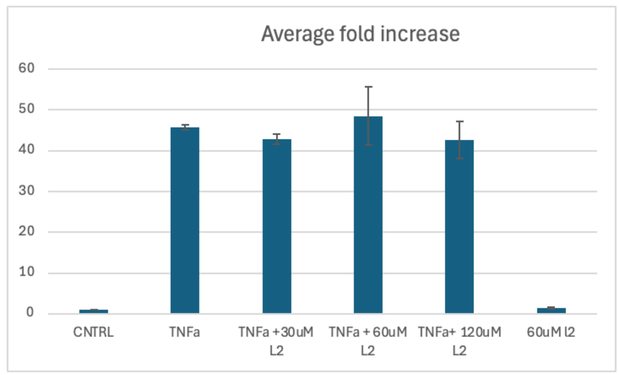
Results

Effect of PD2005 on TNFα induced NFκB activation (fold increase) in HEK293 cells

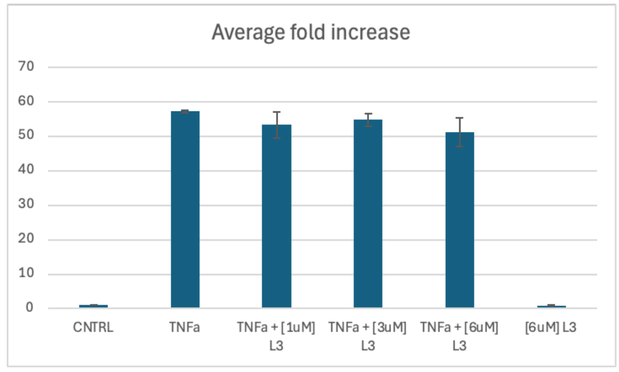


Results

Effect of L2 on TNFα induced NFκB activation (fold increase) in HEK293 cells



Effect of L3 on TNFα induced NFκB activation (fold increase) in HEK293 cells



Conclusions

- PD2005 reduces inflammation in HEK293 cells by inhibiting TNF-α–induced NF-κB activation in a dose dependent manner.
- Compounds L2 and L3 do not inhibit TNF-α–induced NF-κB activation in any significant way.
- Further testing is needed to confirm the mechanism of action for each compound.

Acknowledgements

- PD2005 provided by P2D Biosciences
- L2 and L3 provided by Green Research Lab