Testing the Ability of Macrocyclic Compounds to Reduce Reactive Oxygen Species (ROS)

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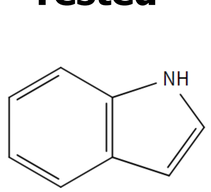
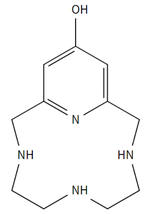
Abstract

In the pathogenesis of neurodegenerative inflammatory diseases, such as Alzheimer's disease, there is an abnormal buildup of redox metal ions that associate with β-amyloid plaques and convert oxygen into oxygen radicals. These radicals are highly reactive with cellular components and lead to oxidative stress that induces damage and death of neuronal cells which is associated with the cognitive decline of Alzheimer's disease. Bifunctional macrocyclic compounds with antioxidant properties are a promising potential therapeutic to reduce levels of reactive oxygen species (ROS) and increase neuronal cell survival via the ability to chelate dysregulated metal ions and radical scavenging. In this project, novel macrocyclic compounds were tested for their efficacy in reducing intracellular levels of H_2O_2-induced ROS and H_2O_2-induced cytotoxicity. Intracellular ROS levels and cell survival were quantified in FRDA and BV-2 cells using the DCFH-DA and MTT cytotoxicity assays.

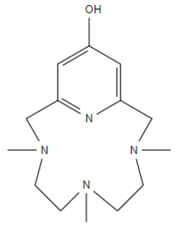
Structures of Compounds Tested



Indole

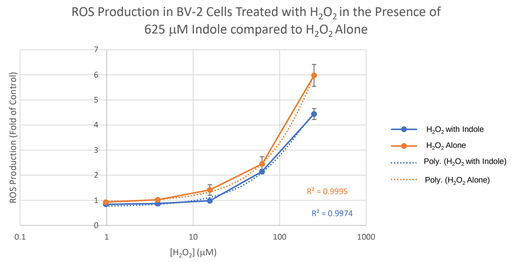


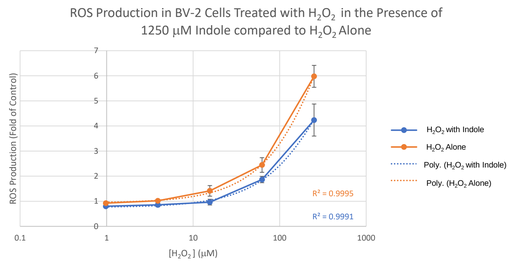
L2



PK95

Indole





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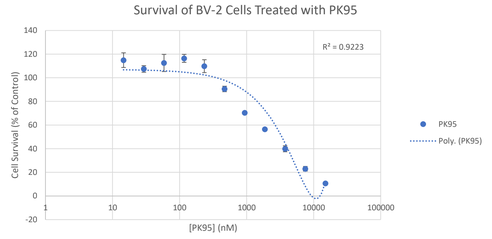
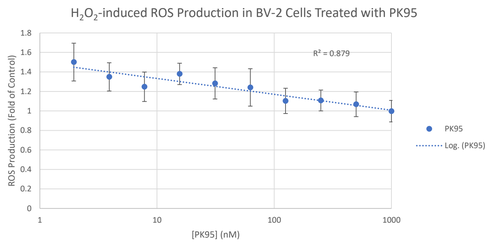




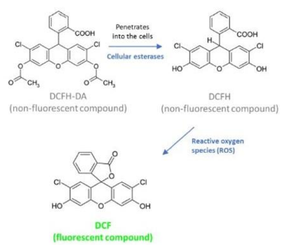
Background

- Alzheimer's Disease (AD), is characterized by the presence of β-amyloid plaques and hyperphosphorylated tau proteins
- Elevated levels of redox metal ions in AD that's correlated with Aβ plaque deposition and ROS generation
- ROS promotes neuroinflammation and leads to cellular damage and death
- FRDA cells are fibroblast cells from patients with Friedrich's Ataxia and are a good model for manipulating intracellular ROS since they have heightened sensitivity to redox state at baseline
- BV-2 cells are microglial cells that are immune cells in the CNS and are a good cell line to test in since in the pathogenesis of Alzheimer's disease, microglial cells release ROS and cytokines onto neuronal cells that lead to their death

Assays

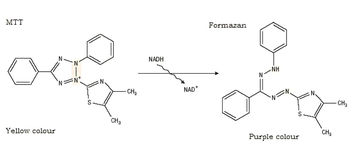
DCFH-DA Assay

- Measures levels of intracellular ROS

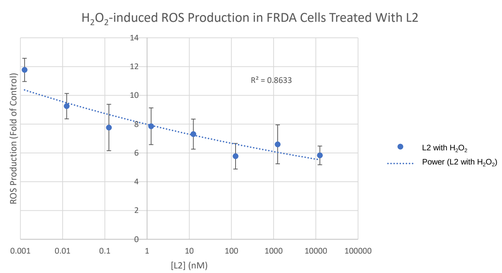


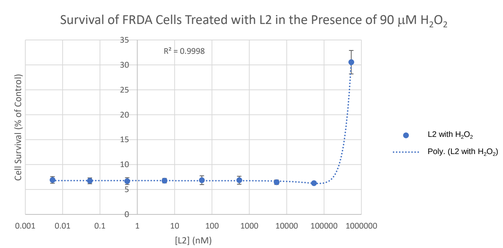
MTT Cytotoxicity Assay

- Measures cell survival



L2





Summary

- Indole reduced the rate of increase of H_2O_2 – induced ROS in BV-2 cells at concentrations of 625 μM and 1250 μM Indole
- L2 reduced intracellular levels of H_2O_2 –induced ROS by 50% in BV-2 cells and increased cell survival from 7% to 30% at the highest concentration of L2 used
- PK95 had no effect on intracellular levels of H_2O_2 – induced ROS in BV-2 cells

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Inflammation has been implicated in the pathology of AD. This inflammation is triggered by the presence of abnormal proteins like Aβ and tau and elevated levels of metals. This results in the formation of high energy oxygen species in the brain leading to damage and death of neurons. In this project, compounds designed to have antioxidant and metal-binding properties are tested for their efficacy in reducing levels of these damaging molecules.