Characterization of BBB Integrity Enhancers as Novel Compounds for the Treatment of Alzheimer’s Disease: In vitro and In vivo studies
K. Elfakhri\(^1\), Q-V. Duong\(^1\), C. Langley\(^1\), A. H. Depaula\(^1\), T. Lebeouf\(^1\), A. Kaddoumi\(^2\)
\(^1\)University of Louisiana at Monroe, \(^2\)Auburn University

**Purpose**
Alzheimer disease (AD) is a neurodegenerative disorder and the most common form of dementia with complex pathophysiology mechanisms. Available approved drugs only treat the symptoms but not the underlying cause of AD. Therefore, in our lab we have recently developed an in vitro high throughput screening (HTS) assay to screen for compounds that are able to increase integrity of a cell-based blood-brain barrier (BBB) model. Results from HTS identified multiple compounds that enhanced the in vitro model integrity. The purpose of this study is to further evaluate these compounds against AD-related pathology.

**Methods**
Dose dependent screening assays to determine the EC\(_{50}\) of identified compounds (AD1-AD6) for their potency to enhance the in vitro BBB model integrity, and for their effect on increasing amyloid beta (A\(_\beta\), a hallmark of AD) transport across the monolayer. Integrity studies were performed by determining compounds effect on Lucifer Yellow permeability, and A\(_\beta\) transport studies were performed to evaluate compounds effect on A\(_\beta\) clearance. The mechanisms by which these compounds altered the integrity and clearance were monitored by Western blot. Finally, most potent compound (AD6) was further studied in vivo to investigate its effect on BBB integrity and brain A\(_\beta\) levels.

**Results**
The in vitro studies demonstrated compounds have different potencies to enhance in vitro BBB model integrity and A\(_\beta\) transport. Such effects were associated with different effect on modulating the expression of tight junction proteins such as claudin-5, occludin and ZO1, and A\(_\beta\) transport proteins LRP1 and P-glycoprotein. Hit compound AD6 was evaluated in vivo as it showed highest in vitro potency to enhance BBB integrity and A\(_\beta\) transport. In vivo findings of AD6 were consistent with in vitro data and overall brain A\(_\beta\) load was significantly reduced when compared to vehicle treatment.

**Conclusion**
Our findings suggest that AD6 is a great candidate for further development to treat, slow or hold the progression of AD