Synthesis and Pharmacological Screening of Novel Chemical Chaperones Against Tunicamycin-Induced Endoplasmic Reticulum Stress and Renal Cell Death

Presented by: Dalia Abdel Sattar

College of Pharmacy, Qatar University, Doha, Qatar

Co-Authors:

Mahtab Noorizadeh — Omaima Mahmoud

Dr. Ashraf Khalil — Dr. Shankar Munusamy

DUPHAT 2015 Conference Dubai, UAE

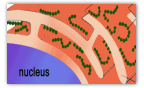
8 - 10 March 2015

ER Stress and Kidney Disease

- Endoplasmic reticulum (ER) plays a central role in protein folding and maturation.
- Metabolic disturbances impair ER function and trigger ER stress in the kidney and leads to renal cell death[1].
- 4-Phenylbutyric acid (4-PBA) - a drug to treat urea cycle disorders – also possess "**chemical chaperone**" properties[2]
  - Shown to protect the diabetic kidney against ER stress-induced renal damage[3]
- **Limitation:** Low potency ➔ Requires high doses (in grams) to exert therapeutic effects.

Aim: *To develop novel, potent derivatives of 4-PBA that would protect the kidney against ER stress and associated cell death*

Metabolic Alterations (⇑Glucose, Albumin, FFA, etc.)

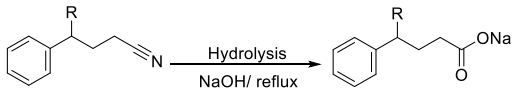


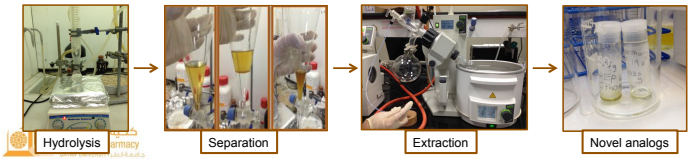
Renal Cell Apoptosis

Kidney Disease

2

Methods: Synthesis of Analogs





Methods: Pharmacological Screening

➢ **NRK-52E** (Normal Rat Kidney) **cells**

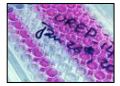
Plate cells	Pre-tx. with 4-PBA or Analogs	Tunicamycin (For 5 hours)	Recovery Phase (For 19 hours)

- 24 h **0 h** **24 h** **48 h**

Western blotting

ER Stress Marker - GRP78

MTT Assay



Cell Viability

4

Results: Synthesis & Characterization

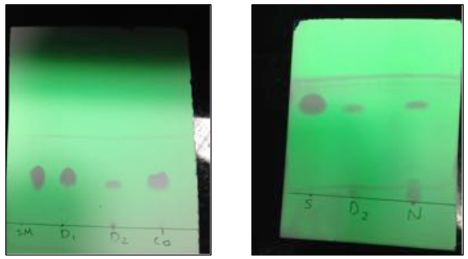
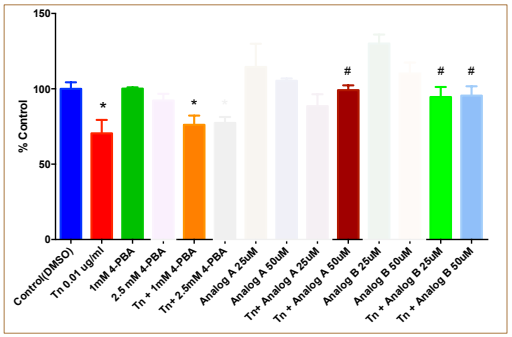


Figure 1 and 2. TLC of 4-PBA analogs A and B respectively

Results: Cell Viability



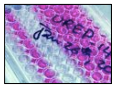


Figure 3: Cell Viabilty Assay. Cells were pre-treated with 4-PBA or analogs for 24h and then exposed to Tunicamycin (Tn) for 5 h. After 5 h, cells were washed and allowed to recover for 19 h. After 24 h of initial exposure to Tn, MTT assay was performed and absorbance was measured at 570 nm using a microplate reader. Values are expressed as Mean±SEM (n = 3). *P < 0.05 compared to control and # P < 0.05 compared to Tn treated group.

Results: ER Stress – GRP78 Expression

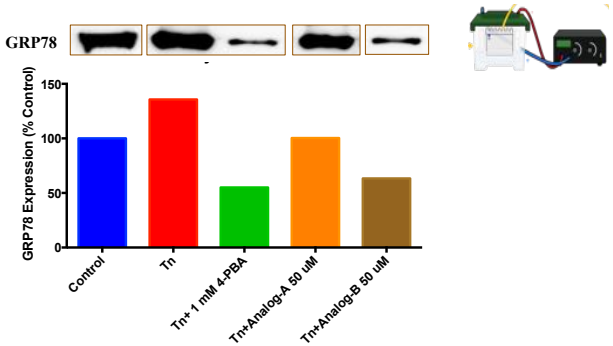


Figure 4. GRP78 Expression. *Upper Panel* – Western blot showing the expression of ER chaperone GRP78 in NRK-52E cells *pre-treated* for 24 h with 4-PBA or analogs and then subjected to Tunicamycin (Tn) mediated ER stress. *Lower Panel* – Densitometry analysis of the western blot with the GRP78 expression of treatment groups normalized to control group (set as 100%).

Summary and Conclusions

- ✓ Two 4-PBA analogs - **4-methoxy-4-phenyl butyric acid** (Analog-A) and **4-ethoxy-4-phenyl butyric acid** (Analog-B) - were synthesized.
- ✓ Both analogs (A & B) possess **cytoprotective properties** and **chemical chaperone potential** to prevent ER stress and associated cell death.
  - ➢ Effective at 25 to 50 µM conc. (vs. 4-PBA used at 1 to 2.5 mM conc.) i.e., **both analogs were 50 to 100 times more potent than 4-PBA**.
- ✓ Our study warrants further investigation of these prepared analogs in animal models to confirm their activity *in vivo*.
- ✓ If proven effective, the *novel 4-PBA analogs could be potentially used to treat ER stress-induced kidney damage in patients with diabetes, obesity and other disorders associated with severe ER stress.*

Acknowledgements

- **Research Mentors**
  - Dr. Shankar Munusamy
  - Dr. Ashraf Khalil
- **Technical Support:**
  - Vinitha Kuruvilla
  - Heba El-Gamal
  - Ayat Hammad
  - Shakila Afroz

Financial Support: *This report was made possible by a UREP award [UREP14-008-3-006] from the Qatar National Research Fund (a member of The Qatar Foundation). The statements made herein are solely the responsibility of the author[s].*

Thank you