A drug discovery pipeline for endoplasmic reticulum stress inhibitors from natural products

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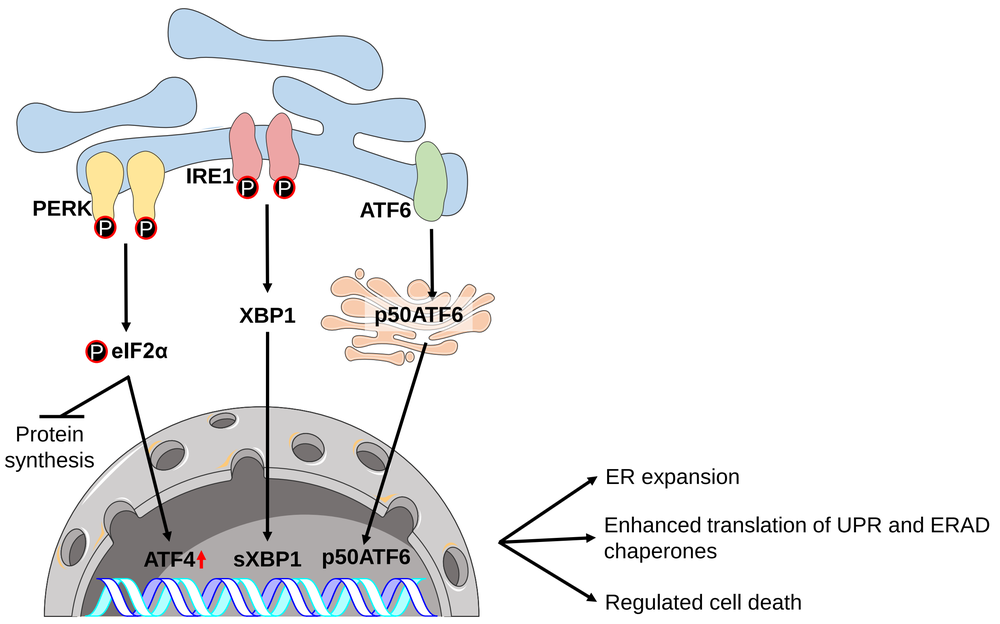
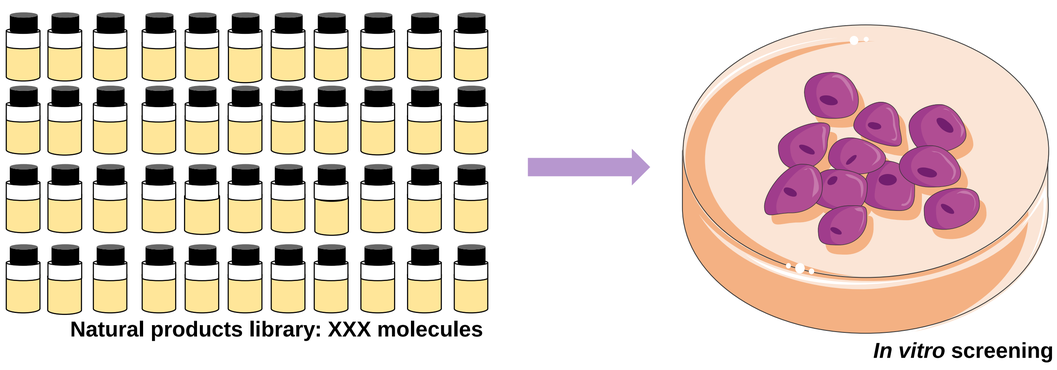
The **endoplasmic reticulum (ER)** is the largest organelle of the eukaryotic cell, comprising an intricate network of tubules and branches that emerge from the nucleus and are distributed throughout the cytoplasm. Analogously to its volume in the cell, the ER plays a role of the outmost importance in the homeostasis of a wide array of cellular processes, even though it is classically associated to its main function, which is the synthesis and folding of proteins. It is in the ER that the synthesis of the majority of the proteins takes place. Furthermore, this organelle is the main calcium reservoir of the cell, being in charge of the tight regulation of its levels in order to be able to maintain the homeostasis of events that include cell proliferation, differentiation, metabolism, apoptosis and gene expression. For these reasons, the ER is crucial for the development of any eukaryotic organism [1].

When the homeostasis of the ER is disturbed, the amount of newly synthesized proteins surpasses the amount which is exiting the ER, building up to the accumulation and aggregation of misfolded and/or unfolded proteins in the ER lumen. At this point, the ER counters by triggering the unfolded protein response (UPR), a chain of molecular events that has evolved towards attempting to restore homeostatic conditions when stress at the ER is recognized.

ER stress and consequent UPR activation have been established as a hallmark of chronic pathologies, such as neurodegenerative diseases. Protein aggregation into amyloid fibrils is a major event in the pathophysiology of these disorders. The accumulation of the cytotoxic precursors of these fibrils in cells creates an environment for chronic UPR activation and eventual loss of neurons *via* regulated cell death mechanisms. For this reason, this study aims to identify molecules that can protect particularly susceptible cells (fibroblasts and neurons) against ER stress [2].



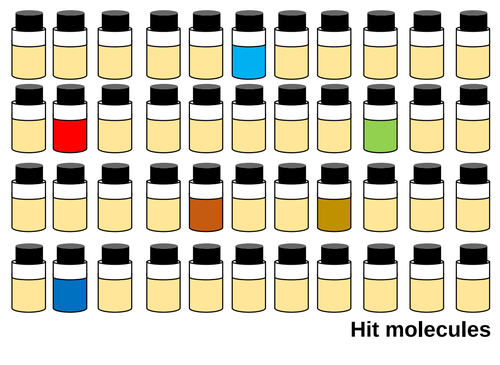


MTT reduction assay: cell viability

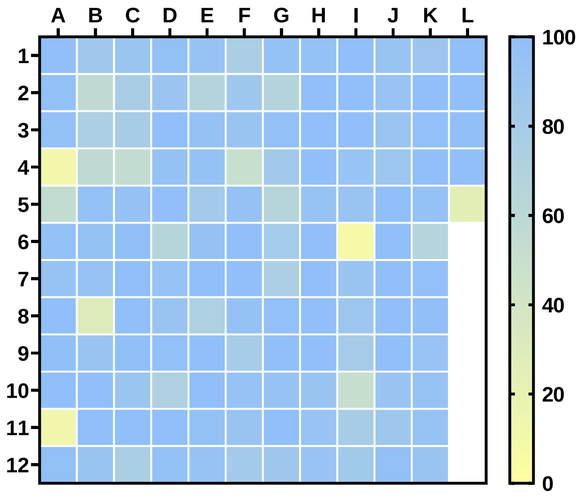
Fura-2/AM fluorescent probe: cytosolic calcium level

RT-qPCR: expression of UPR-related genes

Thioflavin T: $A\beta_{25-35}$-induced protein aggregation



1. Cell viability



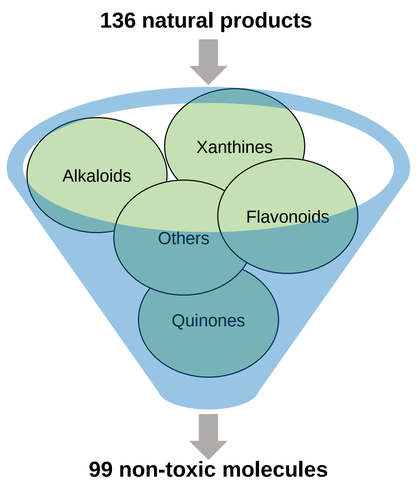


Fig. 1. Impact of a library of natural compounds at 50 µM on the viability of MRC-5 fibroblasts.

Exclusion of cytotoxic molecules → Reduced chemical library → Screening for protective compounds against ER stress

2. Cytosolic calcium levels

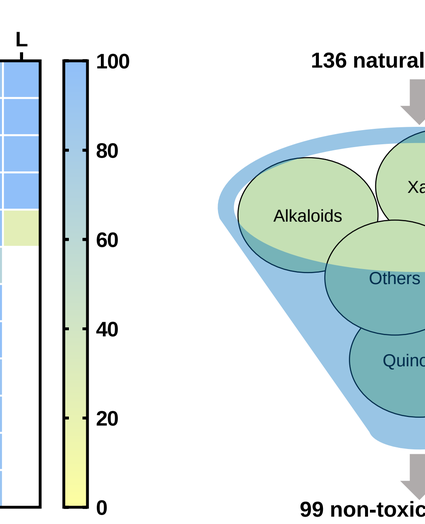


Fig. 2. Impact of non-toxic molecules on calcium ionophore-induced rise of cytosolic calcium levels on MRC-5 cells.

6 polyphenols (5-deoxykaempferol [A10], delphinidin [B12], fisetin [C8], naringenin [F3], naringin [F5], quercetin-3-β-D-glucoside [G4]);
1 anthraquinone (sennoside B [H2]);
1 fatty acid (myristic acid [F1]);
1 polyamine (spermine [H4])

Molecules that preserve calcium homeostasis → Screening for UPR modulators

3. UPR-related gene expression

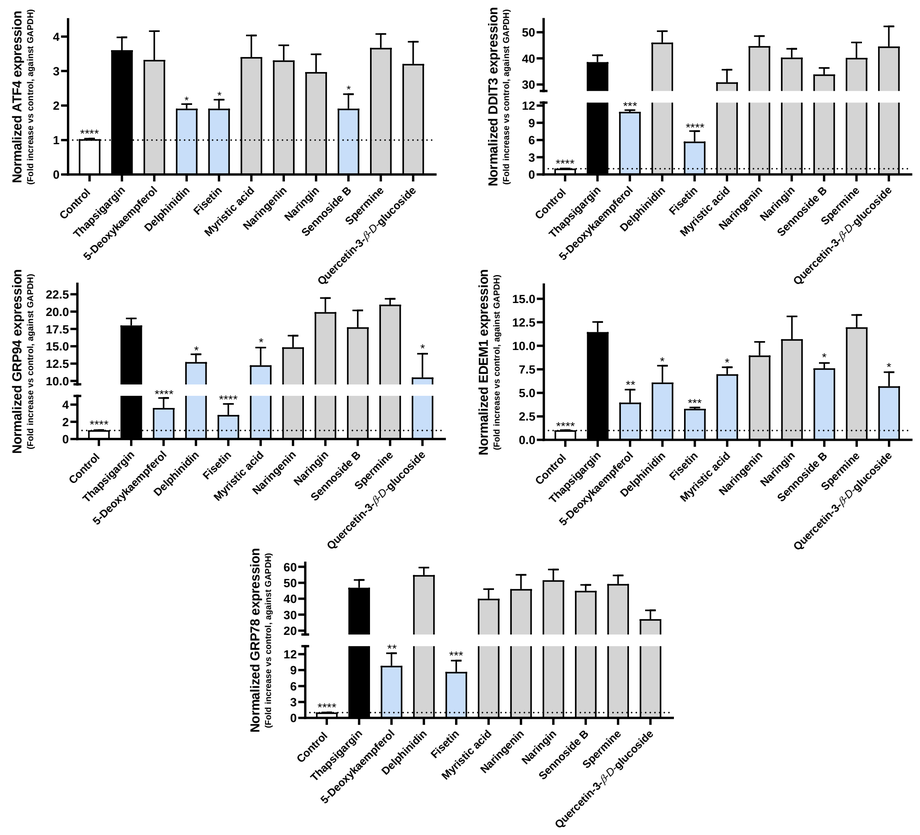


Fig. 3. Effect of selected natural products upon thapsigargin-induced (ER stresser) expression of CHOP, BiP, GRP94, ATF4 and EDEM1 genes on MRC-5 cells.

Identification of molecules that ameliorate UPR activation → Evaluation of their impact on protein aggregation

4. Protein aggregation

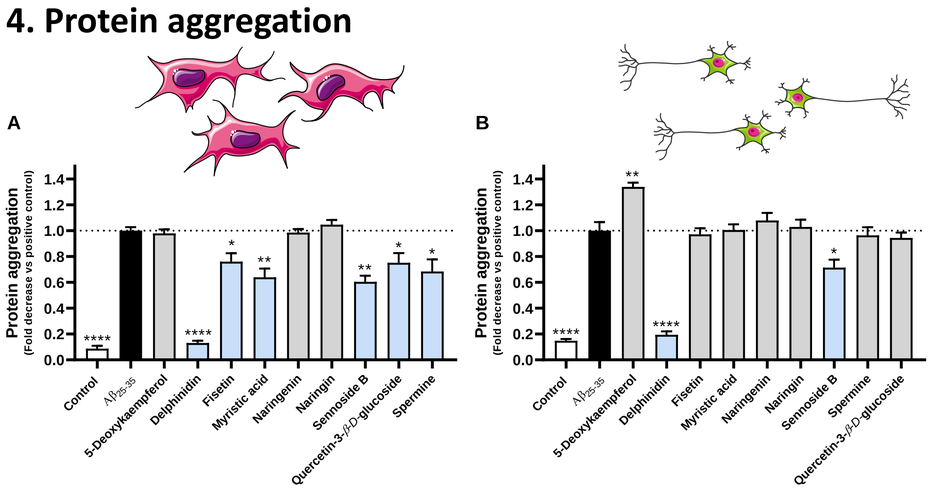


Fig. 4. Effect of bioactive molecules on $A\beta_{25-35}$-induced protein aggregation in MRC-5 (A) and SH-SY5Y (B) cells, as evaluated by determination of the fluorescence of thioflavin T.

The analysis of 136 natural compounds has resulted in the identification of six molecules that prevent protein aggregation in MRC-5 fibroblasts. Two of them, delphinidin and sennoside B, were also active in the neuronal cell line SH-SY5Y, constituting most promising candidates against neurodegeneration.

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References:
1. Pereira, D.M., et al., *Translating endoplasmic reticulum biology into the clinic: a role for ER-targeted natural products?* Natural Product Reports, 2015. **32**(5): p. 705-722.
2. da Silva, D.C., et al., *Endoplasmic reticulum stress signaling in cancer and neurodegenerative disorders: Tools and strategies to understand its complexity.* Pharmacological Research, 2020. **155**: p. 104702.