

Biochemical and Cellular Characterisation of Caspase Inhibitors for Potential Treatment of Huntington's Disease



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Background

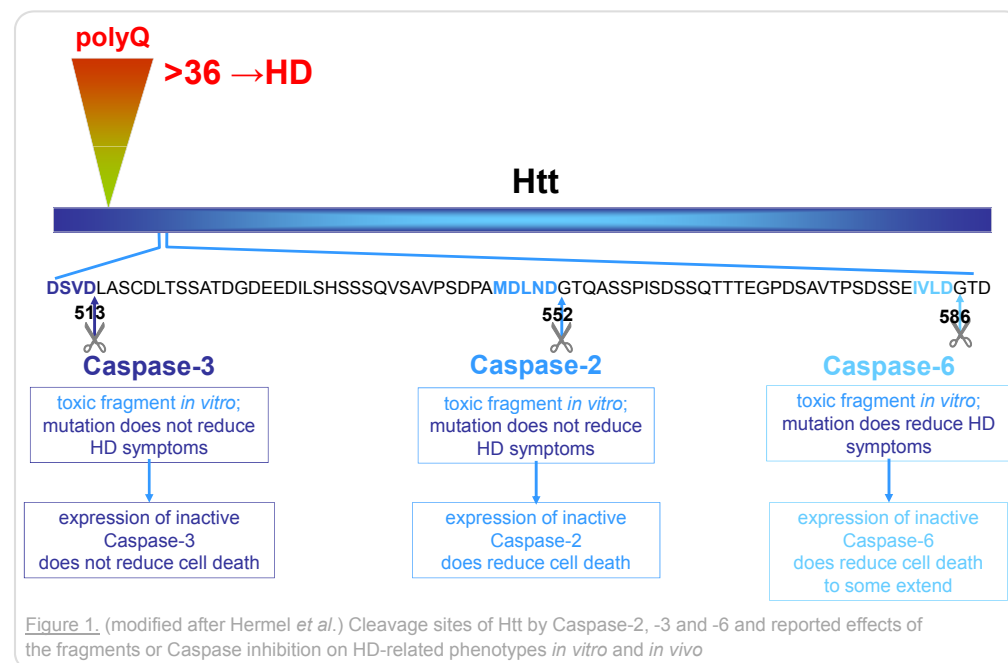
Huntington's disease is an autosomal dominant neurodegenerative disorder that may partly be caused by the proteolytic processing of full length mutant huntingtin (Htt, elongated poly-glutamine stretch).

Htt has been identified as substrate for different caspases; Hermel *et al.*¹ showed that Caspase-2 might play a major role in the initiation of cell death by binding to Htt and selective cleavage at aa 552 in a poly-Q dependent manner. Overexpression of a dominant negative form of Caspase-2 was able to reduce cell-death in primary striatal cells derived from YAC72 mice.

Caspases (cysteine-aspartic-acid-proteases) are a family of Cysteine proteases that cleave proteins after aspartic acid residues. They are the main effectors of apoptosis or programmed cell death.

Caspase-2 is shown to become activated upon stimulation by various reagents/conditions including DNA damaging agents, heat-shock, or nutrient deprivation and the activation depends on localisation of pro-enzyme to specific cellular compartments and formation of a complex with various adaptor molecules.

The biochemical analyses to drive development of structure activity relationship (SAR) together with the assay development of cell based assays for caspase inhibitors will be discussed.



References

- 1: E. Hermel *et al.* (2004): Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease; Cell Death and Differentiation: 11, 424-438
- 2: S. Schaertl *et al.* (2009): Caspases as Drug Discovery Targets for HD; Poster presented 4th Annual HD Conference, Cannes
- 3: J. Andersen *et al.* (2009): Restraint of apoptosis during mitosis through interdomain phosphorylation of caspase-2; The EMBO Journal: 28, 3216-3227
- 4: G. Klaiman *et al.* (2009): Self-activation of Caspase-6 *in vitro* and *in vivo*: Caspase-6 activation does not induce cell death in HEK293T cells; Biochimica et Biophysica Acta: 1793, 592-601

Biochemical Assays

Hit to lead development is an iterative process to design and synthesise structural analogues of compounds, followed by biological and physicochemical evaluation, with the goal to generate lead molecules that can be developed into drug candidates. We characterised the compounds in an assay to discriminate compounds that compete with the substrate from compounds that inhibit the enzyme in an un- or non-competitive fashion. An example for Caspase-2 is depicted below.

Effect of substrate concentration on the IC₅₀ value for a selected compound

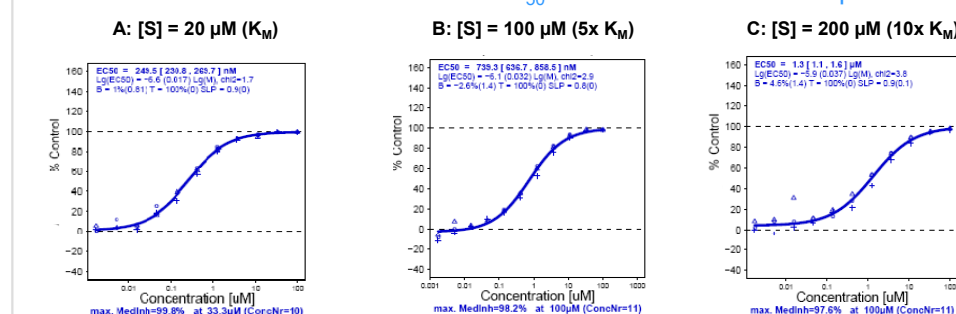


Figure 2. Concentration response curves of a selected compound in the Caspase-2 assay at different substrate concentrations (A-C). The linear increase of IC₅₀ with substrate concentration reveals a competitive inhibition mode (D).

Influence of substrate structure on the IC₅₀ value

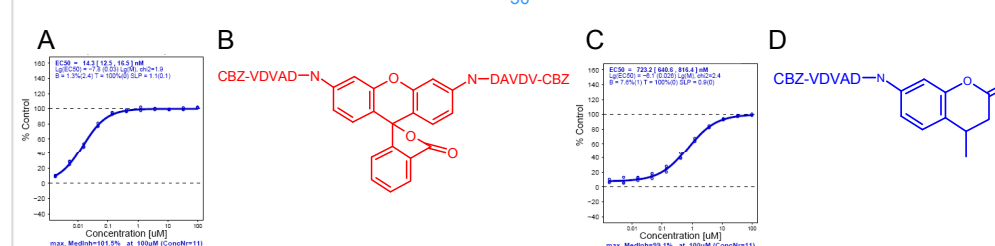


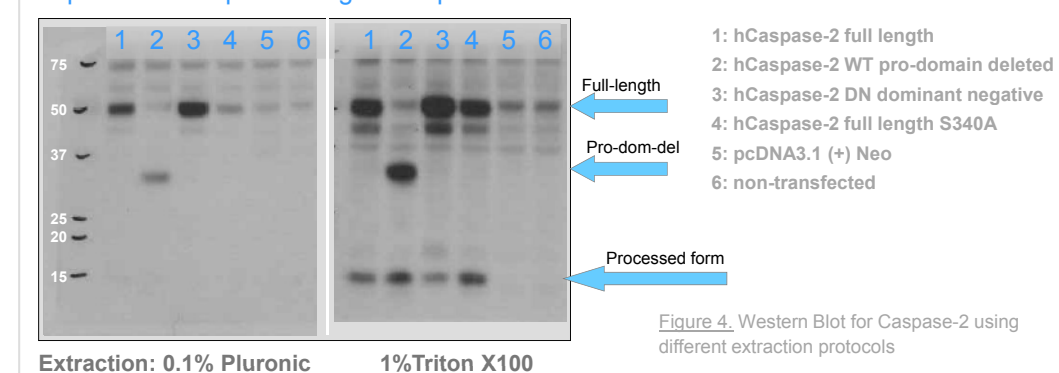
Figure 3. Concentration response curves (A,C) of a selected compound (same as in figure 2) using a commercially available Caspase-2 Rhodamine110-based substrate (B) or a Coumarin120-based substrate (D). Assay conditions were identical for both measurements. A 30-fold lower potency was observed for the Coumarin-based substrate.

Biochemical assays to measure caspase activity are known to be susceptible to assay conditions that affect the oxidative state of catalytic cysteine of caspases. We implemented an extensive triage scheme to run biochemical assays under various assay conditions to filter out potential false positives and promiscuous compounds.² For some compounds, we observed that IC₅₀ can vary depending on the substrate used in the biochemical assay. An example for two commonly used fluorogenic substrates is shown in figure 3. Therefore, we are implementing label-free technologies to re-evaluate the IC₅₀s of the compounds that show inconsistent results in fluorogenic assays.

Cellular Assay Development

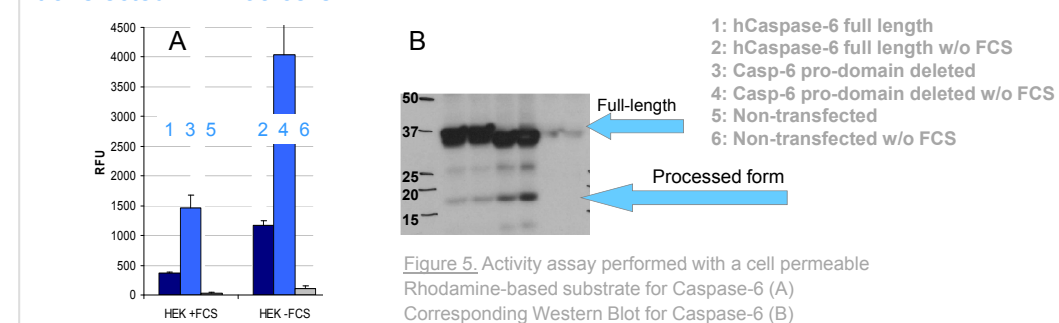
The compounds that passed the series of biochemical assays will be tested in cellular assays to address selectivity and specificity in a cellular context. Therefore, we will evaluate if the Caspase-2 hits can specifically inhibit Caspase-2 activity in a cellular context. A set of Caspase-2 constructs (see figure legend) was used to explore expression and processing of the enzyme in HEK293 cells. A S340A mutant was included to enhance processing of the enzyme as suggested by Andersen *et al.*³

Expression and processing of Caspase-2 in HEK293 cells



In parallel, activity of Caspase-6 is determined in a whole cell assay using transiently transfected HEK293 cells.⁴

Activity of Caspase-6 determined in a whole cell assay using transiently transfected HEK293 cells



Conclusions

- SAR development for proteases should not rely on a single assay format
- hCaspase-2 can be over-expressed and processed in HEK293 cells
- The detection of the processed form of Caspase-2 on Western blots requires harsh extraction conditions, which might indicate that the enzyme has to be released from specific cellular compartments
- Enzymatic activity of full-length hCaspase-6 can be detected in intact over-expressing HEK cells with a fluorogenic substrate
- Increase of hCaspase-6 activity seems to correlate with enhanced processing of the enzyme, induced by serum starvation or by using a pro-domain deleted form of the enzyme (ref. 4 and personal communication by Lisa M. Ellerby)