Design and Synthesis of a New Non-Covalent Caspase-3 Inhibitor with Neuroprotective Property

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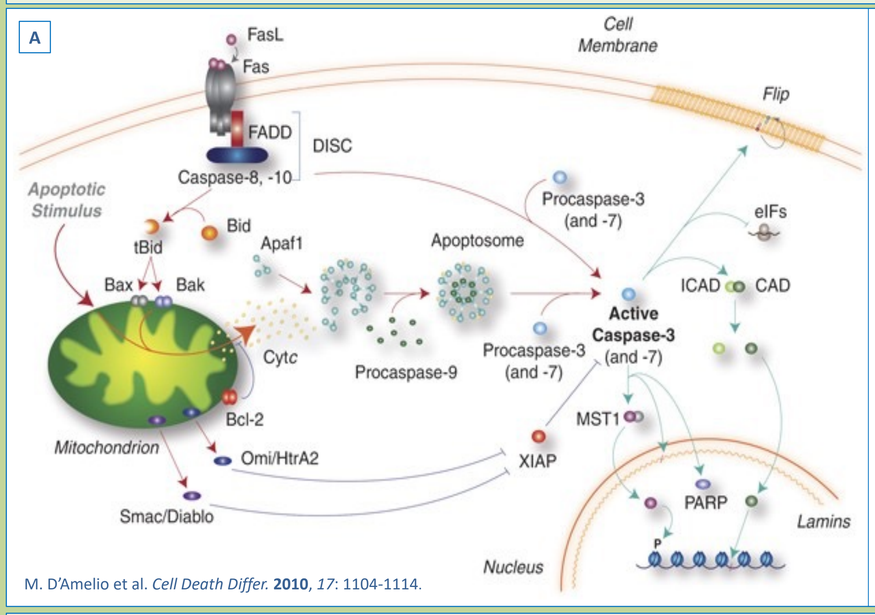
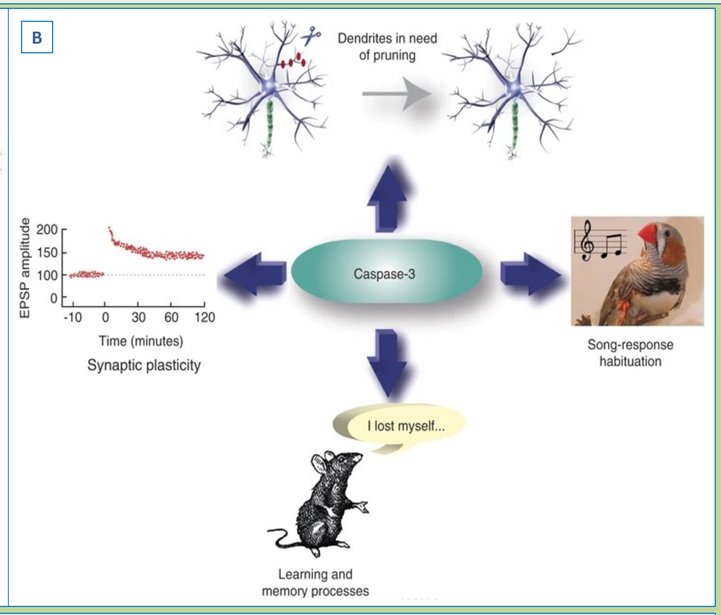
INTRODUCTION

Caspases, the family of cysteine aspartate specific proteases, are well known as killer enzymes driving cell death via apoptosis or pyroptosis.[1] However, the latest findings on the caspases indicate important and non-lethal roles of these enzymes ranging from immune response, cell fate determination, cell proliferation and cellular remodeling.[2] Caspase-3 is the key enzyme in apoptotic processes and when activated executes cell death catalyzing the specific cleavage of many key cellular proteins. It is a key mediator of neuronal programmed cell death and plays an essential role in the development of the nervous system.[3]



M. D'Amelio et al. *Cell Death Differ.* **2010**, *17*: 1104-1114.

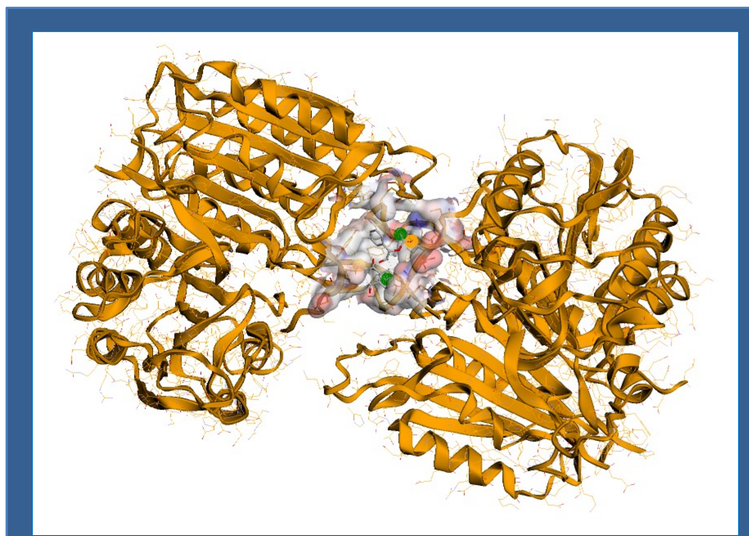


A. Intrinsic and extrinsic pathways of caspase activation in mammals. **B.** Nonapoptotic caspase-3 functions in neuronal cells.[6]

Physiologic non-apoptotic roles have been described for caspase-3 activation in specifics neuronal compartment as in neurite pruning and synaptic plasticity.[4] Its activation is a feature of many chronic neurodegenerative diseases often characterized by perturbations in physiological synapses structure and function as in Alzheimer and Parkinson diseases.[5]

Therefore, these studies validate caspase-3 inhibitors as a novel pharmacological target against multiple diseases.[2b,5]

Many caspase-3 inhibitors have been developed based on covalent mode of action but only few compounds have progressed in clinical trials. Novel, improved, brain penetrable compounds are urgently needed for developing new therapeutics for neurodegenerative pathologies.



Crystal Structure of a Caspase-3 inhibitor bound to the binding site of protein.[7]

Multicomponent reactions.

DESIGN AND SYNTHESIS OF TARGET CASPASE-3 INHIBITORS

We have designed and synthesized via multicomponent reaction (MCR) new non-covalent, non-peptidomimetic, and selective caspase-3 inhibitors.

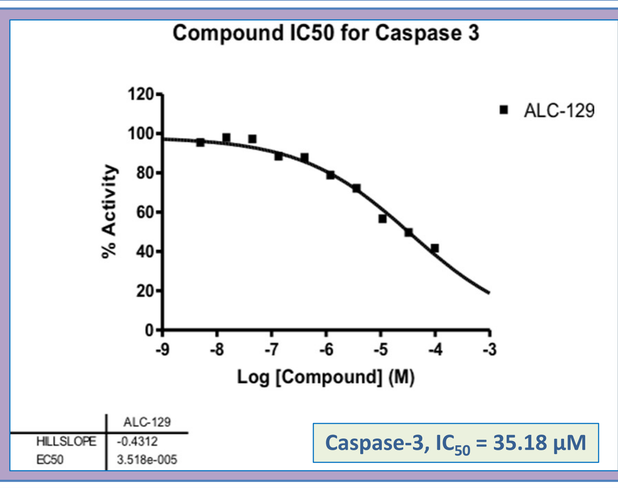
ENZYMATIC ASSAY

The results of enzymatic tests performed on the new series of inhibitors, indicate Caspase-3 activity for compound **ALC-129** and selectivity with respect to Caspase-1.

ALC-129

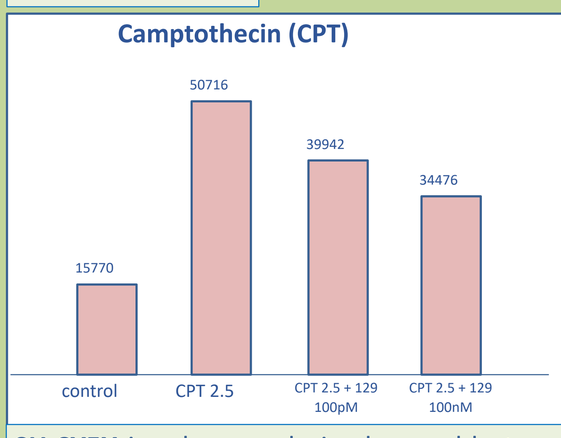
IC_{50} (Caspase-3) = 35.18 µM.

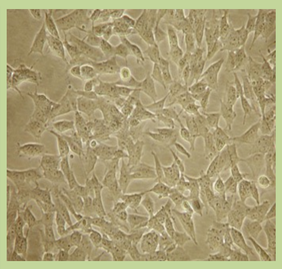
IC_{50} (Caspase-1) > 100 µM.



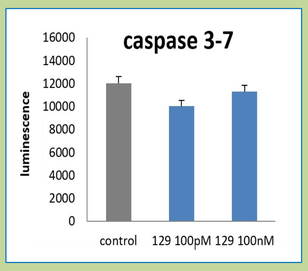
Caspase-3, IC_{50} = 35.18 µM

CELL BASED ASSAY





SH-SY5Y human derived neuroblastoma cell line.

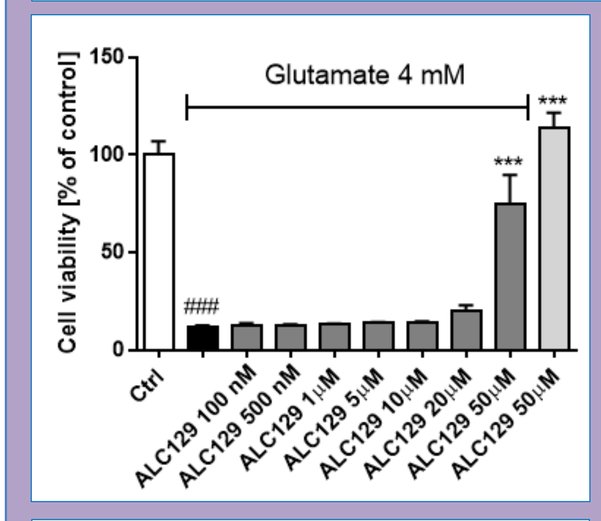


Caspase-Glo® 3/7 Assay System. Luminescent Measure of Caspase-3 activity.

SH-SY5Y is a human derived neuroblastoma cell line used as *in vitro* models of neuronal function.
Deregulation of the intrinsic apoptotic pathway is implicated in various human diseases, such as cancer, autoimmune disorders and neurodegenerative diseases.
Targeting apoptotic components by both enhancing and attenuating apoptosis represents important therapeutic approaches.

NEUROPROTECTION AGAINST GLUTAMATE INDUCED TOXICITY

Caspase-3 role in the mechanism involved in glutamate-induced neurotoxicity via oxidative stress is known.[8] MTT assay results indicate neuroprotective potential in glutamate toxicity for caspase-3 inhibitor ALC-129.



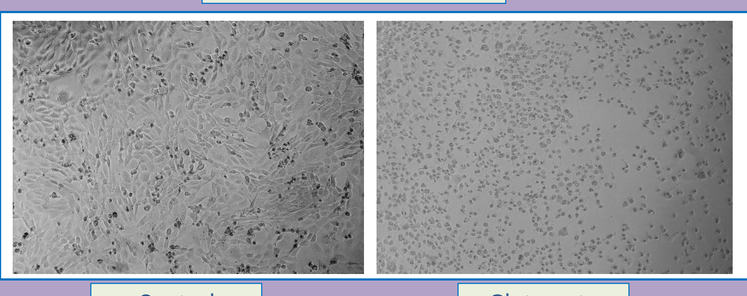
MTT assay, one end-point measurement at 18h following glutamate challenge.

Moreover, ALC-129 alone is not neurotoxic (see final test). Labelled compound ^{11}C-ALC-129 has been prepared for Positron Emission Tomography (PET) preliminary studies.

HT-22 cells

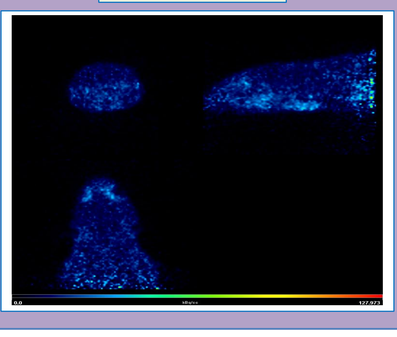
- Immortalized mouse hippocampal cell line
- Glutamate neurotoxicity via oxidative stress, glutathione depletion, mitochondrial dysfunction

HT-22 cell morphology



Control | Glutamate

PET Labelling



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