(-)-Meptazinol-Melatonin Hybrids as Novel Dual Inhibitors of Cholinesterases and Amyloid-β Aggregation with High Antioxidant Potency for Alzheimer's Therapy

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Tanja Krainz

Current Literature Seminar

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What is Alzheimer's Disease?

First described by Dr Alois Alzheimer (1906)

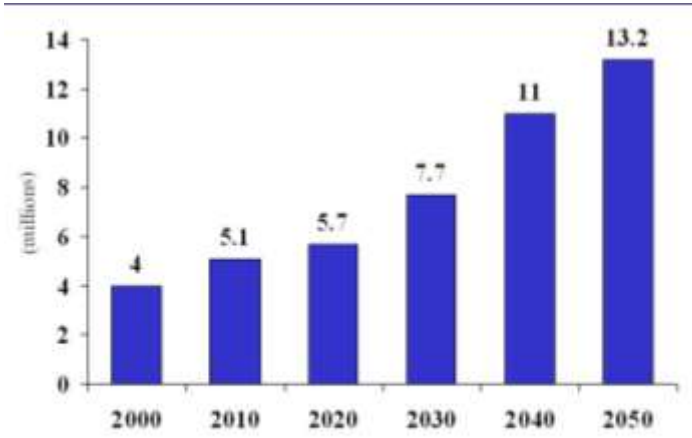
Dr Alois Alzheimer

❑ **Irreversible, progressive brain disorder** that is characterized by impairment of memory and eventually by disturbances in **reasoning, planning**, l**anguage**, and **perception**.

- **Generally diagnosed in people over 65 years of age**
  - *-Early-onset (before 65); only 5-10% of patients*
  - *-Several genetic causes*
- **~ 5 million Americans suffer from it (24 million worldwide)**
  - *-5% of 65-74 years of age*
  - *-Nearly 50% of 85+*
- **1 in 6 women over 55; 1 in 10 men over 55**

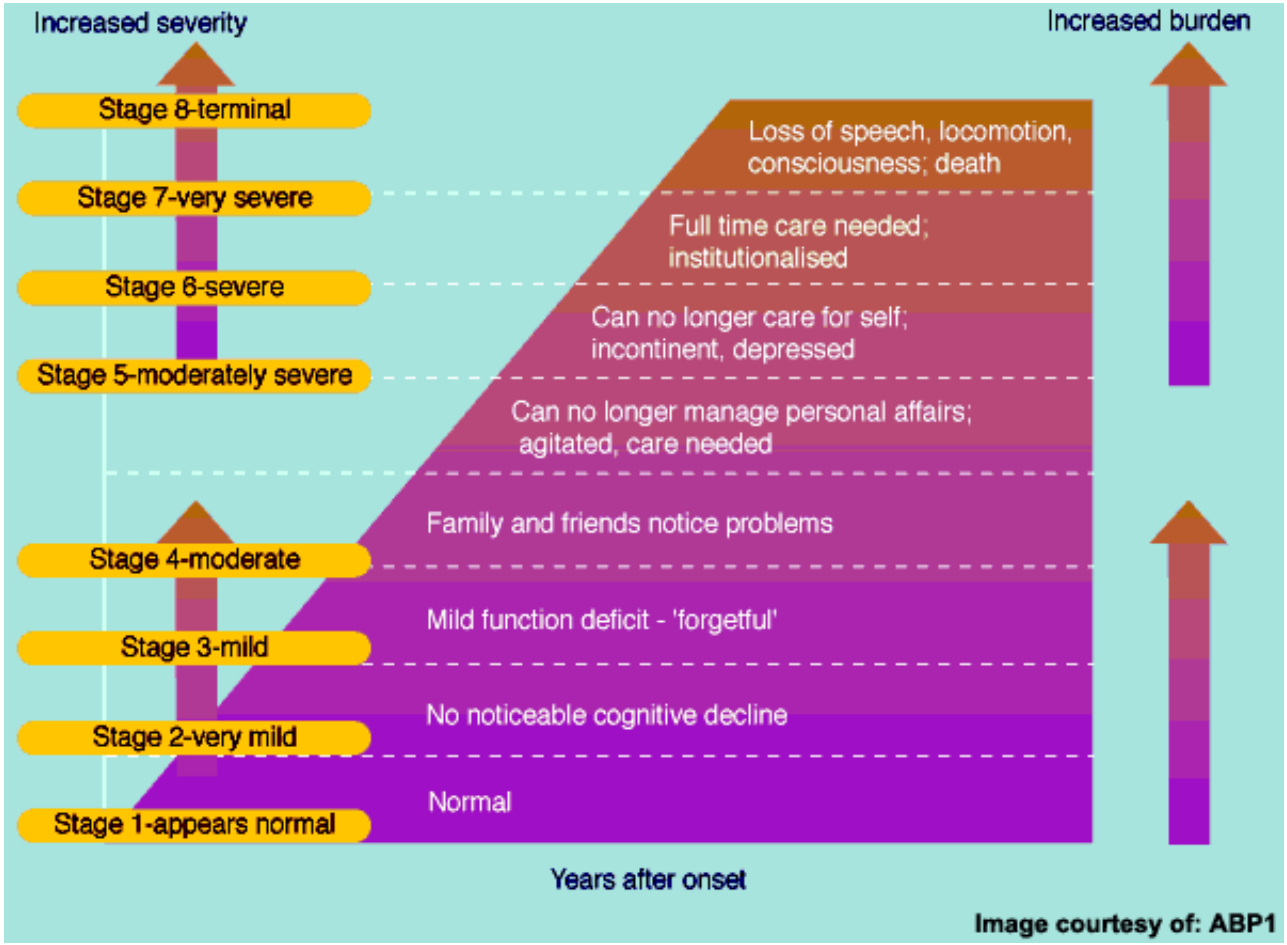
Risk factors:

- Family history, Old age and genetics



The number of Alzheimer's Disease patients is expected to more than triple over the next 50 years

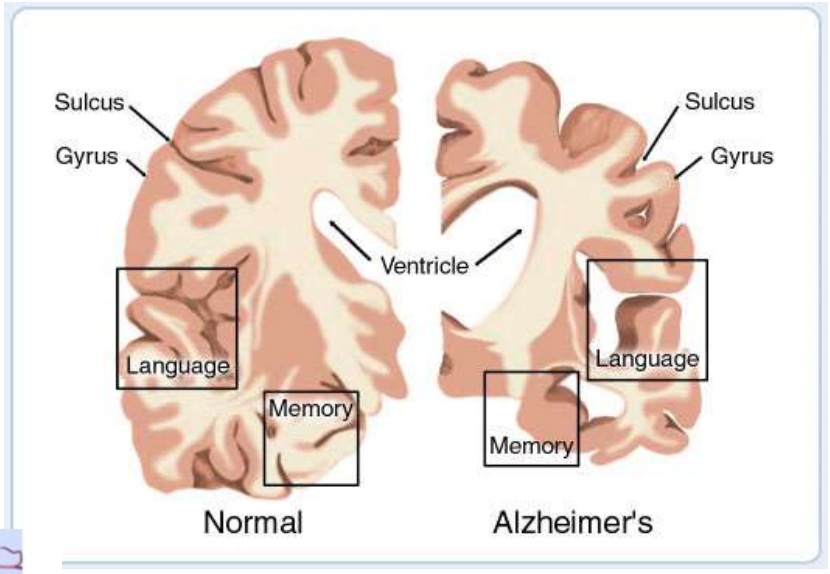
Stages of Alzheimer's Disease

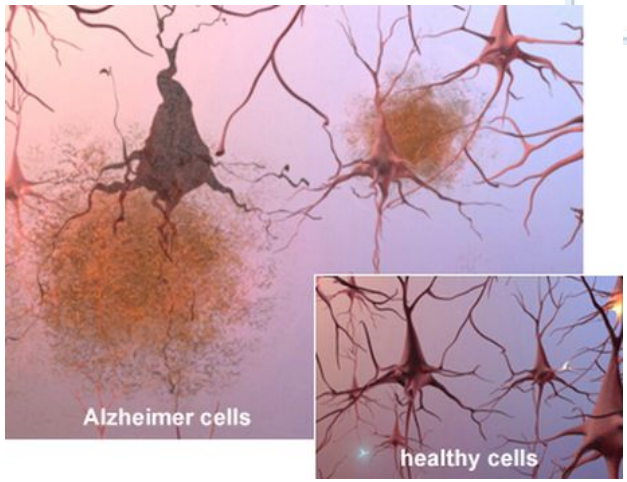


Alzheimer's and the Brain

The brains of people with AD have an abundance of two abnormal structures:

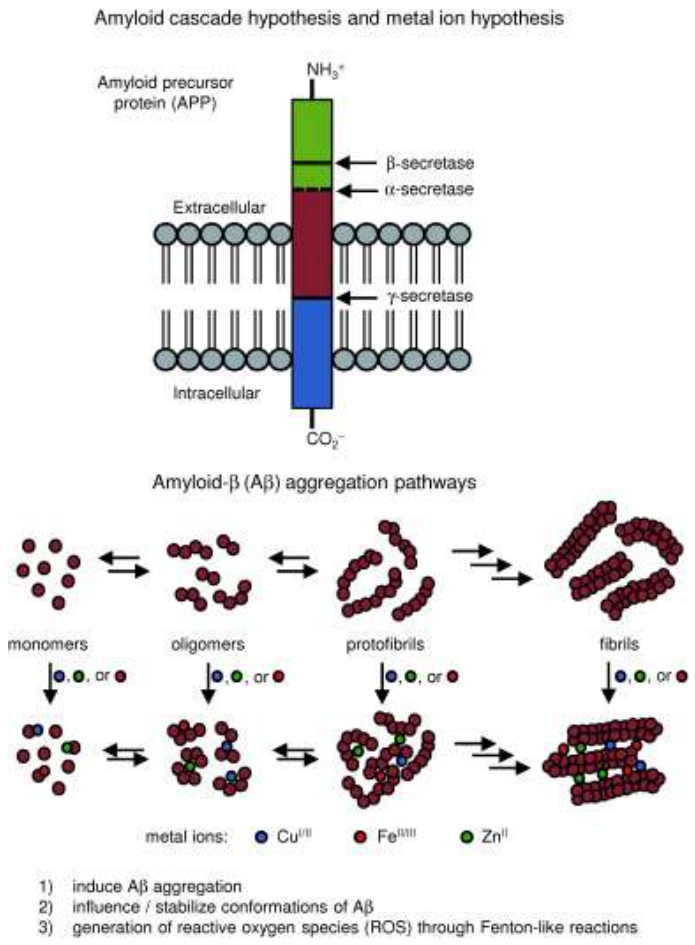
- **β-Amyloid plaques**, which are dense deposits of protein and cellular material that accumulate outside and around nerve cells
- **Neurofibrillary tangles** (NFTs) composed of misfolded, aggregated Aβ peptides and hyperphosphorylated tau protein (ptau)





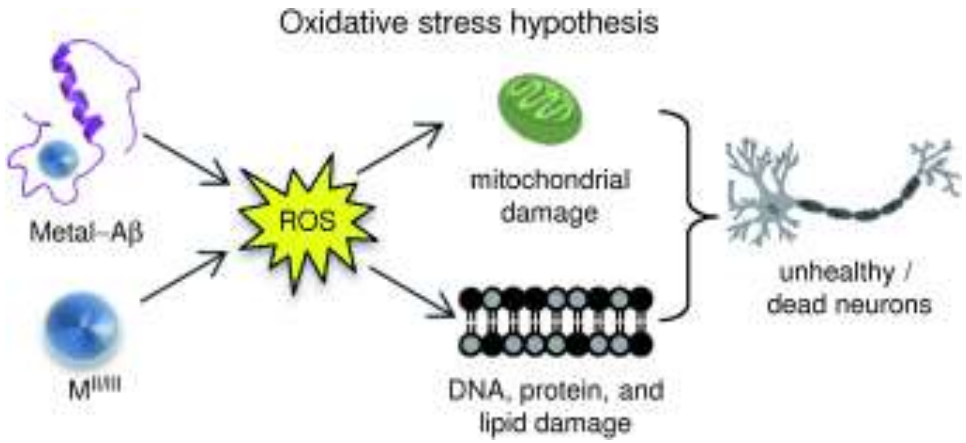
β-Amyloid Plaque Hypothesis

- **Amyloid Cascade Hypothesis**



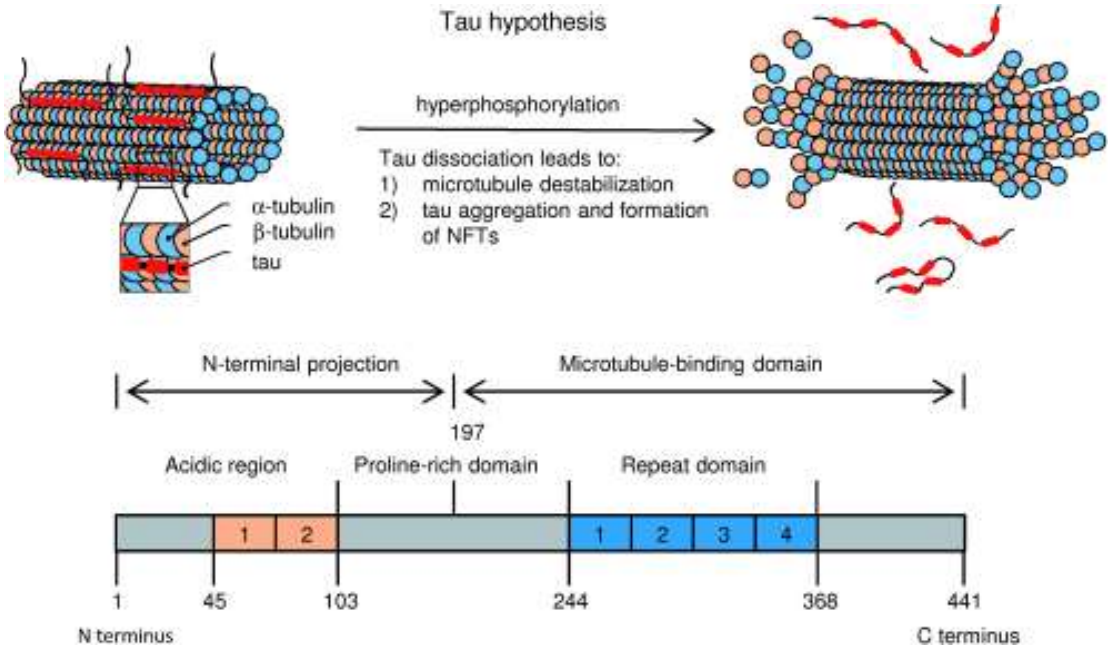
- APP sticks through the neuron membrane.
- Enzymes cut the APP into fragments of protein, including beta-amyloid.
- Isoforms of β-amyloid can go through a slow nucleation stage, followed by fast elongation resulting in the formation of mature aggregated fibrils.
- Overproduction and ineffective clearance of Aβ causes plaque deposition.
- Metal interaction with Aβ species facilitate peptide aggregation pathways, stabilize toxic conformations and generate ROS through Fenton reaction.

Oxidative Stress Hypothesis



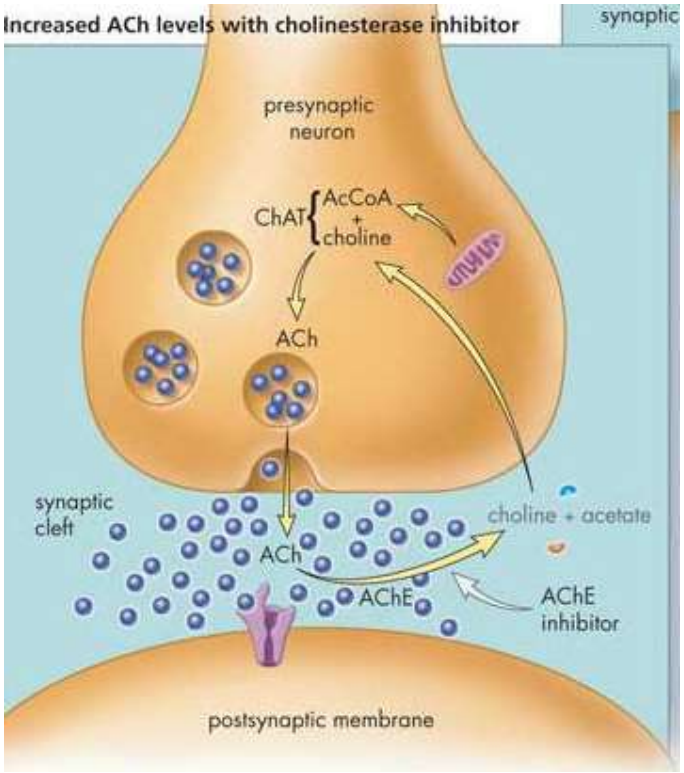
ROS generated and redox-active metal-Aβ can initiate damage of DNA, lipids, and proteins as well as induce mitochondrial dysfunction → induction of neuronal death

Tau Hypothesis in Alzheimer's



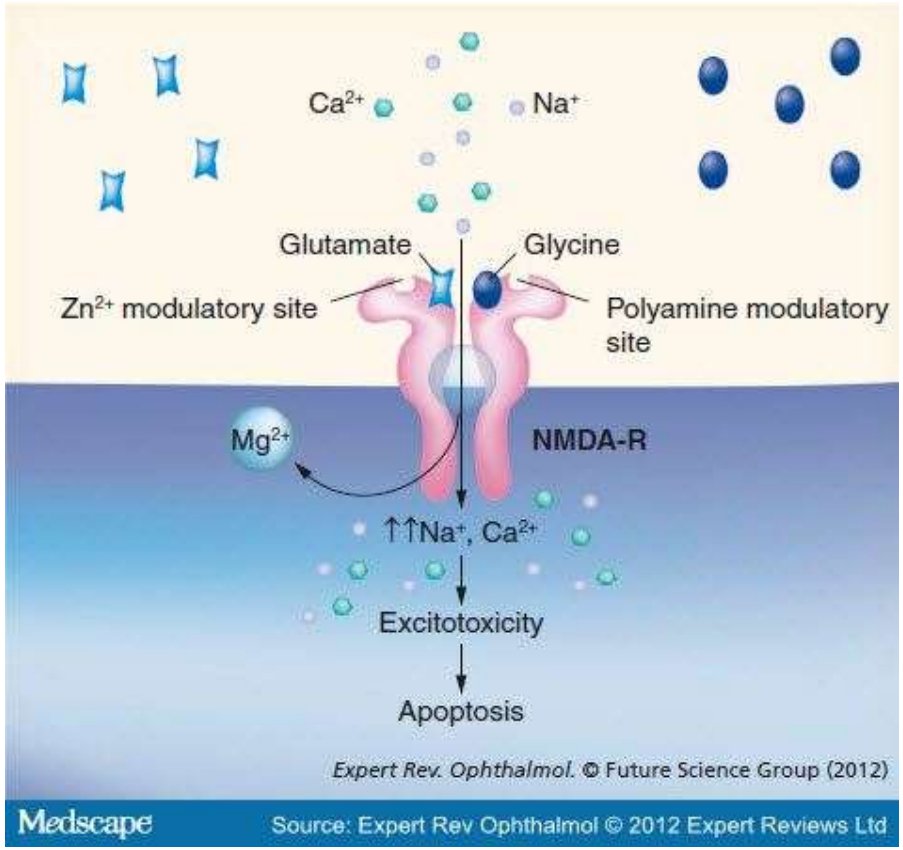
Neurons have an internal support structure partly made up of microtubules. A protein called *tau* helps stabilize microtubules. In AD, *tau* changes, causing microtubules to collapse, and *tau* proteins clump together to form neurofibrillary tangles.

Classical Targets: Cholinesterase Inhibitors



- Acetylcholine (ACh) is a CNS messenger crucial for learning and memory.
- Degradation of ACh by acetylcholinesterase (AChE) → in Alzheimer's levels of ACh are low due to increased degradation
- Cholinesterase-Inhibitors correct the deficit of ACh by blocking AChE, increasing the amount of ACh in synaptic cleft

Classical Targets: NMDA Antagonists

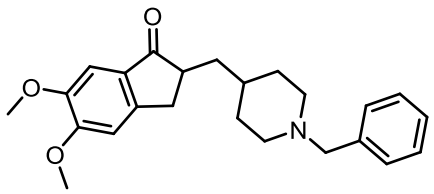


- Glutamate is a neurotransmitter involved in cognition and higher mental function.
- In AD, abnormalities in NMDA and excess glutamate in the synapse due to failure of reuptake
- Excess glutamate causes release of excitatory ions leading to excitotoxicity and neuronal death
- NMDA Antagonist modulates the NMDA receptor during the excessive glutamate stimulation

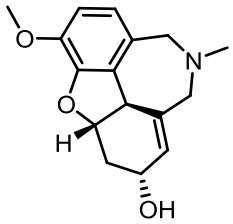
NMDA: ***N*-Methyl-D-aspartic acid** or ***N*-Methyl-D-aspartate** (**NMDA**) is an amino acid derivative that acts as a specific agonist at the NMDA receptor mimicking the action of glutamate, the neurotransmitter which normally acts at that receptor

Current FDA Approved Treatments for AD

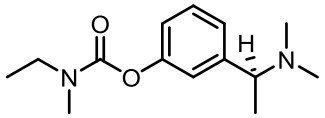
- **Cholinesterase-Inhibitors:**



Aricept
Donepezil
Pfizer/Eisai (1996)
All stages of AD

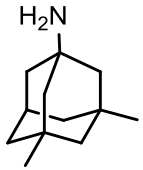


Razadyne
Galantamine
Mild to moderate AD



Exelon
Rivastigmine
Novartis (2000)
All stages of AD

- **NMDA Antagonist**

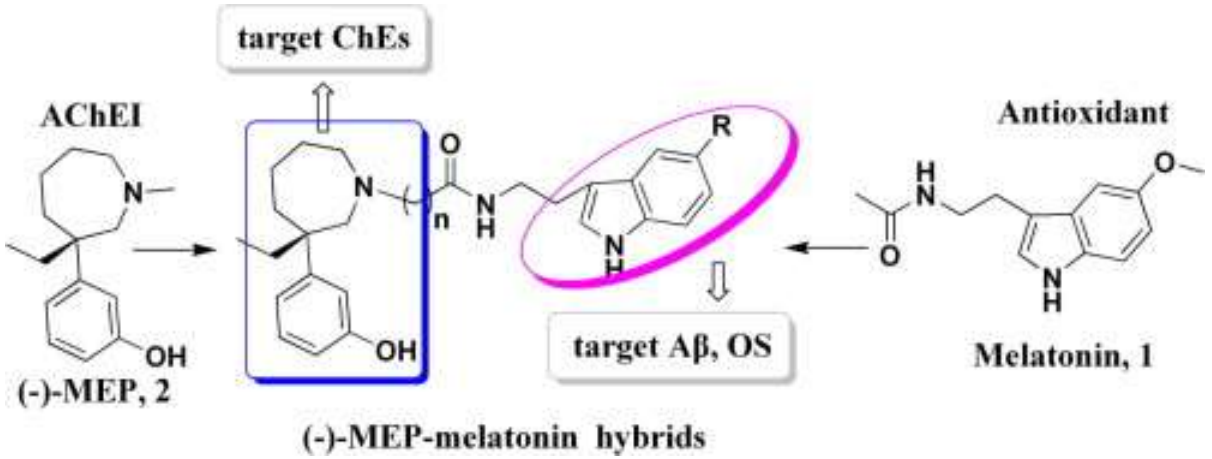


Namenda
Memantine
Eli Lilly (2003)
Moderate to Severe AD

Namzaric (Donepezil and Memantine)

- **Provide only symptomatic relief while not prevent or reverse the pathological process**

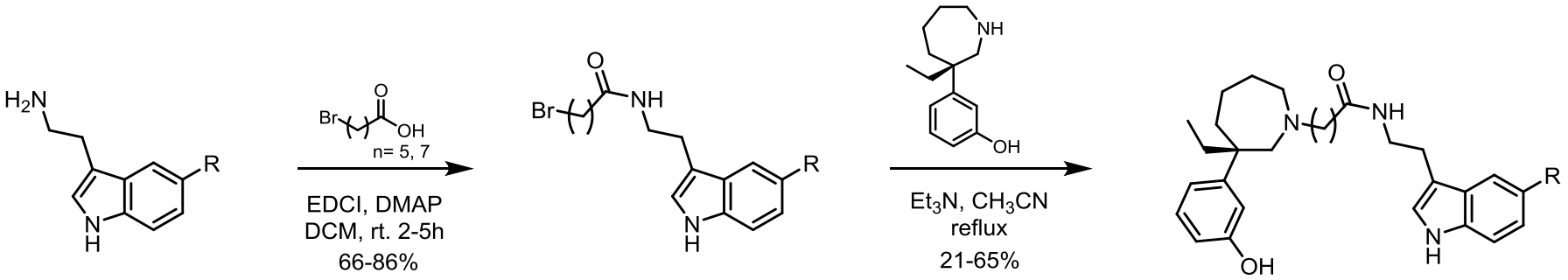
Design of a Novel Dual Inhibitor of AD



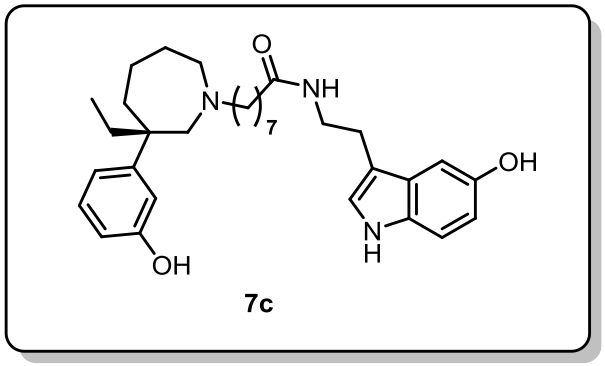
- **Melatonin** is a neurohormone whose levels are decreased in AD patients. **Good antioxidant capacity** and **metal binding** capabilities. In addition, studies have shown that melatonin is a moderate **Aβ aggregation disruptor**.

- **(-)-MEP ((-)-Meptazinol)** is a dual site AChEIs with Aβ aggregation and metal binding properties.

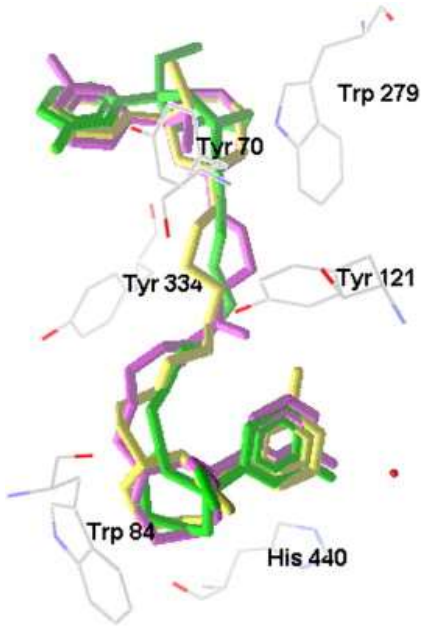
Synthesis of (-)-MEP-Melatonin Hybrid



5 R = H n = 5
6 R = H n = 7
7a R = OMe n = 7
7b R = Cl n = 7
7d R = OTBS n = 7



7c

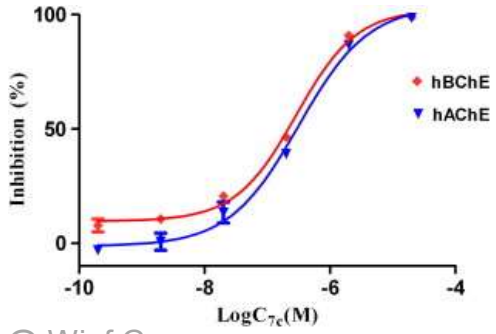


Properties of Newly Designed Hybrids

Oxygen Radical Absorbance Capacity (ORAC)

Compd	ORAC[a]
7	6.346 ± 0.370
7a	6.010 ± 0.521
7b	1.538 ± 0.229
7c	7.235 ± 0.904
5-HT	5.003 ± 0.022
(−)-MEP	0.384 ± 0.177

Values expressed as trolox equivalents: μM of trolox eq/ μM tested cpd (three independent experiments)



Inhibition of Cholinesterases

Inhibitory potency was tested against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) from mice forebrain homogenates

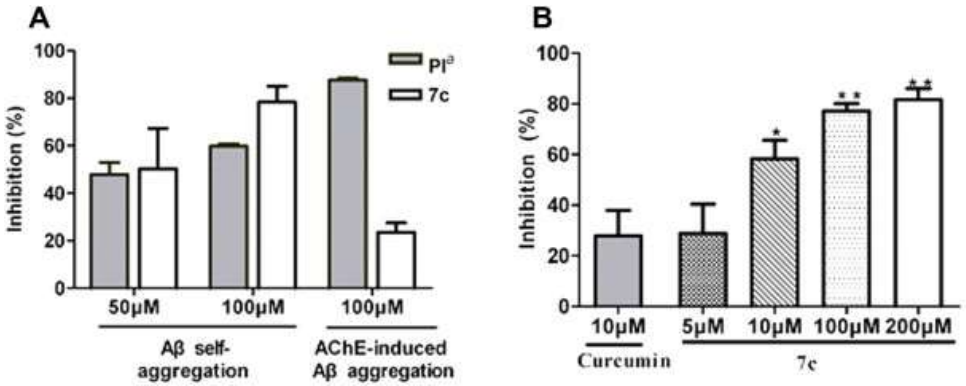
Compd	AChE inhibition (%) at 1 μM	IC_{50} ± SEM[b] (μM) AChE	BChE	Selectivity for AChE[e]
7	62.7[d]	0.67 ± 0.07	0.59 ± 0.01	0.89
7a	69.6	0.44 ± 0.01	0.70 ± 0.05	1.59
7b	55.5	0.92 ± 0.05	1.35 ± 0.11	1.47
7c	72.7	0.31 ± 0.01	0.29 ± 0.01	0.93
5-HT	8.8	nt	nt	nt
(−)-MEP	26.5	6.02 ± 0.81	nt	nt
Donepezil	98.1	0.023 ± 0.001	8.99 ± 0.34	389.69

Selectivity for hAChE=IC_{50}(hBChE)/IC_{50}(hAChE)

Properties of Newly Designed Hybrids

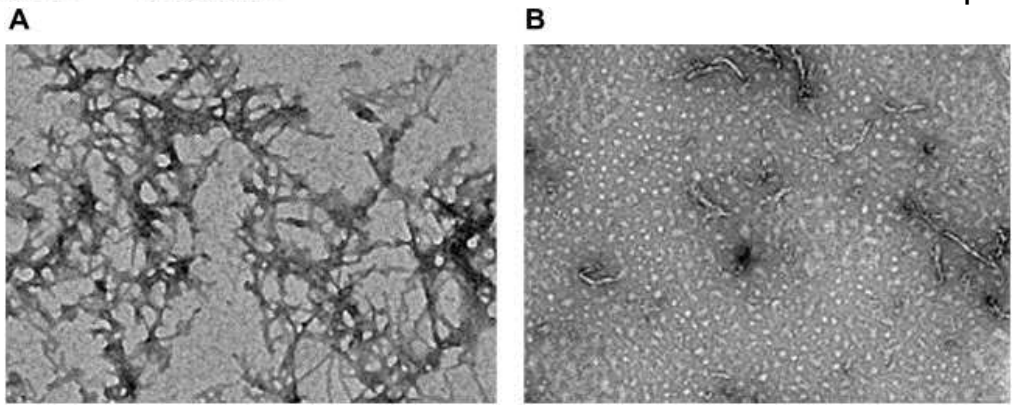
- **Inhibition of Aβ self aggregation and AChE-induced Aβ aggregation**

  ThT assay (Thioflavin T): measures the changes of fluorescence intensity of ThT upon binding to amyloid fibrils



A. Inhibition of $A\beta_{1-40}$ self-aggregation and AChE induced aggregation
B. Inhibition of $A\beta_{1-42}$ self aggregation by 7c

PI: propidium iodide



Summary

- Novel (-)-MEP-melatonin hybrids were developed exhibiting dual inhibitory potency on both hAChE and hBChE.
- Compounds show high oxygen radical absorbance capacity (ORAC)
- Hybrid **7c** can effectively retard $A\beta_{1-40}$ and $A\beta_{1-42}$ self-aggregation