Mining the *A. nidulans* Metabolome for Tau Aggregation Inhibitors

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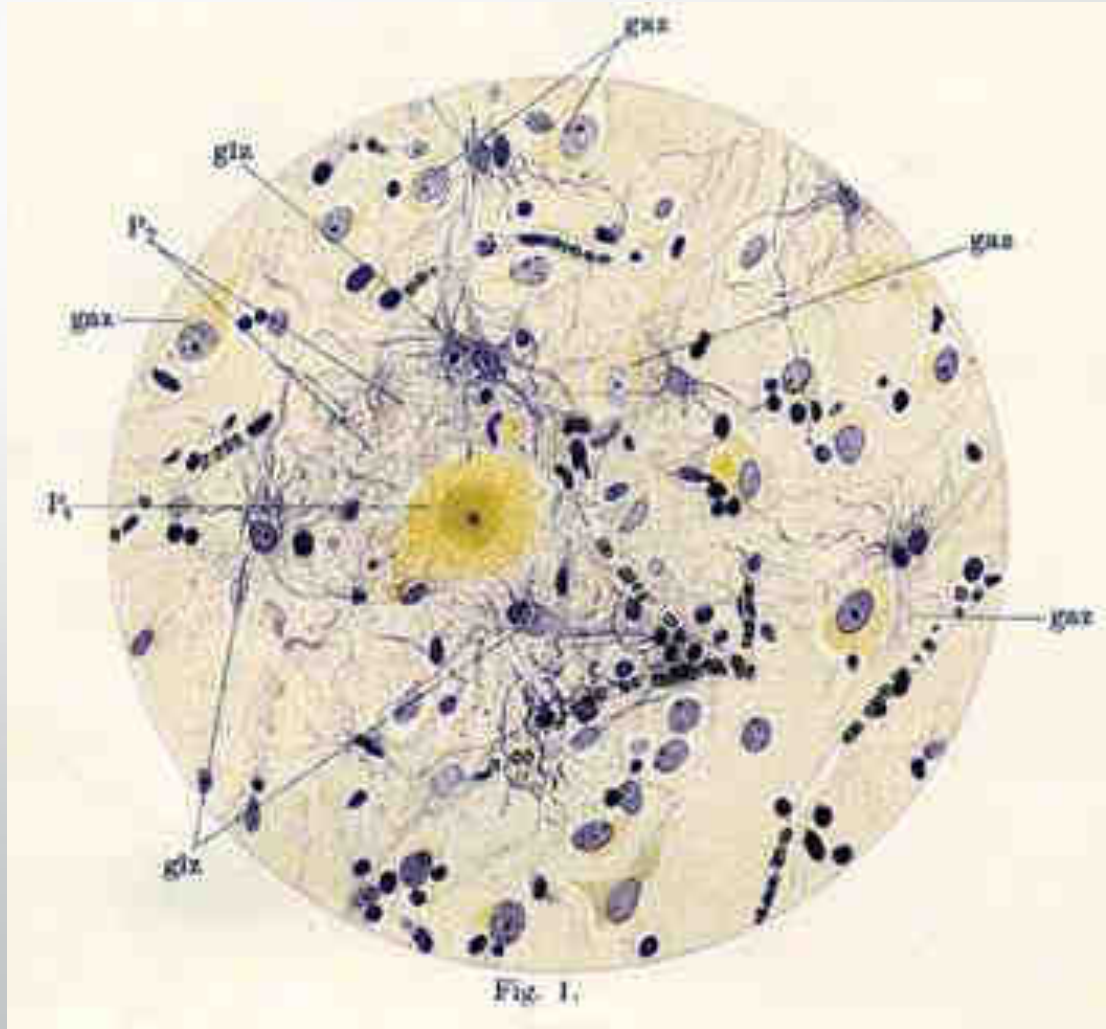
Neurodegenerative Tauopathies

- Alzheimer's disease
- Pick's disease
- Progressive supranuclear palsy
- Corticobasal degeneration
- Agyrophilic grain disease
- FTDP-17
- Dementia pugilistica (CTE)
- Niemann-Pick disease, type C
- Amyotrophic lateral sclerosis/parkinsonism-dementia complex

Alzheimer's disease

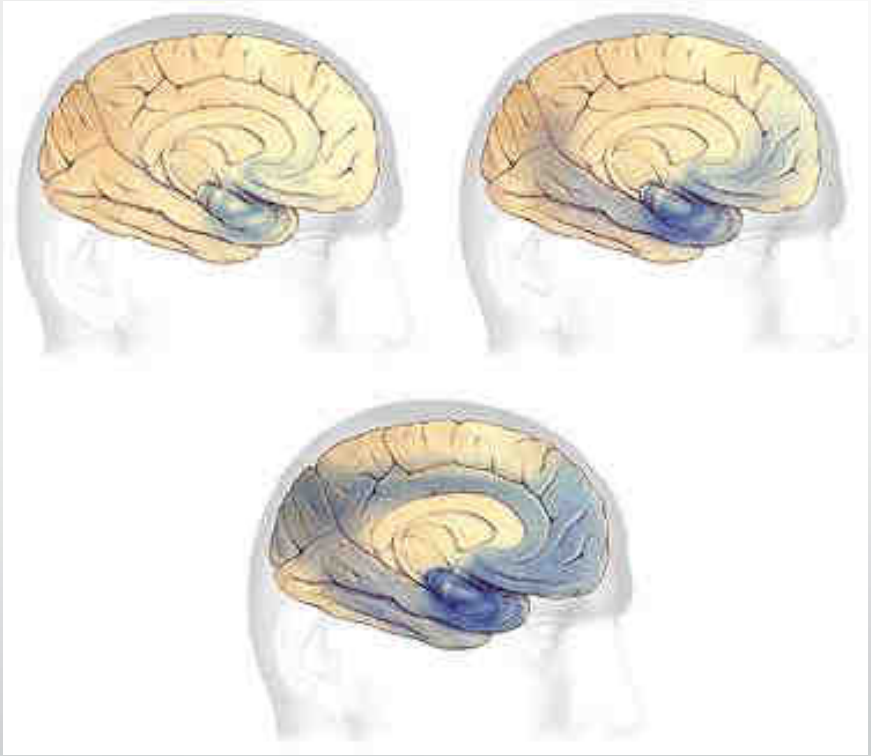
- Progressive amnestic disorder characterized by the abnormal accumulation of proteins in the brain
  - Extracellular Aβ Senile Plaques
  - Intracellular Tau Neurofibrillary Tangles



Stages of Alzheimer's disease

Early (Mild cognitive impairment):
- Memory & Learning
- Thinking & Planning

Mild to moderate (Possible Alzheimer's):
- Language problems
- Sense of your surroundings



Aβ Plaques elevated everywhere in brain

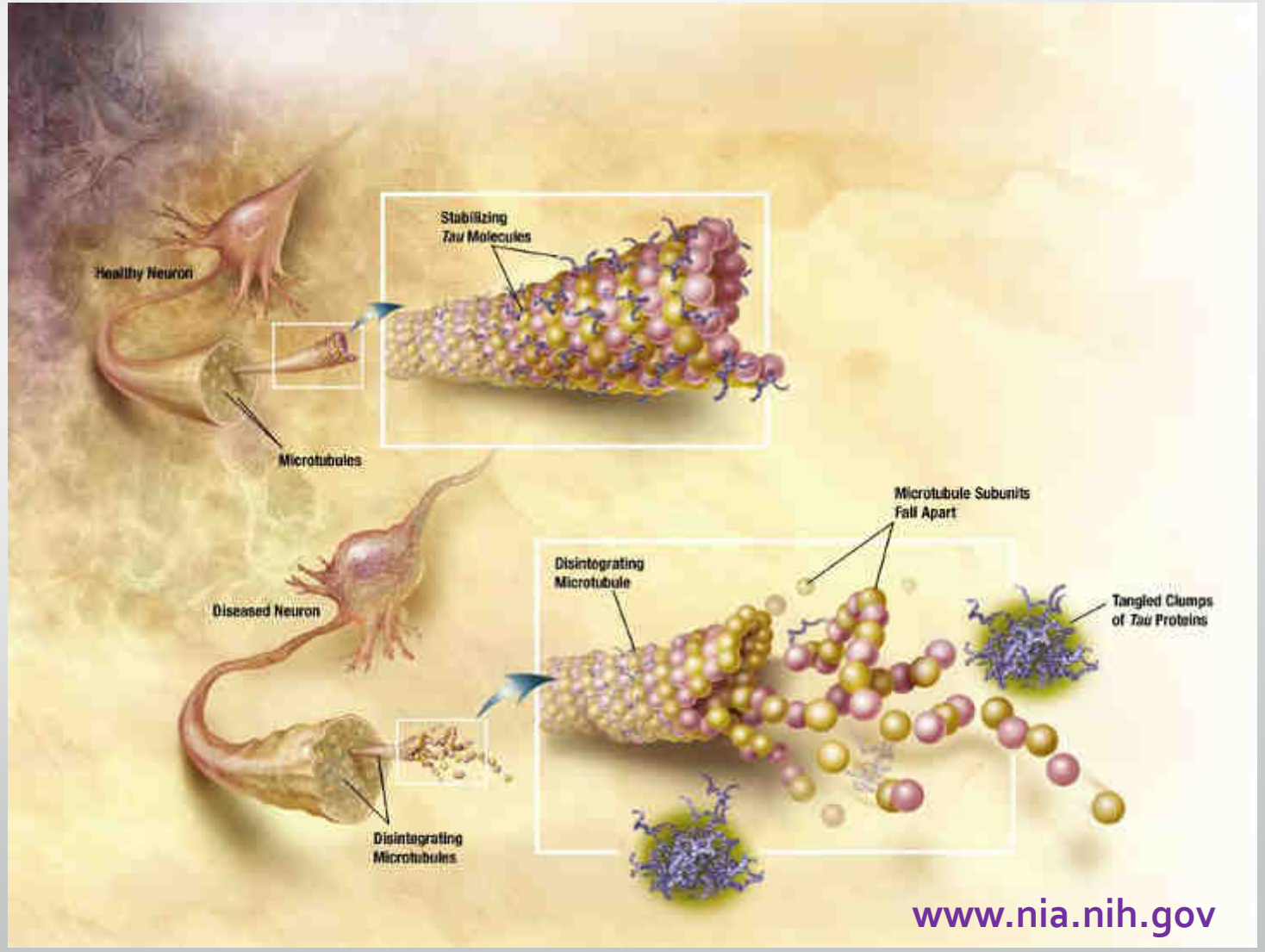
Tau Neurofibrillary Tangles elevated in affected brain regions

Severe (Probable Alzheimer's):
- Lose ability to communicate
- Lose ability to care for themselves
- Lose ability to recognize friends and family

www.alz.org

Tau hypothesis

- Tau normally binds to and stabilizes microtubules in neurons, creating a stable cytoskeleton that can be used for vital transport
- In disease tau is modified by unknown mechanisms that could include phosphorylation, truncation or other changes
- The tau releases from microtubules, destabilizing them
- Tau self-associates into oligomers, fibrils and eventually intracellular neurofibrillary tangles
- Because tau pathology correlates with the severity and type of cognitive impairment, it is hypothesized that tau pathology is responsible for cell death and neuronal dysfunction



Tau Biology –

•Six isoforms (generated by alternative mRNA processing of a single gene on chromosome 17)

•Mainly neuronal expression

•Microtubule-associated protein
binds to and stabilizes microtubules
-helps to maintain cell shape
-MTs serve as tracks for axonal transport

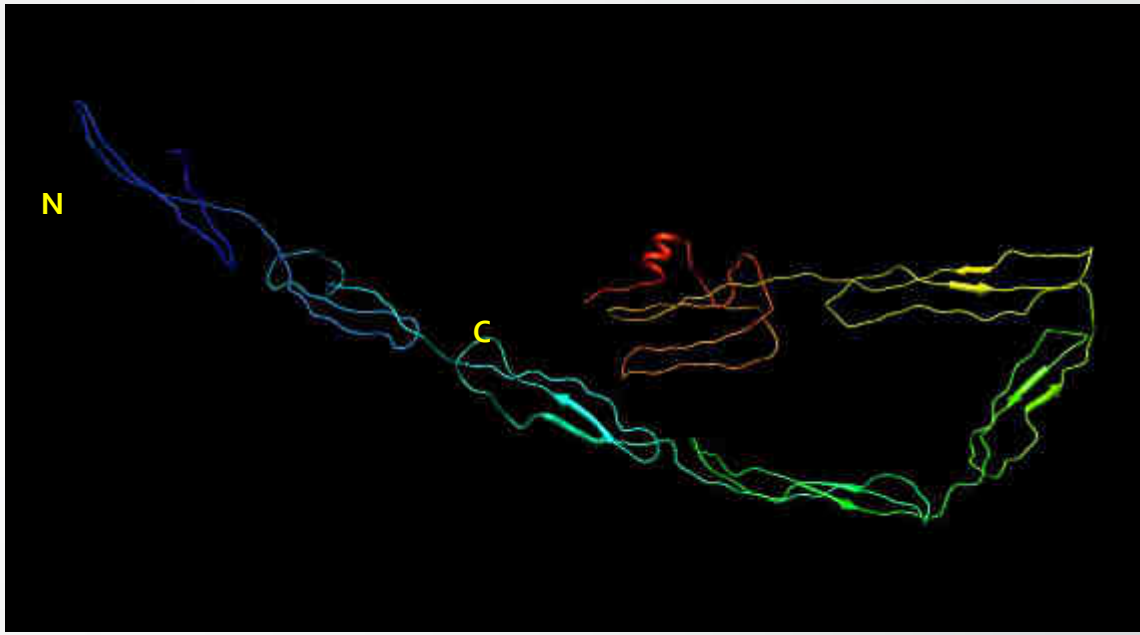
•Activity can be modulated by phosphorylation
17% of amino acids are serine/threonine

Tau Structure –

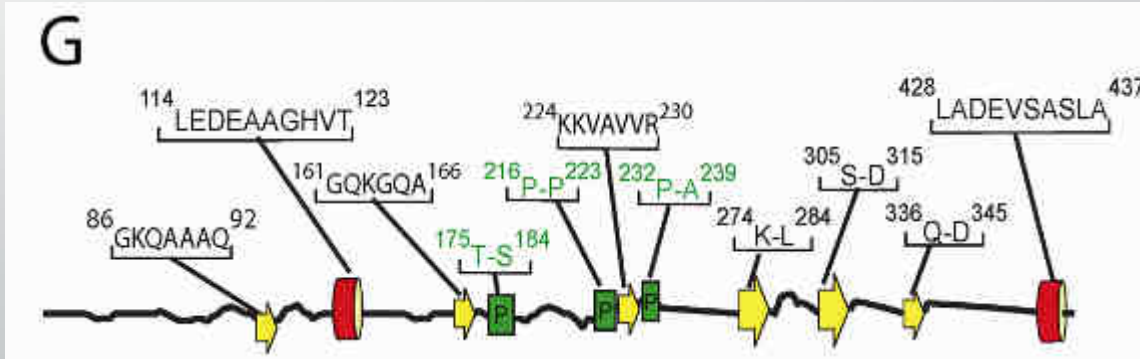
•"Natively Unfolded" in solution

•~12% of residues are predicted to have 2° structure

•Can withstand boiling at pH ~3 and maintain function



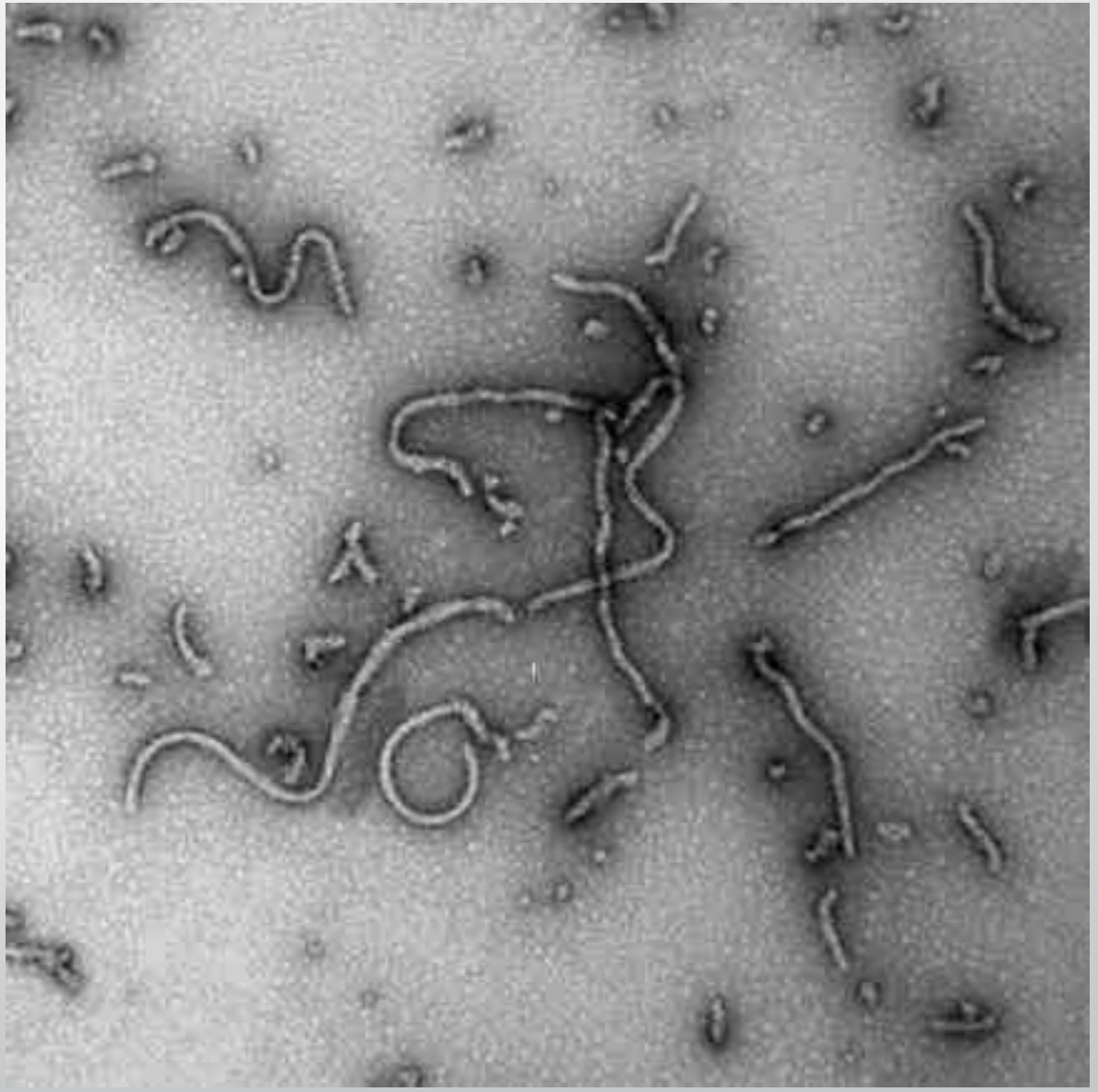
Yang Zhang. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9:40 (2008).
Yang Zhang. Template-based modeling and free modeling by I-TASSER in CASP7. Proteins, 8: 108-117 (2007).
Sitao Wu, Jeffrey Skolnick, Yang Zhang. Ab initio modeling of small proteins by iterative TASSER simulations. BMC Biology, 5:17 (2007).



NMR
Structural polymorphism of 441-residue tau at single residue resolution.
Muckrasch, et al PLOS Biol 2009 Feb 17;7(2):e34

Inhibiting Tau aggregation

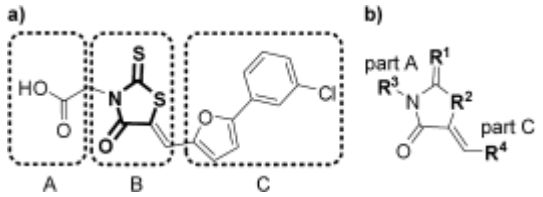
- Tau aggregation can be induced *in vitro* using biochemical approaches
  - *In vitro* filaments resemble authentic filaments from AD morphologically, immunologically, and structurally
- Screen chemical libraries for inhibitors of tau aggregation



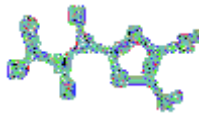
Synthetic tau filaments induced by arachidonic acid (ARA)

Tau aggregation inhibitors

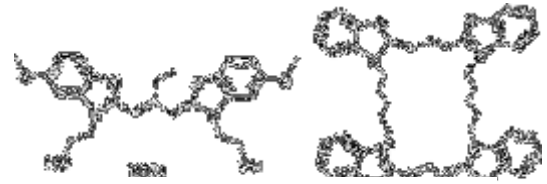
- High Throughput screening of hundreds of thousands of compounds have identified several classes of potential tau aggregation inhibitors
- My laboratory is very interested in identifying lead compounds for therapeutic development



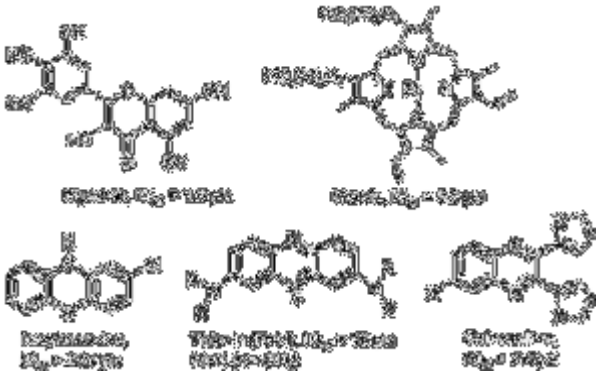
Variations of rhodanine based inhibitors



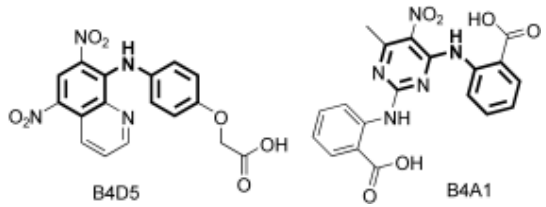
Core structure of thiazolylhydrazide Inhibitors



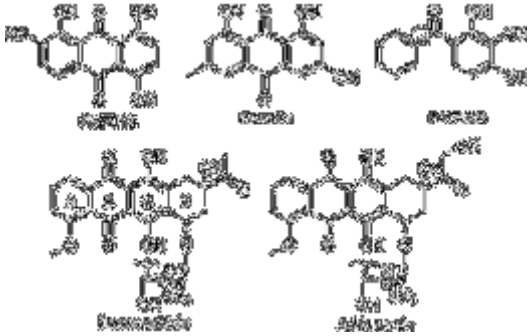
Benzothiazole derivatives



Phenothiazines, Porphyrins, and Polyphenols



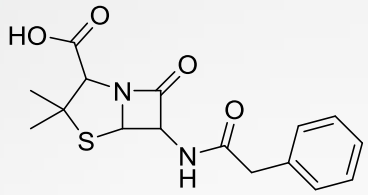
N-Phenylamine-derived compounds

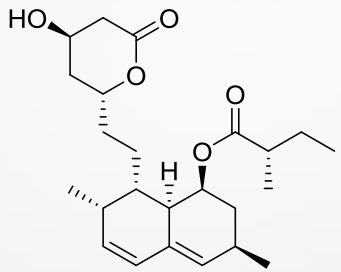


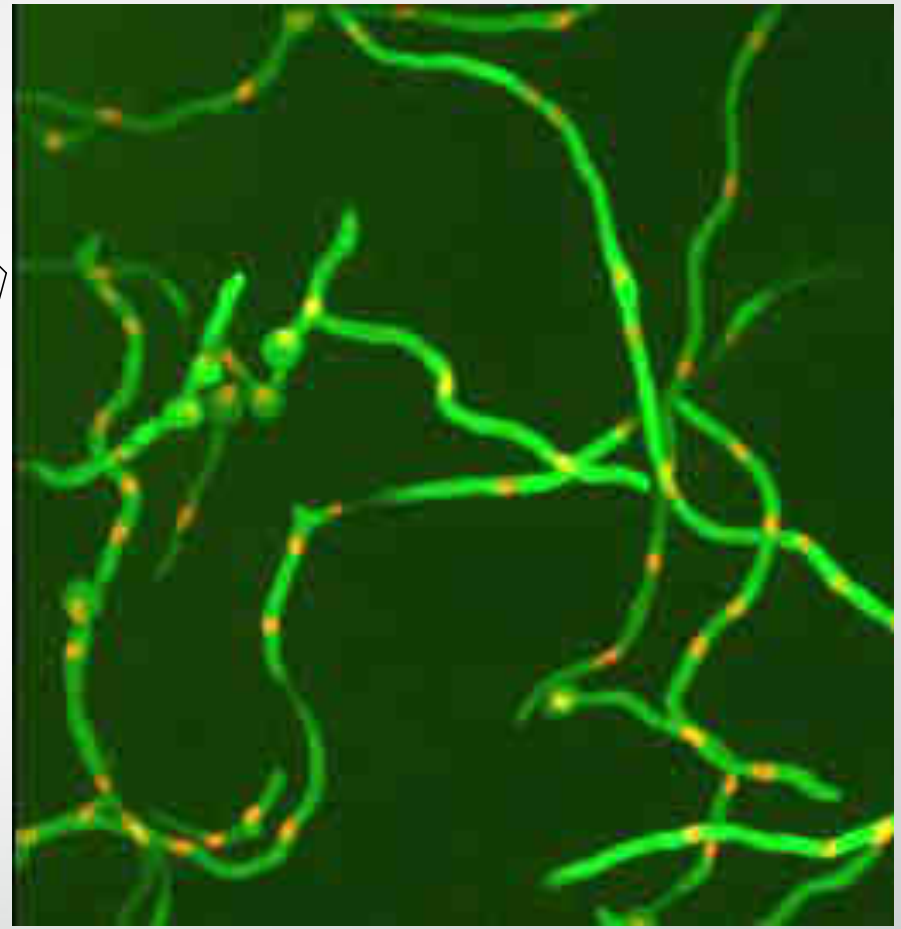
Anthraquinone-derived compounds

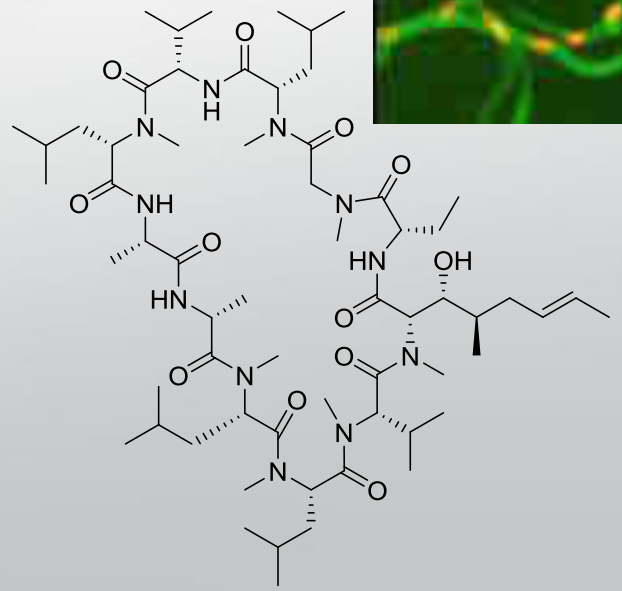
*Bulic, Mandelkow et. al. "Development of Tau Aggregation Inhibitors for Alzheimer's Disease" (Review) *Angewandte Chemie International* 48(10): 1740-1752, 2009

Fungal natural products









- *Aspergillus nidulans*
- Fungi have historically been a rich source of biomedically useful compounds
  - Penicillin
  - Lovastatin
  - Ciclosporin
- Many of these compounds are secondary metabolites, or are not made under normal laboratory conditions

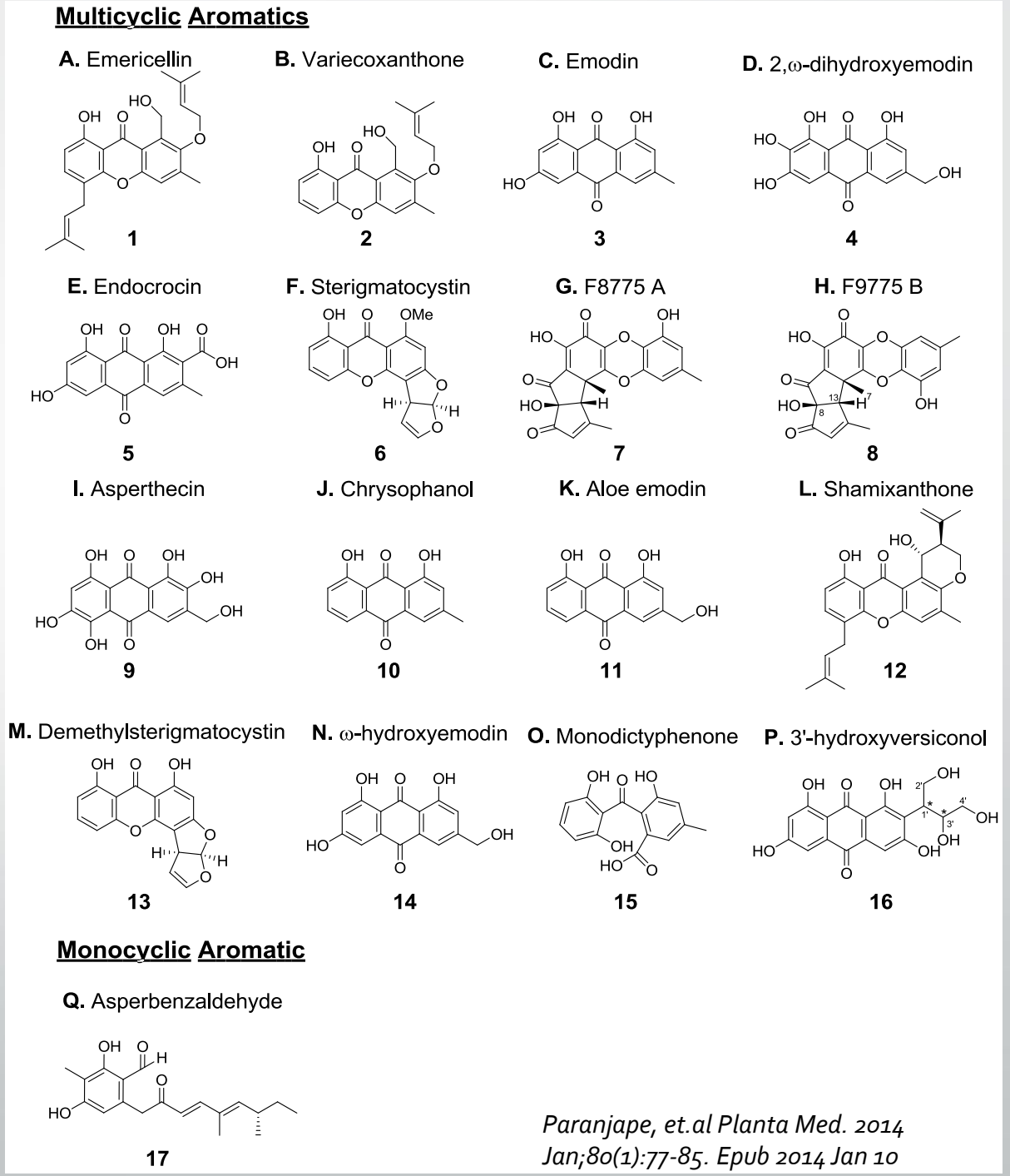
MINING THE *ASPERGILLUS* METABOLOME

- Using advanced genetic techniques, the fungi can be engineered to produce compounds that they normally would only produce under special circumstances
- Silenced gene clusters encoding enzymes such as nonribosomal peptide synthetases and polyketide synthases can be activated genetically
- They produce large amounts of secondary metabolites and their intermediates that can be readily purified and identified

Dr. Berl Oakley
Irving S. Johnson Distinguished Professor of Molecular Biology
Department of Molecular Biosciences

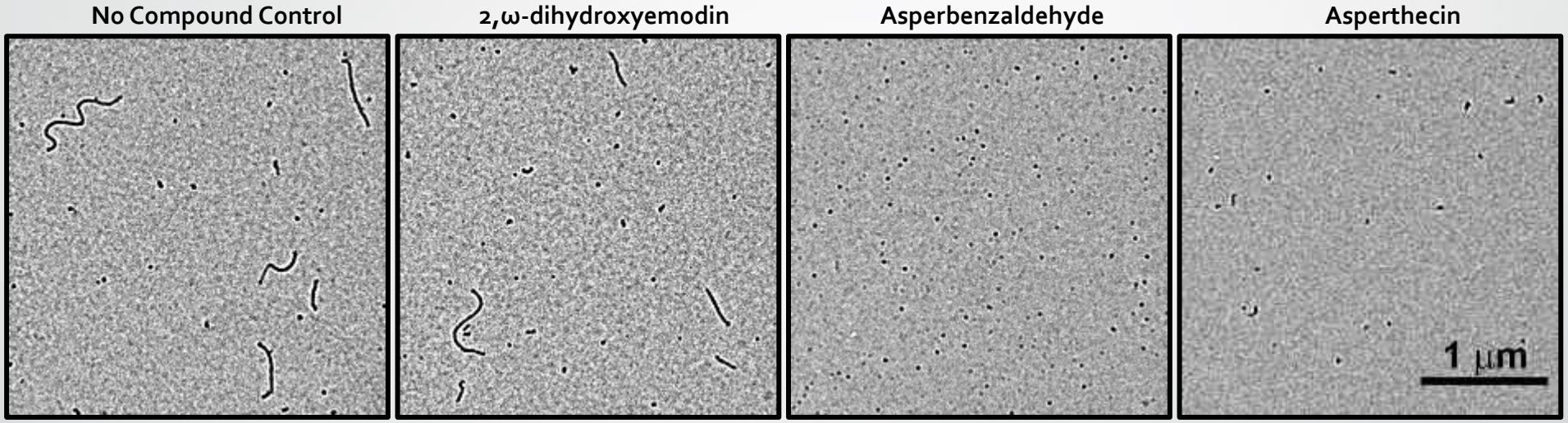
A. nidulans 2° Metabolites

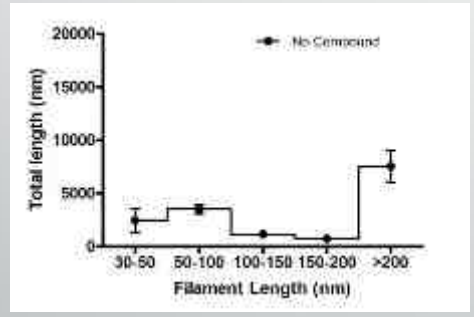
- Many compounds share structural similarity to previously identified inhibitors of tau aggregation (Emodin)
  - Anthraquinones
  - Xanthones
  - Polyketide Cathepsin K Inhibitors
  - Benzophenone
  - Asperbenzaldehyde

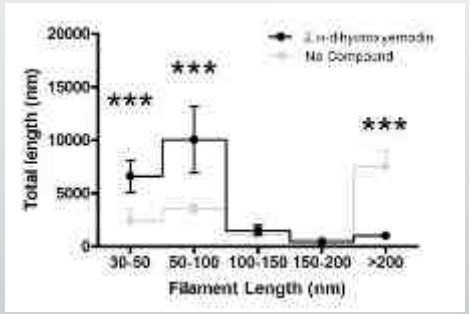


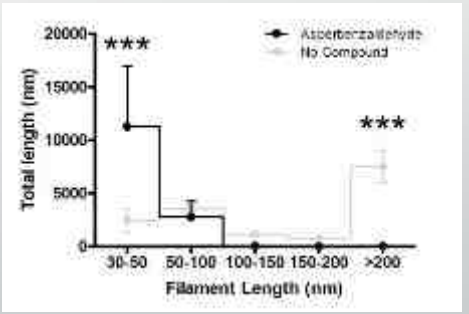
Paranjape, et.al Planta Med. 2014 Jan;80(1):77-85. Epub 2014 Jan 10

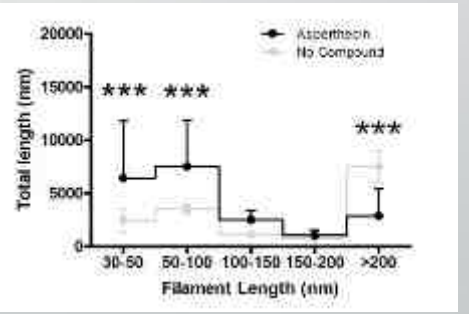
A. nidulans 2° Metabolites Aggregation Inhibition

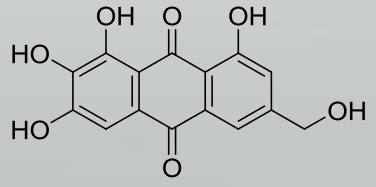


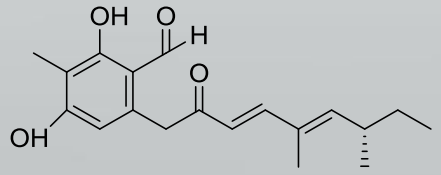


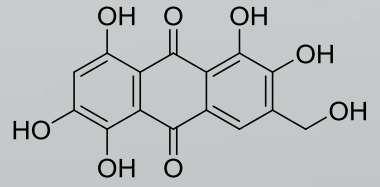












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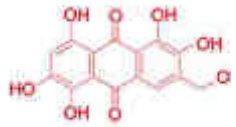
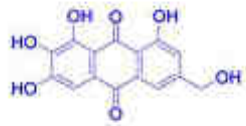
First Generation Compounds

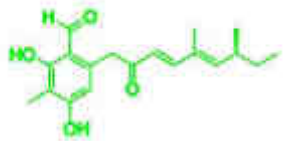
- Compounds were added over a wide range of concentrations
- All 3 compounds show a dose-dependent decrease in tau aggregation
- Under these conditions, Asperthecin is the most potent compound
- Asperbenzaldehyde represents a new structural class of compounds that inhibit tau aggregation



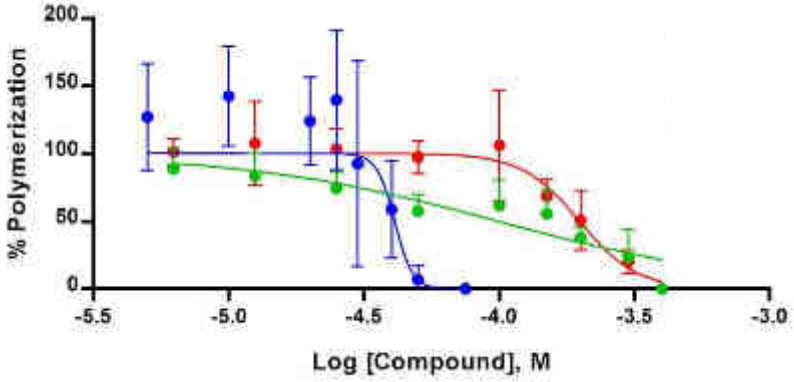
2,ω-dihydroxyemodin
IC_{50} 205 ± 28 µM



Asperthecin
IC_{50} 39 ± 2 µM



Asperbenzaldehyde
IC_{50} 177 ± 103 µM



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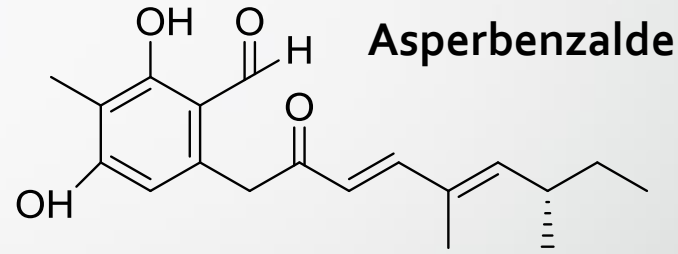
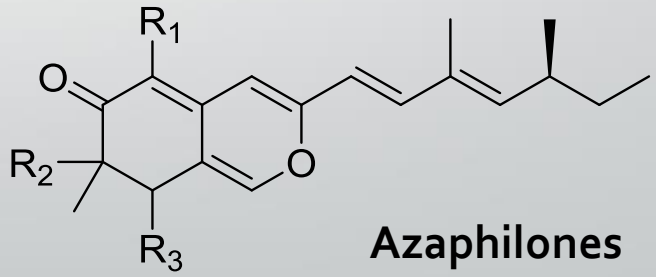
Second generation compounds – Azaphilones

- Asperbenzaldehyde is an intermediate in azaphilone biosynthesis
- We can generate many azaphilone derivatives from asperbenzaldehyde in 2-3 steps
  - Complete chemical synthesis would be more than 15 steps
- Many azaphilone compounds also show 5-Lipoxygenase inhibition activity



Asperbenzaldehyde

2-3 steps

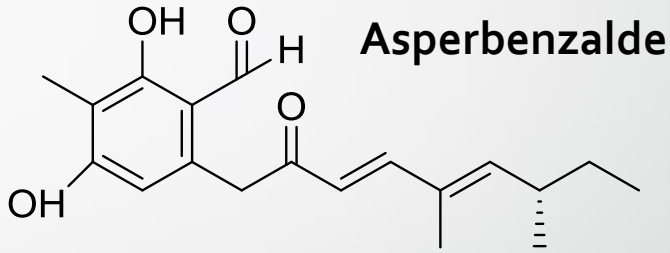


Azaphilones

Second generation compounds – Azaphilones

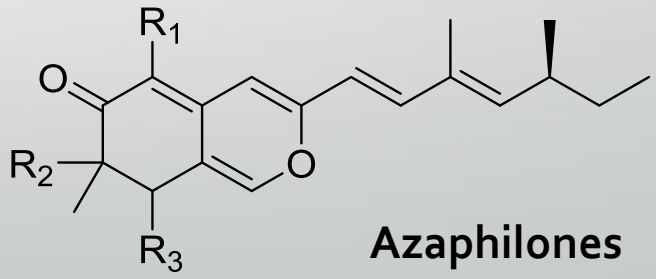
- 4 different groups at X position
- 2 different R1 groups
- 2 different R2 groups

Compounds	R1	R2	R3
Aza 7	-H	-O-CO-CH_3	-Ketone
Aza 8	-Cl	-O-CO-CH_3	-Ketone
Aza 9	-Br	-O-CO-CH_3	-Ketone
Aza 10	-I	-O-CO-CH_3	-Ketone
Aza 11	-H	-OH	-Ketone
Aza 12	-Cl	-OH	-Ketone
Aza 13	-Br	-OH	-Ketone
Aza 14	-I	-OH	-Ketone
Aza 15	-H	-OH	-$CHCO_2Et$
Aza 16	-Br	-OH	-$CHCO_2Et$
Aza 17	-I	-OH	-$CHCO_2Et$



Asperbenzaldehyde

2-3 steps

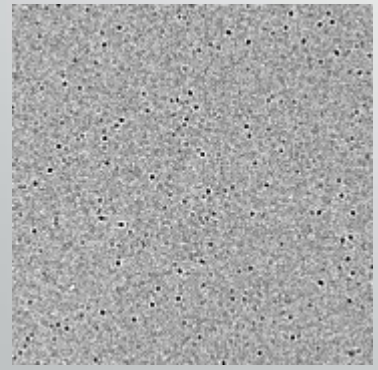


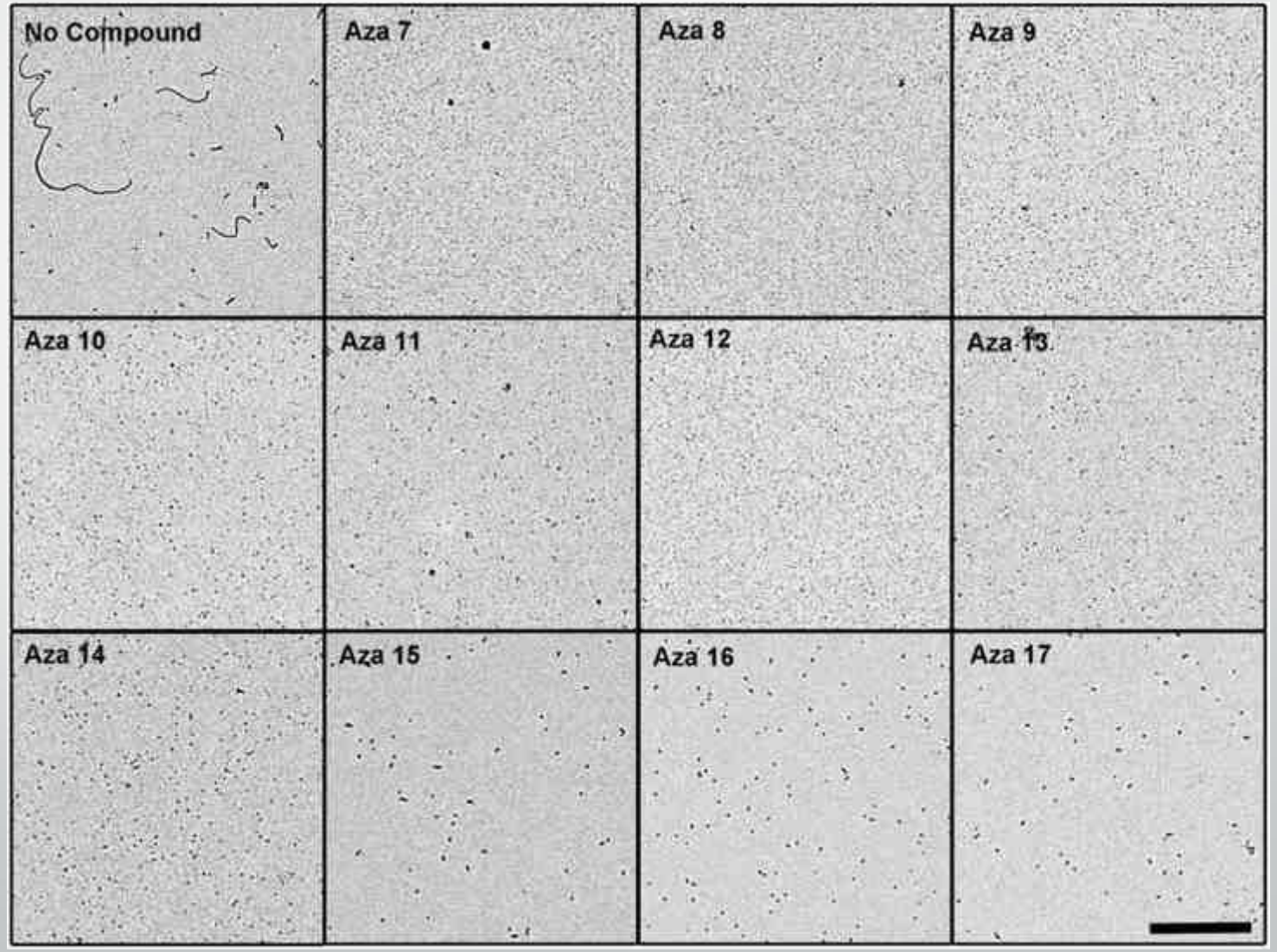
Azaphilones

Azaphilone Aggregation Inhibition

At 200 µM all the azaphilones decreased tau aggregation drastically

Asperbenzaldehyde
(Parent Compound)





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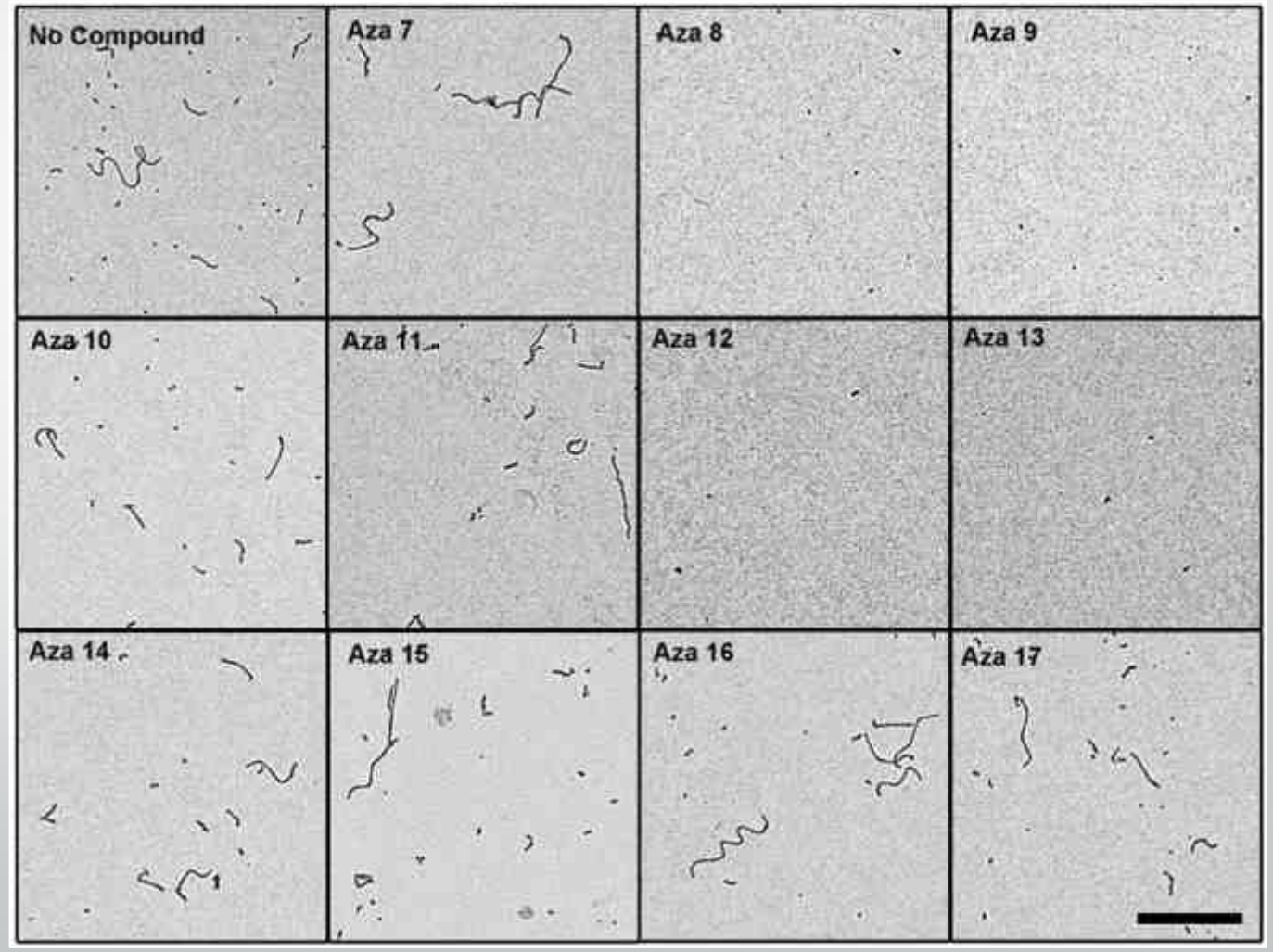
Azaphilone – Aggregate Disassembly

- Filaments were formed in the presence of ARA for 24 hours, compounds were added to 200 µM and incubated an additional 24 hours
- Compounds 8, 9, 12 and 13 had greatly reduced levels of tau filaments



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Disassembly IC50

- Aza 8, aza 9, aza 12 and aza 13 showed a dose dependent disassembly of pre formed tau aggregates
- Aza 9 was the most potent amongst these

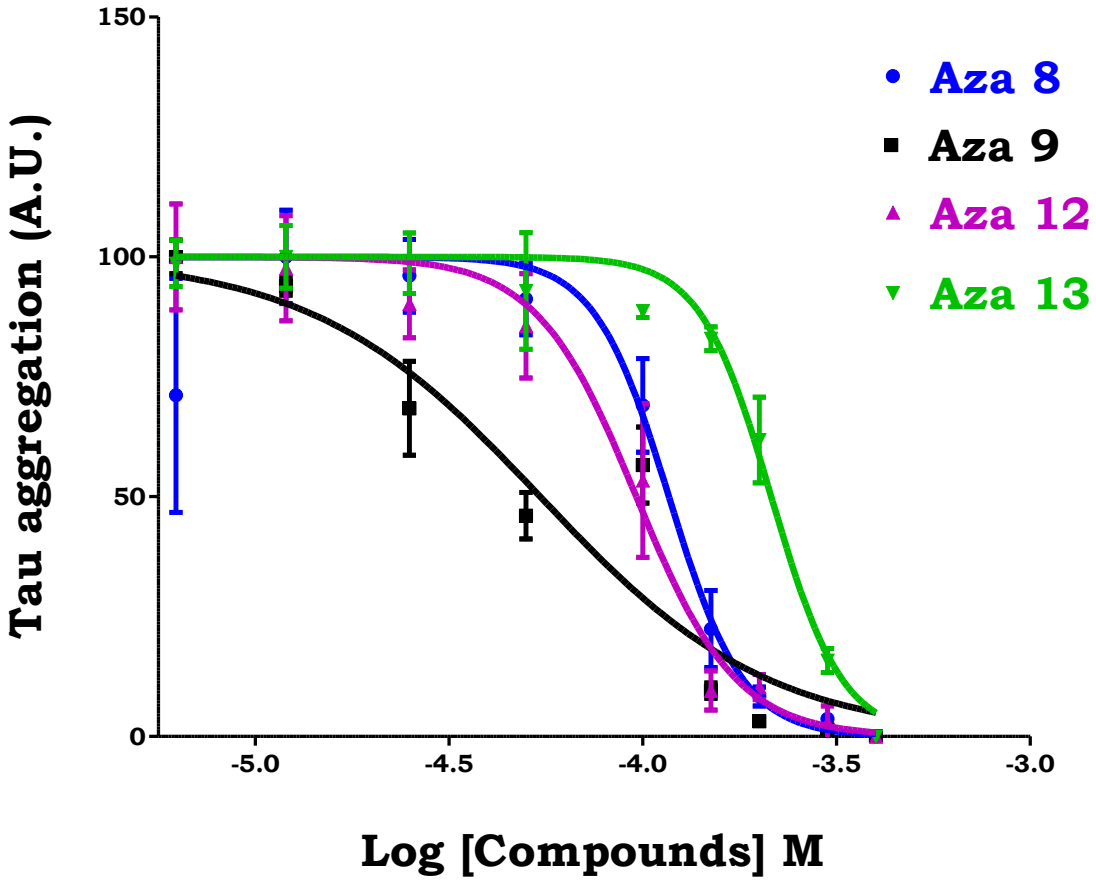
IC_{50} values :

Aza 8 118.47 ± 19.33 µM

Aza 9 : 56.185 ± 14.1 µM

Aza 12 : 97.6 ± 16.2 µM

Aza 13 : 215.5 ± 17.6 µM



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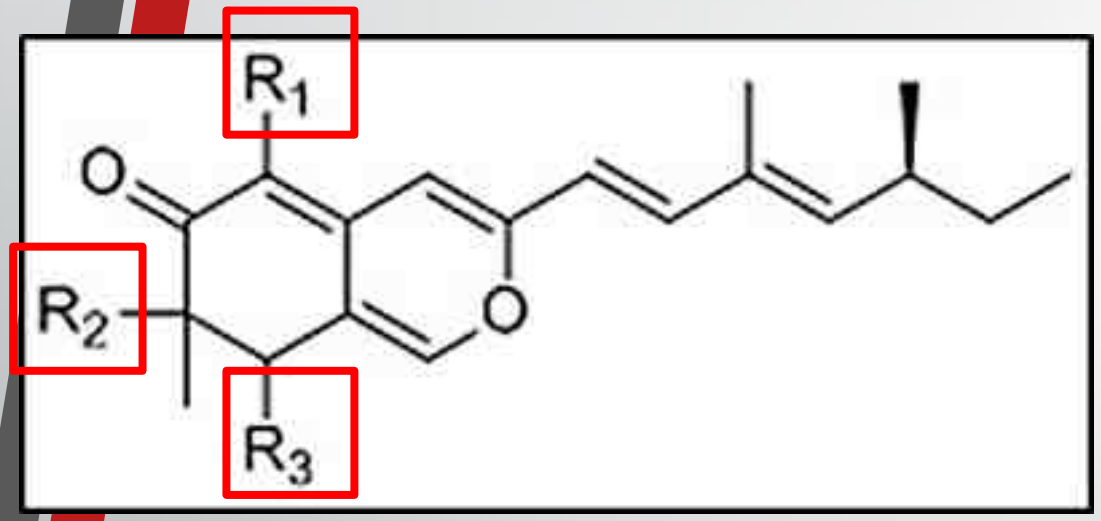
Structure activity relationship studies

- Cl and Br better than I – Increased electronegativity at R1 has significant impact on disassembly



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Aza 13	-Br	-OH	-Ketone
Aza 14	-I	-OH	-Ketone
Aza 15	-H	-OH	-$CHCO_2Et$
Aza 16	-Br	-OH	-$CHCO_2Et$
Aza 17	-I	-OH	-$CHCO_2Et$

- Acetate group at R2 in presence of Br at R1 important for disassembly
- CHCO2Et moiety at R3 – eliminates disassembly even with halogenation
- Ketone group present in all 4 disassembly molecules

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Mining the *A. nidulans* Metabolome for Tau Aggregation Inhibitors

- 14 of 28 compounds have Tau Aggregation Inhibition Activity
- 4 of 28 compounds also disassemble pre-formed tau filaments

- We are working with a medicinal chemist to optimize the probes to get biologically useful IC50 values and then move into pre-clinical testing
- We are also continuing to screen additional *A. nidulans* 2° metabolites

Acknowledgements

Gamblin Lab

- Dr. Smita Paranjape
- Dr. Mythili Yenjerla
- Yamini Mutreja
- Bryce Blankedfeld
- Adam Miltner
- Dakota Bunch

Collaborators

- Dr. Berl Oakley, KU
- Dr. Tom Prisinzano, KU
- Dr. Clay Wang, USC

Funding

- NIH/NINDS R01NS083391
- HL Snyder Medical Foundation