Far Red Amyloid Binding Fluorophores for Alzheimer's Disease Drug Development

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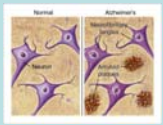
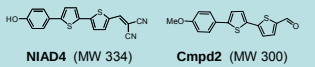
Abstract

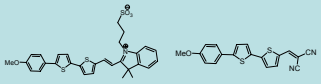
Alzheimer's disease (AD), the most common type of dementia, is a progressive degenerative disorder affecting tens of millions of people worldwide. Confirmation of AD still relies on *post-mortem* examination of amyloid senile plaque in brain tissue. Amyloid beta (A-beta) aggregates are an important indicator of AD. Hence, analytical methods to accurately assess aggregate formation are useful for monitoring the efficacy of AD therapeutic drugs. We present the development and evaluation of a unique class of small molecule fluorophores that can cross the blood-brain barrier, specifically bind to A-beta aggregates and emit strong red or near-infrared fluorescence in the APP transgenic mouse model. These non-antibody based molecular imaging agents promise to facilitate preclinical Alzheimer's disease drug development by enabling researchers to monitor amyloid-beta aggregate formation *in vivo*.



NIAD structures



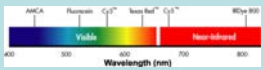
NIAD4 (MW 334) **Cmpd2** (MW 300)



Cmpd4 (MW 564) **Cmpd8** (MW 348)

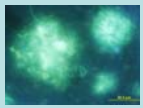
Optical properties

In MeOH	Abs Max (nm)	Ex. Coeff. ($M^{-1}.cm^{-1}$)	Em Max (nm)	Stokes shift (nm)
NIAD4	475	29,000	625	150
Cmpd2	396	38,000	554	158
Cmpd4	560	54,000	734	174
Cmpd8	470	54,000	628	158

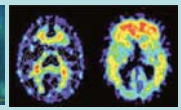


Introduction

In neurodegenerative Alzheimer's disease (AD) the abnormal decay of brain memory and cognitive function occurs many years, perhaps decades, before the first symptoms. *In vivo* imaging of Amyloid-beta plaque, an important AD biomarker, using positron emission topography (PET) and the Pittsburgh Compound (PIB), has provided a major thrust for understanding AD pathology, disease diagnostics and AD drug development. Deep tissue *in vivo* optical imaging of amyloid plaque in AD transgenic mice model is now achievable with multi-photon microscopy. In addition, the amyloid plaque load can be measured through the intact mice skull using a near-infrared (NIR) fluorophore. **NIAD4** is first in a series of novel class of fluorescent probes that is designed to have good blood-brain barrier permeability, specificity to amyloid-beta plaques, and "turn-on" bright far-red emission upon A-beta binding.



Thioflavin S stained human AD brain (40 x, DAPI4200 filter)

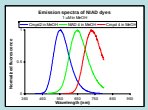


PET imaging of human AD brain using radioactive PIB (Klunk W. *Science* 2002;297:752)

Advantages of NIAD dyes

✓Cross blood-brain barrier
✓ Specific binding to beta-amyloid plaque and cerebral amyloid angiography (CAA)
✓ Large Stokes shift (>150 nm) that minimizes autofluorescence interference
✓Much brighter than Thioflavin-T
✓Greater array of fluorophores provides greater flexibility in multiplexing molecular imaging
✓Enhanced contrast due to planarization upon binding to amyloid plaque
✓Emission sensitive to environment

Molecular modeling

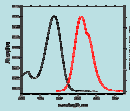


Emission spectral

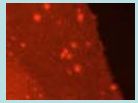
multiplex emission colors (in water)

multiplex emission colors (in MeOH)

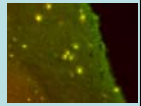
Amyloid histochemical imaging



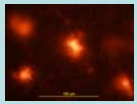
NIAD4 spectral



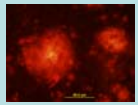
NIAD4 stained Tg mouse brain



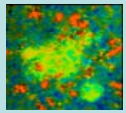
Color merge with Thioflavin-S



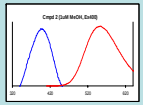
NIAD4 (mouse, 40 x, cy3 filter)



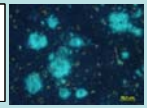
NIAD4 (human AD, 40 x, cy3 filter)



NIAD4 (human AD, 20x, FLIM imaging)

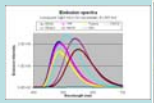


Cmpd2 spectral

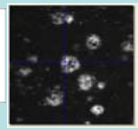


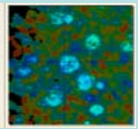
Cmpd2 (human AD brain, 20 x, DAPI4200 filter)

Cmpd2 (human AD brain, 4 x, DAPI4200 filter)

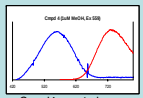


Cmpd2 emissions are sensitive to solvents

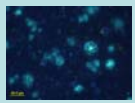


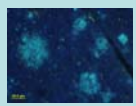


Cmpd 2 (human AD brain, FLIM imaging)

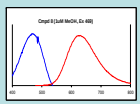


Cmpd4 spectral





Cmpd4 (human AD, 20 x, DAPI4200 filter)

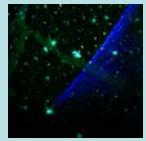


Cmpd8 spectral

Cmpd8 (Tg mouse, 20x, Cy3 filter)

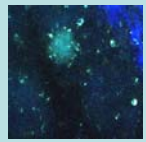
Cmpd8 (human AD, 20x, Cy3 filter)

In vivo amyloid Imaging



NIAD4 crosses blood-brain barrier and labels amyloid plaque *in vivo* after systemic administration in a mouse imaged with multiphoton microscopy.

NIAD4 labels cerebral amyloid angiography (CAA) *in vivo* with multiphoton microscopy.



Cmpd4 labels amyloid plaque *in vivo* after systemic administration in a mouse imaged with multiphoton microscopy.

Cmpd8 labels cerebral amyloid angiography (CAA) *in vivo* after systemic administration in a mouse imaged with multiphoton microscopy.

Conclusion

Current development of NIAD dyes has shown the utility of these novel fluorescent small molecules as the molecular imaging probes for β-amyloid plaque and cerebral amyloid angiography. The flexibility in structural modifications achieved by rational design and synthesis enables tunability of optical properties, i.e. the absorption and emission wavelength and the quantum yield, so as to accommodate various microscopic imaging hardware settings. Most significantly, several NIAD fluorophores (NIAD4, compound 2, 4 and 8) have shown to cross the blood-brain barrier in transgenic mouse and bind amyloid-β plaques *in vivo*. Upon excitation with a two-photon laser, they emit bright fluorescence which can be detected by multiphoton microscopy through a cranial window on the mouse skull. Our ongoing research and development of NIAD fluorophores toward visualizing amyloid-beta aggregates in preclinical animal models of Alzheimer's disease will facilitate the understanding of the role played by amyloid plaque in AD and drug development efforts based on the amyloid cascade hypothesis of Alzheimer's disease.

References

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3. Skoch, J., G.A. Hickey, S.T. Kajdasz, B.T. Hyman and B.J. Bacskai. 2005. In vivo imaging of amyloid-beta deposits in mouse brain with multiphoton microscopy. Methods Mol. Biol. 299:349-363.
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