[[5'-(4-Hydroxyphenyl)[2,2′-bithiophen]-5-yl]methylene]-propanedinitrile NIAD-4

Kenneth T. Cheng, PhD[1]

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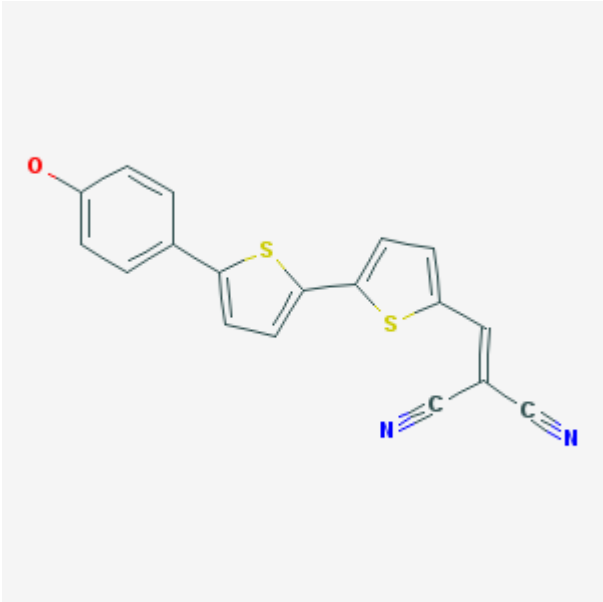
Chemical name:	[[5'-(4-Hydroxyphenyl)[2,2'-bithiophen]-5-yl]methylene]-propanedinitrile
Abbreviated name:	NIAD-4
Synonym:	
Backbone:	Compound
Target:	Amyloid-β (Aβ)
Mechanism:	Specific binding
Method of detection:	Optical, red fluorescence imaging
Source of signal:	NIAD-4
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

[1] National Center for Biotechnology, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.gov.

Corresponding author.

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[[5'-(4-Hydroxyphenyl)[2,2'-bithiophen]-5-yl]methylene]-propanedinitrile (NIAD-4) is a fluorophore that was developed for optical imaging of amyloid-β (Aβ) in the central nervous system (CNS) in basic and pre-clinical research for Alzheimer's disease (AD).

Aβ-peptide was sequenced from the meningeal blood vessels of AD patients and individuals with Downs syndrome (1). Aβ peptides contain 40-42 amino acid residues and are metabolic products of Aβ precursor protein (APP) from cleavage by β– and γ –secretases . Aβ is also identified as the primary component of the neuritic plaques of AD patient brain tissue (2). The cloning of the gene encoding the APP and its localization to chromosome 21 led to the hypothesis that Aβ accumulation is the primary event in AD pathogenesis (1, 3). This hypothesis proposes that neuronal death in AD is related to toxic effect of Aβ on the adjacent cell bodies or cell processes (4). AD is a progressive, neurogenerative disorder of the CNS, and is characterized by a common set of clinical and pathological features . In addition to Aβ, the microtubule-associated protein, tau (τ), is also found in the cell body and axons of neurons as neurofibrillary tangles.

The search for a cure or effective treatment of AD requires *in vivo* detection and quantification of Aβ in the brain for evaluation of the efficacy of AD therapy . Various amyloid-imaging probes have been developed based on positron emission tomography, single-photon emission computed tomography, and optical imaging. Optical imaging is an imaging method that utilizes light photons emitted from bioluminescence and fluorescence probes (5). Fluorescence imaging in the visible light range of 395-600 nm can penetrate only to a depth of 1–2 cm and has significant background signal because of tissue autofluorescence. Depth penetration is one major limiting factor in *in vivo* optical imaging. Currently, *in vivo* optical imaging has wide applications in small animal imaging but only limited applications in large animal and human studies (6). Near-Infrared (NIR) (light range 650–900 nm) fluorescence imaging has the advantages of relatively higher tissue penetration and lower autofluorescence from nontarget tissue. One promising approach of developing optical imaging probes is based on the development of small-molecules of Aβ–staining compounds, such as Congo red and thioflavin T. The specific binding of these compounds to Aβ aggregates is believed to be a combination of electrostatic interactions of the compounds with the Aβ protein, and the compound's shape is complementary to Aβ's surface whichinvolves hydrophobic interactions with the planarized aromatic Л system of the compound (7, 8). In an effort to produce an *in vivo* optical imaging probe which gives specific far-red fluorescence, Nesterov et al. (7) developed NIAD-4 which has a relatively low molecular weight of 334 Da and a dithienyl-ethenyll Л-conjugated bridge between donor and acceptor. NIAD-4 has a structure geometry that is readily planarized and results in a red shift upon binding to Aβ. The highly polarizable bridge provides a substantial bathochromic shift in both absorption and emission spectra of the agent. The rigid rodlike aromatic core of the agent is designed to achieve high specific binding to Aβ aggregrates. NIAD-4 is commercially available for *in vitro*/*in situ* applications and *in vivo* imaging in animal research.

Synthesis

[PubMed]

Nesterov et al. (7) reported the preparation of NIAD-4 in a three-step synthesis based on Stille coupling. Briefly, 4-iodo-1-tert-butyl-dimethylsiloxylbenzene was reacted with 5-tributylstannyl-2,2'-bithiophene at 70°C for 36 h with a yield of 57%. The resulting product was converted to the silyl-protected aldehyde using N,N,N',N'-tetramethylethylenediamine and malononitrile with a yield of 84%. The final step involved *in situ* deprotection using hydrochloric acid at 60°C for 6 h with a yield of 77%. The chemical purity of the final product was >95%. The final product was a dark-red material that exhibited a bright red fluorescence (625 nm) in methanol solution (quantum yield = 15%). NIAD-4 stored in the dark at −20°C was stable for 6 months.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Nesterov et al. (7) characterized the properties of NIAD-4 *in vitro*. The agent was only slightly soluble in aqueous media. At concentrations above ≈10 μM, NIAD-4 formed micelles/aggregates. The aqueous solutions of NIAD-4 only had trace fluorescent emission of quantum yield ≈ 0.008%. In the presence of aggregated Aβ protein (≈ 10 μM), there was a dramatic enhancement of the fluorescent emission with a quantum yield of 5% (NIAD-4 = 4.1 μM in phosphate-buffered saline). There was also a significant red shift (Δ70 ≈ נ nm) and a 1.3-fold increase in the extinction coefficient. *In vitro* binding studies with artificially aggregated Aβ protein indicated that NIAD-4 bound to the same Aβ sites as BTA-1 (9), a known high-affinity Aβ-binding compound. The studies showed that NIAD-4 had a binding affinity (K_i) of 10 nM. In comparison, thioflavin T had K_i of 580 nM.

Nesterov et al. (7) studied the specificity of NIAD-4 binding to Aβ by *In situ* histochemical staining of fixed sections from transgenic mouse brain. Brain sections were obtained from aged APP transgenic mice with AD-like pathology. The brain sections were labeled with a NIAD-4 (10 μM) solution in DMSO/propylene glycol for 15 min at room temperature. *In vitro* fluorescence imaging showed high-specificity labeling of NIAD-4 which revealed the exact position and size of the aggregated Aβ deposits.

Animal Studies

Rodents

[PubMed]

Nesterov et al. (7) demonstrated the *in vivo* Aβ binding of NIAD-4 in aged APP transgenic mice. Mice were prepared with cranial windows to allow direct monitoring of the brain surface and then administered 10 μM of 2 mg/kg NIAD-4 solution by i.v.

injection. Red fluorescence imaging using multiphoton microscopy showed that the agent readily crossed the blood-brain barrier and labeled specifically both the plaques and cerebrovascular amyloid angiopathy.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

NIAD4 for Alzheimer's Disease Poster 1.

NIAD4 for Alzheimer's Disease Poster 2

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References

1. Hardy J., Selkoe D.J. *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science. 2002;297(5580):353–6. PubMed PMID: 12130773.
2. Masters C.L., Multhaup G., Simms G., Pottgiesser J., Martins R.N., Beyreuther K. *Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels.* Embo J. 1985;4(11):2757–63. PubMed PMID: 4065091.
3. Kang J., Lemaire H.G., Unterbeck A., Salbaum J.M., Masters C.L., Grzeschik K.H., Multhaup G., Beyreuther K., Muller-Hill B. *The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor.* Nature. 1987;325(6106):733–6. PubMed PMID: 2881207.
4. Carter J., Lippa C.F. *Beta-amyloid, neuronal death and Alzheimer's disease.* Curr Mol Med. 2001;1(6):733–7. PubMed PMID: 11899259.

5. Massoud T.F., Gambhir S.S. *Molecular imaging in living subjects: seeing fundamental biological processes in a new light.* Genes Dev. 2003;17(5):545–80. PubMed PMID: 12629038.
6. Bremer C., Ntziachristos V., Weissleder R. *Optical-based molecular imaging: contrast agents and potential medical applications.* Eur Radiol. 2003;13(2):231–43. PubMed PMID: 12598985.
7. Nesterov E.E., Skoch J., Hyman B.T., Klunk W.E., Bacskai B.J., Swager T.M. *In vivo optical imaging of amyloid aggregates in brain: design of fluorescent markers.* Angew Chem Int Ed Engl. 2005;44(34):5452–6. PubMed PMID: 16059955.
8. Khurana R., Uversky V.N., Nielsen L., Fink A.L. *Is Congo red an amyloid-specific dye?* J Biol Chem. 2001;276(25):22715–21. PubMed PMID: 11410601.
9. Klunk W.E., Wang Y., Huang G.F., Debnath M.L., Holt D.P., Shao L., Hamilton R.L., Ikonomovic M.D., DeKosky S.T., Mathis C.A. *The binding of 2-(4′-methylaminophenyl)benzothiazole to postmortem brain homogenates is dominated by the amyloid component.* J Neurosci. 2003;23(6):2086–92. PubMed PMID: 12657667.