Bioorganic & Medicinal Chemistry Letters 21 (2011) 3273–3276

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationship of cyclic thiacarbocyanine tau aggregation inhibitors

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ARTICLE INFO

Article history:
Received 12 December 2010
Revised 4 April 2011
Accepted 8 April 2011
Available online 17 April 2011

Keywords:
Alzheimer's disease
Tau protein
Neurofibrillary tangle
Cyanine dye
Aggregation

ABSTRACT

Macrocyclic bis-thiacarbocyanines are efficacious inhibitors of tau protein aggregation. To extend the structure–activity relationship of this inhibitor class, *N,N'*-alkylene bis-thiacarbocyanines linked by chains of three to eight methylene carbons were synthesized and examined for inhibitory activity against recombinant human tau aggregation in vitro. At 10 micromolar concentration, inhibitory activity varied with linker length, with four methylene units being most efficacious. On the basis of absorbance spectroscopy measurements, linker length also affected compound folding and aggregation propensity, with a linker length of four methylene units being optimal for preserving open monomer conformation. These data suggest that inhibitory potency can be optimized through control of linker length, and that a contributory mechanism involves modulation of compound folding and aggregation.

Aggregation and accumulation of the microtubule-associated protein tau into neurofibrillary lesions accompanies several neurodegenerative disorders, including Alzheimer's disease (AD) and certain forms of frontotemporal lobar degeneration.[1] Because lesion formation correlates with neurodegeneration and cognitive decline, inhibitors of tau aggregation are being investigated as potential therapeutic agents for slowing progression of these disorders.[2] Although small-molecule tau aggregation inhibitors have been reported, including acridines, phenothiazines, carbocyanines, and many other scaffolds,[3–7] the structural features responsible for inhibitory potency are not fully understood. To clarify the mechanism of tau aggregation inhibition, we have been characterizing the structure–activity relationship (SAR) of carbocyanine derivatives, which are capable of inhibiting tau aggregation in vitro and in ex vivo brain slices prepared from transgenic animal models of tauopathy.[5,8–10] We found that inhibitory potency was influenced by the polarizability of constituent cyanine heterocycles and by the length of the polymethine bridge that connected them.[5] Potency could be further augmented by linking carbocyanine units with alkyl chains to create macrocyclic, bis-thiacarbocyanines.[11] Although a previous report on bis-acridine inhibitors of prion conversion found that linker length represents an additional variable that can influence inhibitory potency,[12] the mechanism underlying the observation was not examined. Moreover, whether such an approach may be effective against tau protein has not been reported. Here, we address these issues by characterizing the SAR of bis-thiacarbocyanines in an in vitro assay for tau aggregation inhibition.

To create a novel library of inhibitors, *N,N'*-alkylene bis-thiacarbocyanines were synthesized from 2-methylbenzothiazole in two steps as described previously[11] except that dibromo alkanes (Sigma–Aldrich Co) varying in length from 3 to 9 carbon atoms were used to prepare intermediate bis-quaternary salts **1a**–**1g** (Scheme 1). The final bis-thiacarbocyanines **2a**–**2g**, which contained linkers of length 3–9 methylene units (Scheme 1), were purple/pink solids except **2g** which was a brown oil with poor solubility in aqueous solution. Therefore, only compounds **2a**–**2f** were analyzed further as described below.

To quantify their tau aggregation inhibitory activity, compounds **2a**–**2f** were incubated in the presence of full-length human tau isoform 2N4R and octadecyl sulfate (ODS) inducer under near-physiological conditions of pH, ionic strength, bulk tau concentration (4 μM), and reducing environment. The ODS inducer was included to greatly accelerate aggregation of full-length tau without the need for agitation.[13] Near complete inhibition of aggregation was found for **2b** at 1 μM final concentration (Fig. 1), whereas at least partial inhibition was found for all bis-thiacarbocyanines at 10 μM (Fig. 2). Compounds **2b** and **2d**, which contained linkers of even-numbered methylene units, were the most efficacious inhibitors at this concentration. These data reveal that linker length can in fact influence inhibitory potency of bis-thiacarbocyanines.

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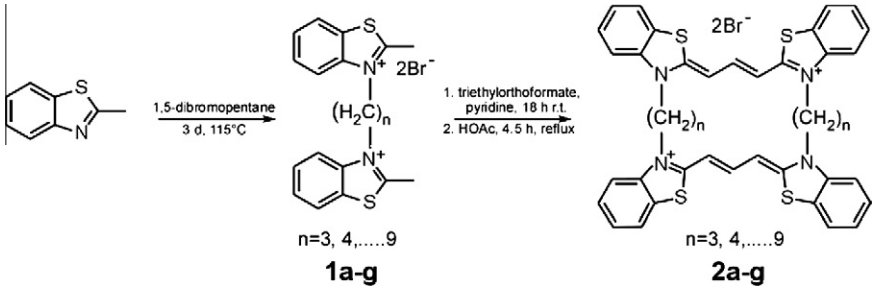
doi:10.1016/j.bmcl.2011.04.039



Scheme 1. General synthetic scheme for the cyclic cyanines reported herein.[11]

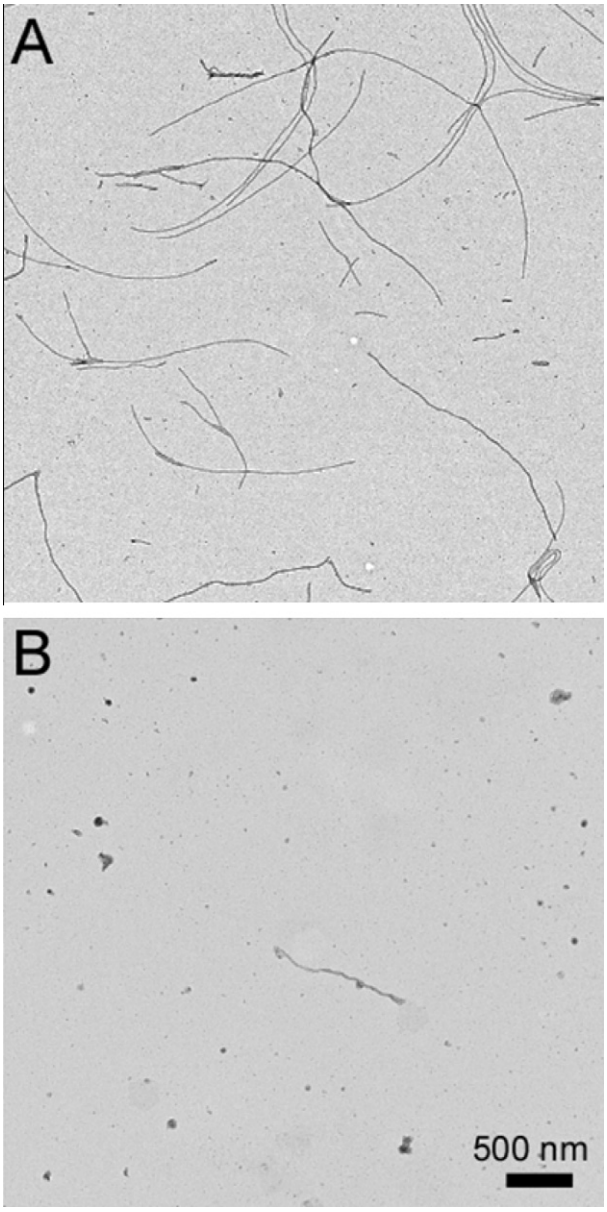


Figure 1. Macrocyclic cyanines inhibit tau aggregation. Tau (2N4R isoform; 4 μM) was incubated (37 °C for 22 h) without agitation in assembly buffer (10 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM dithiothreitol) in the presence or absence of fibrillization inducer ODS (50 μM) and macrocyclic inhibitors. Control reactions contained DMSO vehicle (2% (v/v) final concentration). Reactions were stopped with glutaraldehyde, stained with uranyl acetate, and subjected to electron microscopy as described previously.[15] (A) In the presence of DMSO vehicle control, 2N4R tau formed abundant filaments. (B) In the presence of 1 μM **2b**, 2N4R tau aggregation was inhibited almost completely.

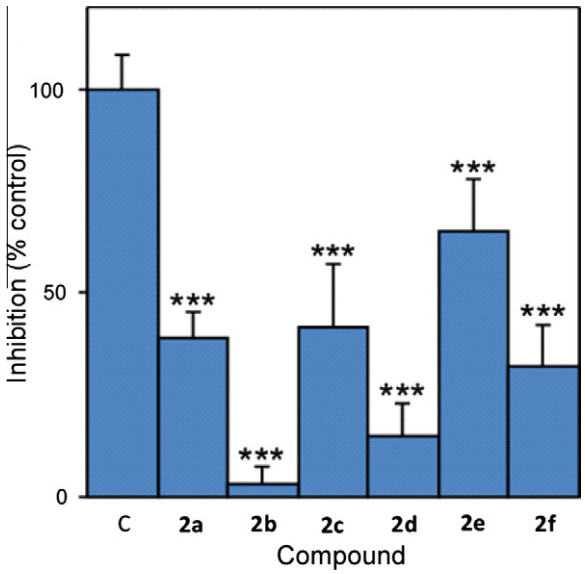


Figure 2. Compound efficacy varies with linker length. Tau (2N4R; 4 μM) was incubated without agitation in assembly buffer containing 50 μM ODS inducer and 10 μM **2a**–**f**. Reaction products were then stained with uranyl acetate and viewed by electron microscopy. Each bar represents total filament length expressed as the normalized percentage of filament length measured in DMSO vehicle alone (quadruplicate determinations ± SD) as described previously.[5] Data were analyzed by one-way ANOVA and Dunnett's multiple comparison test (***p <0.001 compared to DMSO control reaction).

Macrocyclic thiacarbocyanines undergo complex folding and aggregation reactions[14] that may influence their tau aggregation antagonist activity.[10,11] Therefore, the effects of linker length on **2a**–**f** folding were investigated using absorption spectroscopy over visible wavelengths 400–650 nm.[10] In methanol solvent, which depresses compound aggregation,[11,14] all compounds showed two absorption optima: a sharp peak at 560 nm corresponding to the open monomer conformation, and a broader peak centered at 520 nm corresponding to the closed conformation (Fig. 3A).[11,14] Quantitatively, however, the compounds differed in relative peak heights, with some favoring the 560 nm (open) monomer conformation relative to the 520 nm (closed) clamshell conformation. Compound **2b**, the most efficacious inhibitor at 1 and 10 μM concentrations, adopted primarily open monomer conformation under these conditions (Fig. 3A). In contrast, **2a**, a compound that was inactive at 1 μM and weakly inhibitory at 10 μM adopted primarily closed conformation (Fig. 3A). Compounds with longer linkers (**2c**–**2f**) populated both closed and open conformations that varied over a more narrow range (Fig. 3A). These data show that linker length influences compound folding, with the shortest linkers favoring discrete conformations.

To examine the effects of linker length on aggregation propensity, the experiment was repeated in aqueous solution containing