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Fibrillogenesis in ADan peptides is inhibited by biphenyl ethers

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ABSTRACT

In this study, biphenyl ethers of diverse functionality were used to assess their effect on fibrillogenesis of both the oxidized and reduced ADan peptides, *in vitro*. It was noted that these compounds not only stalled fibrillogenesis but were also able to disrupt pre-formed fibers. The EC_{50} values for the inhibition of this process lie in the nanomolar range for 50 μM of peptide concentration, indicating the high potency of these compounds as inhibitors. It was found that these compounds impart to the peptides, an α-helical conformation which does not allow them to aggregate and form fibrils. These studies also point out that the transition of peptides through α-helical conformation may be a prelude to the onset of fibrillogenesis for oxADan peptides.

Of late, the search for effective therapeutic strategies to combat neurodegenerative diseases has attracted a great deal of attention [1–3]. Development of therapeutic strategies requires an idea of the mechanism of the development of fibrillar aggregates, which are considered as a significant trait of these diseases. A host of theories are proposed for the delineation of the hierarchy of the formation of these aggregates [4–6]. These reports can be unified to a common theme wherein the basic components of the fibers first combine together to form small protofibrillar masses, which then associate together to form the protofilaments. These protofilaments, in due course of time, entwine together to produce filamentous masses. Presently, a repertoire of about two dozen proteins and peptides are reported to be involved in the pathogenesis of these disorders including Alzheimer's disease. Although the final forms of the aggregates that are involved in the pathogenesis are apparently similar, there are some differences in the chronology of progression of the protofilaments and other fibrillar aggregates into mature fibers.

Of the many therapeutic strategies designed, the ones involving retardation of the growth of the aggregated mass by small molecular weight inhibitors appear quite promising, as they arrest the peptides in a non-aggregating state. Rational development of therapeutic agents requires a rigorous understanding of the assembly of amyloid fibrils. Knowledge about the early conformational and associative events, particularly those leading to the formation of toxic pre-fibrillar structures, should allow the targeting of critical steps in the fibrillogenesis process. Biphenyl ethers, for example, have recently been noted by us to stall the process of β-sheet aggregation in TTR, by stabilizing the native tetrameric form [7]. As a result, the dissociation of the tetramer into monomers, a crucial step in TTR aggregation, is hindered and fibril formation is prevented.

Recently, the process of fibrillogenesis in ADan peptides (Danish dementia peptides) has been probed by us by a number of biophysical and biochemical techniques. It was shown by molecular modeling that the dimeric form of the peptide is stabilized by a combination of hydrogen bonding and hydrophobic interactions. The diverse functionality of the biphenyl ethers prompted us to use these compounds (listed in Table 1) as inhibitors of fibril formation by ADan peptides. Probing the process of inhibition and disruption unveils two important aspects of the event of aggregation of the ADan peptides. These compounds were found to inhibit the process with very low EC_{50} values (in mid nanomolar ranges). It was noted that though the compounds were very good inhibitors of aggregation process, disruption of pre-formed fibrils was possible only up to a certain extent.

Materials and methods

Preparation of ADan peptide solutions. The ADan peptides were purchased from Bachem AG (Switzerland) and purified using a C-18 HPLC column on a WATERS HPLC and their mass was evaluated by MALDI mass spectrometer (Bruker Daltonics). For disaggregation, the peptides were dissolved in HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) and sonicated in a water bath for 1 h, followed by vortexing for 15 min. This solution was left in HFIP overnight. Finally this solution was lyophilized and then taken for fibrilization. The lyophilized peptides thus obtained were dissolved in a minimum volume of DMSO (dimethyl sulphoxide) under the flow of liquid nitrogen. These were the stock solutions. In this manner peptides can be

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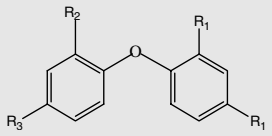
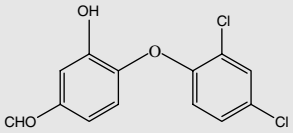
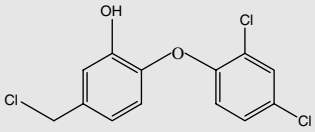
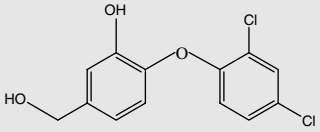
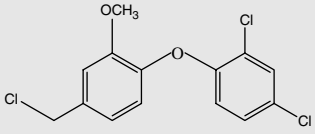
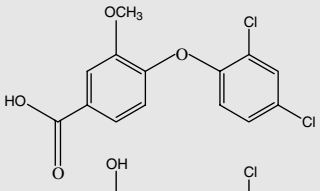
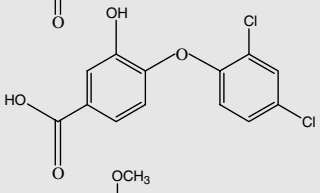
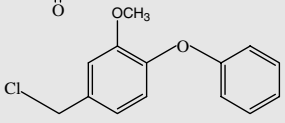
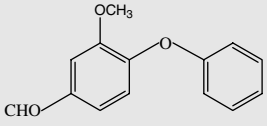
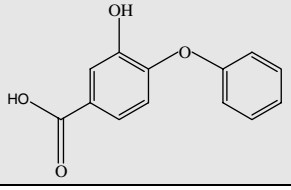
Table 1
Structure of inhibitors I1–I9 and the extent of inhibition of fibril formation by the inhibitors at 100 μM and 1 nM concentration



R1= H, Cl, NO2
R2= OH, OCH3
R3= CHO, COOH, CH2Cl, CH2OH, CH2COOH

Compound	Structure	% Inhibition at 100 μM concentration	% Inhibition at 1 nM concentration
I1		83.03	7.32
I2		80.52	26.51
I3		84.67	13.53
I4		87.16	25.07
I5		98.74	39.96
I6		96.43	35.42
I7		84.73	20.58
I8		80.81	15.36
I9		95.73	32.99

stored in DMSO for a period of almost a month without any aggregation. All the inhibition studies were done with a peptide concentration of 50 μM. The aggregates used for disruption of the process were prepared by aging a solution of 70 μM of the peptides in acetate buffer (sodium acetate buffer 5 mM, 10 mM sodium chloride and 0.03% sodium azide, pH 4.8) for desired time intervals [7].

Preparation of inhibitors. Stock solutions of the compounds to be tested (10 mM) were prepared in DMSO. All the compounds to be tested were analyzed by mass spectrometry, combustion analysis and H^1 and C^{13} NMR for purity. Synthesis of these compounds is detailed in [8].

Thioflavin-T fluorescence. Thioflavin-T (ThT) binding assays were performed as described previously [9]. Briefly, the ADan samples (oxidized and reduced) were excited at 444 nm and emission was taken in the range 460–560 nm on a Jobin Yvon Fluoromax spectrofluorometer using excitation and emission slit width of 2 and 5 nm, respectively. Prior to each fluorescence measurement, 10 μL of 100 μM ThT