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3-*p*-Toluoyl-2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran and 2-[4′-(3-Diethylaminopropoxy)-phenyl]-benzofuran Do Not Act as Surfactants or Micelles when Inhibiting the Aggregation of β-Amyloid Peptide

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Abstract—The cmc and IC_{50} values of the β-amyloid (Aβ) aggregation inhibitors, 3-*p*-toluoyl-2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **1**, and 2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **2** have been determined. After comparison of the cmc data and biological data (IC_{50} values), we conclude that these active benzofurans do not act as surfactants or micelles at the concentration required to inhibit β-amyloid-peptide aggregation.

The formation of abnormal fibrillar protein aggregates is an important pathological feature of approximately 20 different systemic and neurodegenerative (brain) amyloid diseases or 'amyloidoses'.[1] Alzheimer's disease (AD) is a prominent member of the neurodegenerative amyloidoses. In this disease, deposits of a 39–43 amino acid peptide, called β-amyloid (or Aβ), accumulate in the brain.[2] This process is thought by many researchers to be the underlying cause of AD, and so inhibition of Aβ aggregation is a novel approach to potential therapy.[3]

We have recently reported a synthesis[4] of two inhibitors of Aβ aggregation, 3-*p*-toluoyl-2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **1** and its synthetic precursor 2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **2**. We have also shown that simple benzofurans, such as **1** and **2**, are effective inhibitors of Aβ aggregation at μM concentrations.[5] A typical inhibition curve, obtained in our laboratory for benzofuran **1**, is shown in Figure 2. For this data, the aggregation of Aβ was monitored by an immunoassay method, where the same anti-Aβ monoclonal antibody (6E10) was used for capture and detection in a sandwich format.[6,7] A strong signal is obtained in this immunoassay only with multimeric forms of the Aβ peptide.[6,7] IC_{50} values of 20 and 78 μM were determined for benzofurans **1** and **2**, respectively, where this is the concentration of benzofuran required to inhibit aggregation of 50 μM Aβ 1–40 peptide by 50%, over a 24 h peptide incubation period, at 37 °C, as monitored by the aggregation-dependent immunoassay. Interestingly, the results show that the acylated side chain in **1** is not essential for compounds of this type to act as Aβ aggregation inhibitors. Despite these encouraging results, it is possible that, due to their amphiphilic structure[8] these benzofurans may be inhibiting protein aggregation by means of a simple micellar/surfactant-based mechanism rather than via a traditional ligand/protein binding interaction.[8,9] In order to test this hypothesis, the critical micelle concentration (cmc) of the active benzofurans **1** and **2** was measured using conductance titration experiments.[10] These measurements were carried out in aqueous solutions containing 10% DMSO to replicate the conditions used for the Aβ aggregation experiments. In addition, ^{1}H NMR spectra of benzofuran **1** at various concentrations were also recorded in an attempt to confirm, or otherwise, that micellisation was taking place.

The conductivity titration plots are shown in Figure 3. These show that a drop in the rate of change of con-

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ductance occurs for benzofurans **1** and **2** at the onset of micelle formation. From these points, cmc values of 225μM (±10 μM) and 220 μM (±15 μM) were determined for benzofurans **1** and **2**, respectively. This indicates that both benzofurans have almost identical cmc values. Since the IC_{50} values for inhibition of Aβ aggregation were significantly different (20 and 78 μM, respectively) and well below the cmc values, a micellar/surfactant mechanism cannot be used to explain the biological properties of these benzofuran molecules. For example if we examine benzofuran **1** more thoroughly, we note that its cmc value is 10 times greater than its concentration required to inhibit 50% of Aβ peptide aggregation. Further evidence against a micellar/surfactant mechanism also comes from the inhibition curve shown in Figure 2. This shows that at 100 μM, which is well below the cmc value of benzofuran **1**, virtually no aggregation of Aβ 1–40 peptide can be detected. Overall this indicates that a traditional ligand/peptide binding interaction is the main mechanism for inhibition of the β-amyloid peptide aggregation. Our conclusions are also consistent with the report of Howlett et al.[6] that certain benzofuran molecules (including **1**) can selectively bind to Aβ peptide, as determined by a scintillation proximity assay.

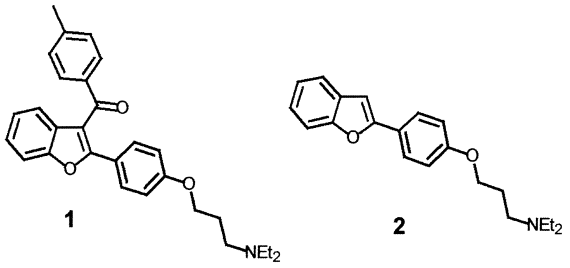


Figure 1. The two active benzofurans, 3-*p*-toluoyl-2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **1** and 2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **2**.

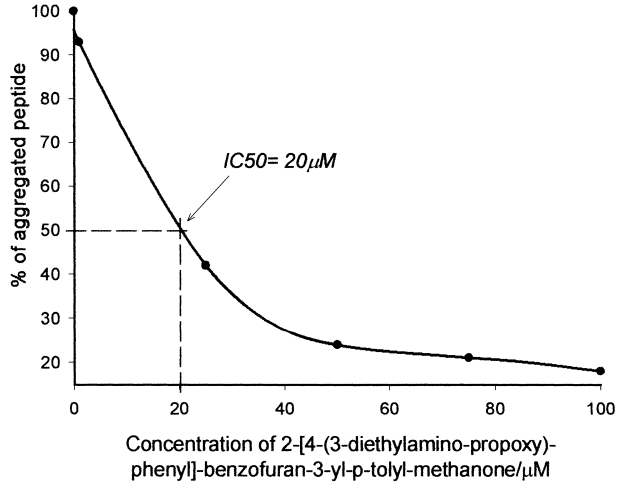


Figure 2. Determination of IC_{50} value for benzofuran **1** (plot of peptide aggregation versus concentration of benzofuran **1**, see text for assay).

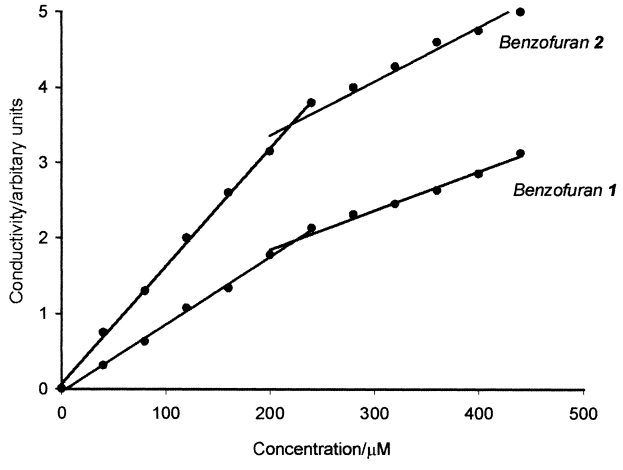
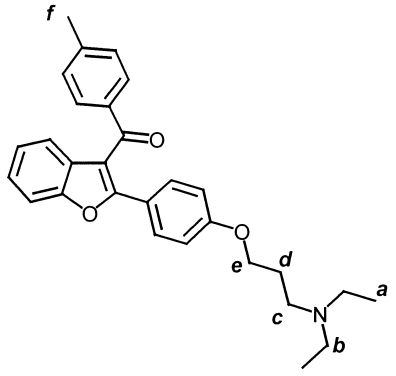


Figure 3. Conductance titration plots used to determine the cmc values for benzofurans **1** and **2**.

In an effort to further establish the surfactant nature of benzofuran **1**, ^1H NMR spectra were recorded below and above its cmc value (solutions of benzofuran **1** were recorded at 100 μM and 300 μM in D_2O containing 10% DMSO-d_6). These spectra revealed large high field shifts for the alkyl resonances of the aggregated form of benzofuran **1** (indicating a less shielded environment for these protons). In addition, as the concentration of the benzofuran was increased from 100 μM to 300 μM, the resolution of all peaks decreased until only broad unassignable peaks could be seen. However, on dilution of the same 300 μM sample to 100 μM, an assignable (reasonably) well-defined spectrum once again appeared: these results are summarised in Figure 4.

In conclusion, we have shown, using conductivity and ^1H NMR measurements, that the Aβ aggregation inhibitors, 3-*p*-toluoyl-2-[4′-(3-diethylaminopropoxy)-phenyl]-benzo-



Proton	*ppm at 100μM*	*ppm at 300μM*
a	1.15	1.27
b+c	3.07	3.22
d	1.99	2.18
e	3.78	4.11
f	2.21	2.35

Figure 4. NMR shifts of benzofurans **1** at 100 and 300 μM.

furan **1**, and 2-[4′-(3-diethylaminopropoxy)-phenyl]-benzofuran **2** can form micelles at high concentrations relative to their IC_{50} values for inhibition of Aβ aggregation. In addition, we have measured the cmc values for benzofurans **1** and **2**, and after comparison with their IC_{50} values, conclude that these benzofurans do not inhibit Aβ aggregation via a micellar/surfactant-based mechanism.

Acknowledgements

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8. It has been suggested in referee's comments that these benzofurans may be acting as rigid amphiphiles. For examples see (a) Barrelt, D. G.; Gellman, S. G. *J. Am. Chem. Soc.* **1993**, *115*, 9343. (b) Carey, J. C.; Montet, J. C.; Phillips, M. C.; Armstrong, M. J.; Mazer, N. A. *Biochemistry* **1981**, *20*, 3637.
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10. Conductivity experiments were performed using a WPA CMD630 digital conductivity meter equipped with a Kent EIL2000, 2022/670 type conductivity probe. The initial solution of benzofuran was made up as follows; 5.011×10^{-5} mol of benzofuran in 50 mL of a water/DMSO (10% v/v) solution. This solution was then titrated into 50 mL of water (at 2 mL intervals) to give a final benzofuran concentration of 500 μM.