LC-UV-MS\textsuperscript{n} Studies of Selective β-Amyloid Aggregation Inhibitor BTB01473

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Overview

Drug design studies targeting soluble oligomers of amyloid β-protein (Aβ), which are one of the primary toxic agents in Alzheimer’s disease, have been complicated by the rapid, heterogeneous aggregation of Aβ and the resulting difficulty in characterizing the peptide. A previous study using a ligand-based design approach identified a small molecule which inhibited the aggregation of Aβ with high selectivity. This molecule – BTB01473 (1) – thus potentially provides a novel tool to study the structure of Aβ oligomers\textsuperscript{1}.

Introduction

Degradation studies of a selective β-amyloid inhibitor (1) were undertaken: A liquid chromatography-UV-ion trap mass spectrometry (LC-UV-MS) method described here was applied to investigate the stability of 1 in PBS buffer, followed by structural elucidation of the degradation products and a proposed degradation mechanism. Collision induced dissociation (CID) was applied to structural elucidation.

Methods

Sample preparation: A stock solution of molecule 1 (10 mM) was prepared in DMSO solution. Molecule 1 (100 μM) was incubated in PBS buffer for 0, 15, 30, 60 minutes and 24 hours at room temperature. Samples were diluted to 20 μg/ml, with acetonitrile/water (1:9), and 10 μl aliquots were injected for LC-UV-MS analysis.

Results

FIGURE 1. Representative chromatograms of a freshly prepared solution of 1 in PBS buffer.

At all time points from 0 to 24 hours, UV peak areas showed no changes in relative amounts of the 5 major components.

FIGURE 2. Full mass spectra of 1 and 1-20. A closely eluting molecule has a mass 20 Da lighter than 1. The modification site was assigned based on MS\textsuperscript{3} data. Both 1 and 1-20 may undergo in vitro hydrolysis to produce degradation products 2 and 2-20 respectively, along with 3.

FIGURE 3. MS\textsuperscript{3} of 1 and 1-20. In A, a loss of 175 results in the formation of m/z 294. In B, MS\textsuperscript{2} of 1-20 parallels that of 1, with a loss of 155 to form m/z 294.

FIGURE 4. MS\textsuperscript{3} of 1 and 1-20. Further fragmentation of m/z 294 for 1 and 1-20 generates nearly identical MS\textsuperscript{3} spectra, revealing losses of 36 (HC\textsubscript{1}) and 161 (free radical fragment). Aromatic peaks are known to stabilize free aryls\textsuperscript{3,4}.

FIGURE 5. Full scan mass spectra of 2 and 2-20. Molecules 2 (A) and 2-20 (B) may be formed via in vitro hydrolysis of 1 and 1-20, respectively.

FIGURE 6. MS\textsuperscript{5} Spectra of 2 and 2-20. The CID fragmentation pattern for 2-20 is similar to that of 2. In A (MS\textsuperscript{5} of 2), a loss of 175 results in the formation of m/z 122. In B (MS\textsuperscript{5} of 2-20), a loss of 155 also results in formation of m/z 122.

Conclusions

- Degradation kinetics and products of molecule 1 in PBS buffer were investigated using LC-UV and ion trap mass spectrometry with CID.
- The study observed stable ratios of 1 and four related products.
- Structures and a degradation scheme consistent with the observed mass spectrometric data were proposed.

Future considerations

- The absence of kinetic trends is a concern, as is the unusual nature of some structures necessarily proposed to fit the degradation model of the data.
- NMR and direct MS of molecule 1 prior to contact with aqueous or proton solution should provide key information.
- High-resolution mass spectra will be extremely informative: in defining the elemental composition of the 20 Da difference in assisting with structural elucidation, e.g. distinguishing oxidative dechlorination from dechlorination plus methylation, indicating the presence or absence of sulfur in the various fragments.

References

2) Xu G et al., Thermo Scientific Application Note, 496, 2007

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