LC-UV-MS^n Studies of Selective β-Amyloid Aggregation Inhibitor BTB01473

Ludmila Alexandrova[1], Paul A. Novick[2],Vijay S. Pande[2], and Allis Chien[1]

[1] Vincent Coates Foundation Mass Spectrometry Laboratory, [2] Department of Chemistry; Stanford University

Overview

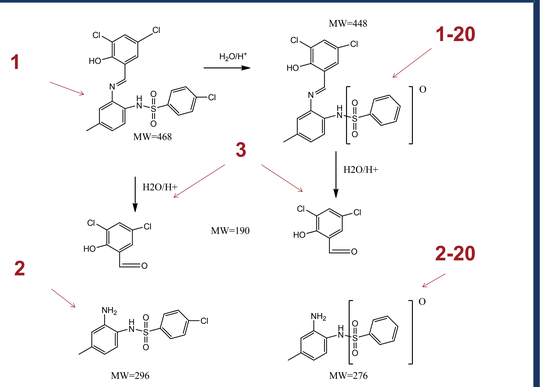
Drug design studies targeting soluble oligomers of amyloid β-protein (Aβ), which are one of the primary toxic agents in Alzheimer's disease, have been complicated by the rapid, heterogeneous aggregation of Aβ and the resulting difficulty in characterizing the peptide. A previous study using a ligand-based 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[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.

SCHEME 1. Putative structures of BTB0147 (1) and four related molecules: 1-20, 2, 2-20, and 3. Proposed scheme: Oxidative dechlorination of 1 results in the formation of a molecule of mass 20 Da lighter than 1. The modification site was assigned based on MS/MS data. Both 1 and 1-20 may undergo imine hydrolysis to produce degradation products 2 and 2-20, respectively, along with 3.



Methods

Sample preparation: A stock solution of molecule 1 (10 mM) was prepared in DMSO solution. Molecule 1 (100μM) 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.

Instrumentation: A unified LC-UV-MS method was developed for assessing both qualitative kinetics and structural elucidation of degradation products, using an 1100 series HPLC-UV (Agilent Technologies) integrated with an LTQ XL ion trap mass spectrometer (Thermo Fisher Scientific). Liquid chromatography was performed on a Hypersil Gold C18 column (100 x 2.1 mm), using 0.1% formic acid in acetonitrile (B) and 0.1% formic acid in water (A) and eluting with a linear gradient from 40% B to 95% B in 11 minutes. Flow rate was 250 uL/min and UV detection was at 254 nm.

Qualitative comparison: Qualitative assessment was based on UV peak area ratio calculations. MS^n data for structural elucidation were acquired using electrospray positive/negative ion switching with data dependent acquisition in dynamic exclusion mode.

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.

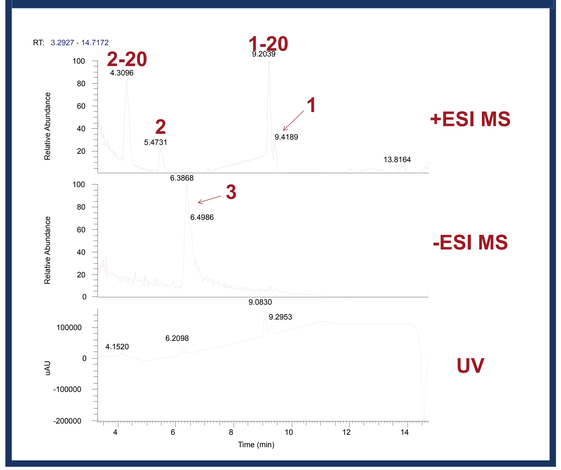


FIGURE 2. Full mass spectra of 1 and 1-20. A closely eluting molecule has a mass 20 Da less than 1. The isotope pattern for 1-20 indicates the presence of two chlorines, in contrast to the three chlorines in 1.

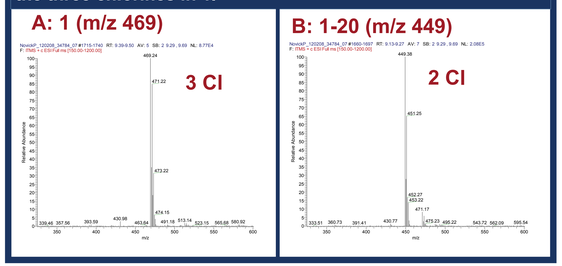


FIGURE 3. MS^2 of 1 and 1-20. In A, a loss of 175 results in the formation of m/z 294. In B, MS^2 of 1-20 parallels that of 1, with a loss of 155 to form m/z 294.

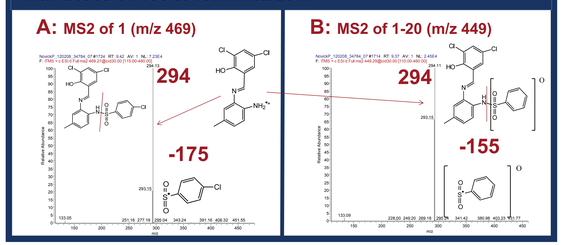


FIGURE 4. MS^3 of 1 and 1-20. Further fragmentation of m/z 294 for 1 and 1-20 generates nearly identical MS^3 spectra, revealing losses of 36 (HCl) and 161 (free radical fragment). Aromatic rings are known to stabilize free radicals[2,3,4]

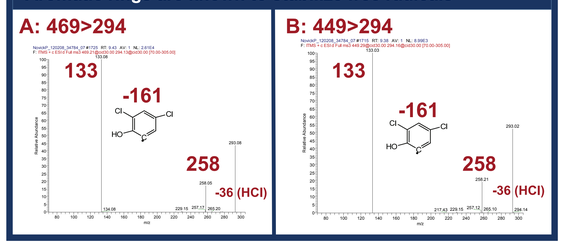


FIGURE 5. Full scan mass spectra of 2 and 2-20. Molecules 2 (A) and 2-20 (B) may be formed via imine hydrolysis of 1 and 1-20, respectively.

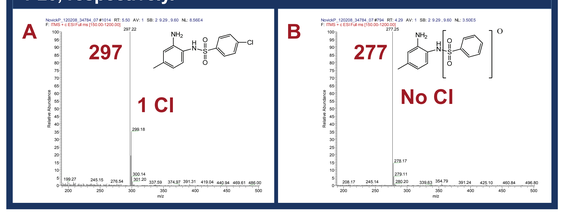


FIGURE 6. MS^2 Spectra of 2 and 2-20. The CID fragmentation pattern for 2-20 is similar to that of 2. In A (MS^2 of 2), a loss of 175 results in the formation of m/z 122. In B (MS^2 of 2-20), a loss of 155 also results in formation of m/z 122.

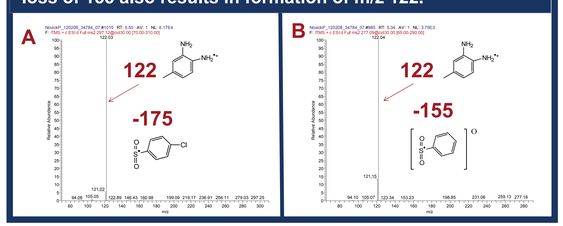


FIGURE 7. MS^3 of 2 and 2-20. Further fragmentation of m/z 122 for 2 (A) and 2-20 (B) generates parallel spectra.

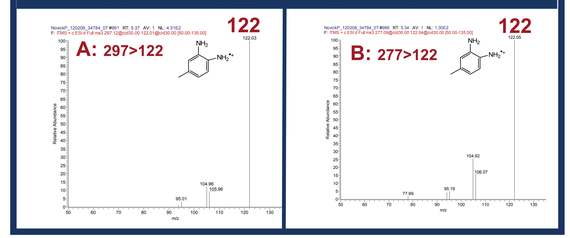
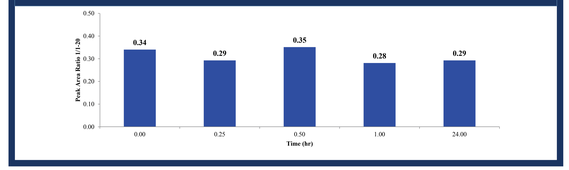


FIGURE 8. Qualitative kinetics assessment for degradation of BTB0147 (1) to 1-20 in PBS buffer.

Molecule 1 incubated in PBS buffer up to 24 hours did not show any trend in increasing 1-20. A parallel analysis of molecule 2 did not show formation of 2-20.



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 static 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 protic 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

1) Novick P et al., Journal of Medicinal Chemistry, 2012, *55*, 3002-3010
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3) Xu G. et. al., Rapid Commun. Mass Spectrom. 2010; 24: 321–327
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Acknowledgements

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