Novel dimers as inhibitors of alpha-synuclein aggregation

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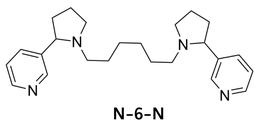
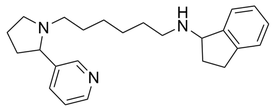
Introduction

- Parkinson's Disease is characterized by the death of dopaminergic neurons in the substantia nigra as a result of the aggregation of alpha-synuclein (AS)
- Nicotine from smoking and 1-aminoindan (a metabolite of Rasagiline) seem to be neuroprotective compounds and both have been associated with the reduction of the risk to develop Parkinson's disease
- These compounds bind to AS at both the N- and C-terminus, forcing the protein to adopt a loop conformation, which appear to contribute to the neuroprotective activity of the drugs[1]
- Dimer molecules linked with two neuroprotective compounds should increase the binding constants to AS and increase the efficacy to prevent AS aggregation



N-6-N



N-6-I

- Phase 1 metabolic studies using hepatic microsomes *in vitro* are needed to determine the susceptibility of the compounds to biotransformation

Objectives

1) Synthesize, purify, and characterize: N-6-N, N-4-N, N-6-I and N-4-I
2) Assess the inhibition of alpha-synuclein aggregation by N-4-N, N-6-N, N-4-I and N-6-I
3) Identify *in vitro* hepatic mouse and human microsomal metabolites of N-4-N, N-6-N, N-4-I and N-6-I

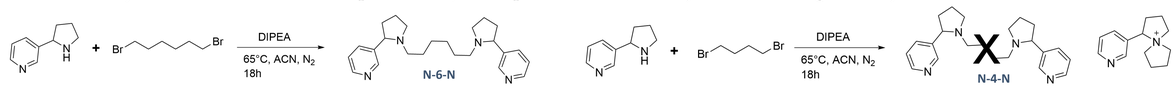
Methodology

- N-6-N was synthesized with nornicotine and 1,6-dibromohexane (2:1) using DIPEA as base and dry ACN as solvent. The reaction was done under N2 at 65 °C for 18h
- The Thioflavin T Assay was done according to a previously established and optimized protocol[2]
- *In vitro* metabolism studies were done following an established and optimized method[3]
- LC-MS/MS was used to identify metabolites by their molecular weight and fragmentation pattern[4]

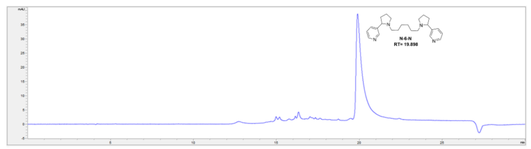
Results

N-6-N, N-4-N and N-6-I Synthesis

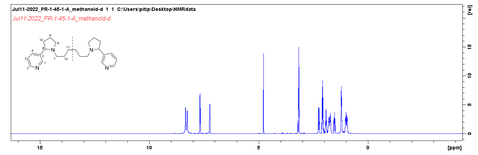
- The solvent system used in the column for purification, can improve or decrease the yield obtained significantly



- Purity of the final N-6-N product was determined by HPLC, and structure was confirmed by H^1, C^{13}, COSY and HMQC NMR

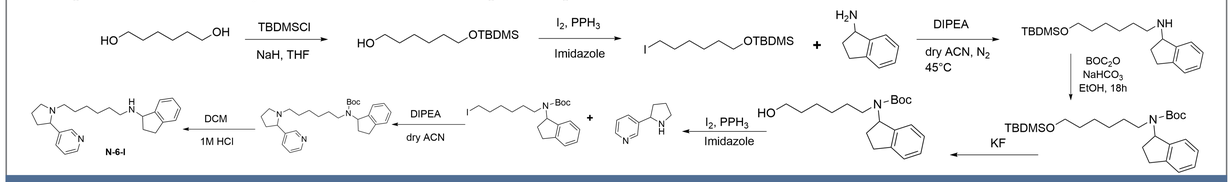


HPLC Chromatogram of N-6-N



^{1}H NMR Spectrum of N-6-N

- Proposed scheme for the synthesis of N-6-I. The first step needs optimization



Alpha-synuclein aggregation studies: Thioflavin T assay

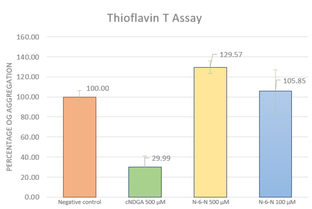
Alpha-syn 1.5 mg/mL
PBS, pH 7,4
100 µM/500 µM of drug

Add to the thermomixer at 37 °C and 1400 rpm for 5 days

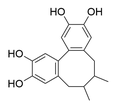
144 µL ThT working solution (26 µM)

Fluorescence of the wells was measured by excitation at 444 nm and emission at 484 nm with a plate reader.

Cyclized nordihydroguaiaretic acid (cNDGA) was used as positive control



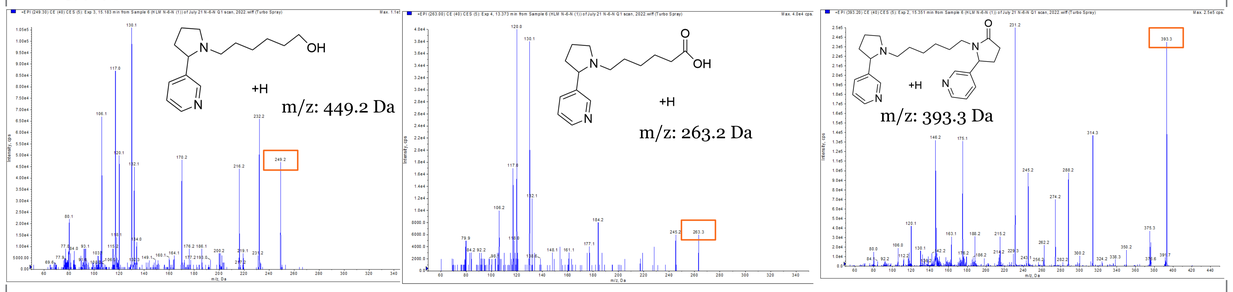
Thioflavin T Assay rusluts



The resulting averages from tubes of the same drug were compared with the control using a t-test assuming equal variances.

LC-MS/MS Analysis of N-6-N in HLM

m/z 379.4 $[N\text{-}6\text{-}N + H]^+$



LC-MS/MS Spectra of metabolites of N-6-N in HLM. Three metabolites were detected.

Conclusions

- N-6-N was successfully synthesized, purified, and characterized
- The results of the Thioflavin T Assay are congruent with other experiments done with similar molecules in our lab
- Three metabolites of N-6-N were identified by LC-MS
- N-6-N undergo similar metabolism in MLM and HLM
- Synthesis of N-4-N based on the method proposed for N-6-I will be attempted

Future Work

- Realize alpha synuclein aggregation studies of N-4-N, N-6-I and N-4-I
- Analysis of the microsomal metabolism of N-4-N, N-6-I and N-4-I in mouse liver microsomes and human liver microsomes by LC-MS/MS
- Determine the binding constants of N-6-N, N-4-N, N-6-I and N-4-I to alpha-synuclein
- Determine the metabolic kinetics of N-6-N, N-4-N, N-6-I and N-4-I using a validated bioanalytical method

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References

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2) Moser, B. *HARVEST*. **2020**,
3) Nwabufo, C. K., Aigbogun, O. P., Allen, K. J. H., Owens, M. N., Lee, J. S., Phenix, C. P., & Krol, E. S. Xenobiotica. **2021**, 51(8), 885–900
4) Nwabufo, C. K., El-Aneed, A., & Krol, E. S. Rapid Communications in Mass Spectrometry, **2019**, 33(23), 1792–1803