Synthesis of PADK Derivatives for the Treatment of Alzheimer's Disease

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ABSTRACT

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Alzheimer's disease is a progressive neurodegenerative disease that slowly destroys memory, thinking capacity, body function, and eventually leads to death. No cure is currently known for this disease and the best medications only slow its inevitable progression. The most prominent theories about the root cause of the disease are the accumulation of either B-amyloid or tau protein plaques. Research is currently underway to develop methods of reducing plaque buildup and hopefully reversing the disease. Previous research endeavors have shown that derivatives of the lysosomal modulator Phe-Ala-Diazomethylketone (PADK) can confer some level of protection against typical Alzheimer's protein accumulation pathology in transgenic mice. Through this research endeavor we have synthesized derivatives of PADK that will be tested for biological activity after confirming that they are structurally different than previously synthesized compounds.

Introduction

Alzheimer's disease is believed to be caused by the accumulation of protein plaques within the brain that eventually cause neurodegeneration and, eventually, death. Amyloid-beta-42 (Aβ-42) and tau are the two proteins that are most suspected of being the root cause of Alzheimer's. The Aβ precursor protein can be cleaved into several different lengths (38, 40, etc.), but the only plaque-forming length is Aβ-42.(Butler et al.) The tau protein is a protein associated with the microtubules within a cell, and hyperphosporylation of these proteins gives a form of tau that forms polymers and neurofibrillary tangles.(Lasagna-Reeves et al.) In the brain these tangles interfere with the functions of the cell and neuronal signaling. In a normally functioning brain the lysosomes of the cells degrade these bad proteins before they can cause any harm. What is believed to happen in Alzheimer's disease is that the turnover rate of the lysosomes decreases significantly over time. As lysosomal activity decreases, the possibility that bad proteins, such as Aβ-42 and tau, will form plaques increases drastically. It is important to note that the lysosomes are still functional, however their rate of catalysis is lower than the rate of formation of the protein plaques, which leads to an overall accumulation of these plaques. Once plaques form they interfere with the normal signaling of the brain and begin the process of neurodegeneration.

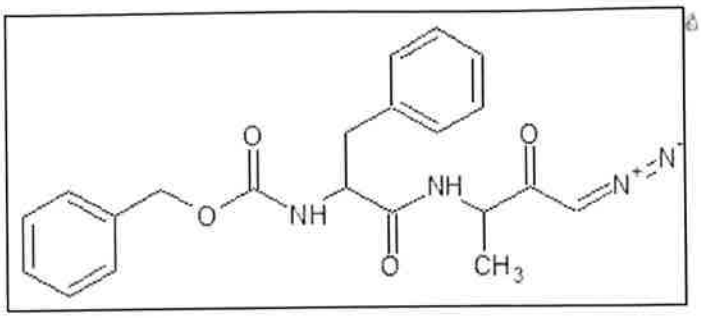


Figure 1: PADK

Z-Phe-Ala-Diazomethylketone, or PADK (Figure 1), is a compound that has been shown to act as a lysosomal modulator that up-regulates lysosomal activity allowing for the clearing of these abnormal proteins and protein plaques.(Zheng et al.) In addition to lysosomal modulation PADK can also directly bind to the early stages of the Aβ-42 monomers and prevent their aggregation.(Viswanathan et al.) Since PADK has the property of directly inhibiting the formation of a major component of Alzheimer's disease as well as up-regulating processes that can reverse the disease, the potential of this compound to become a drug must be explored. One process that nearly all new drug-like compound go through is the development of derivatives, compounds of similar structure that can have different biological properties. Our research has focused on the development and characterization of new derivatives of PADK. After synthesizing the compounds we attempt to characterize them to determine what makes them different from PADK.

Experimental

Synthesis:

TB2: A round-bottom flask with a magnetic stir bar was suspended inside of a beaker with a clamp. Approximately 125 mg of PADK (purchased from BACHEM) was added to the flask

along with 5 mL of acetic anhydride. The flask was fitted with a rubber stopper and the stirrer turned on to a moderate rate. The reaction was left for 2-3 weeks to ensure a complete reaction.

TB5: As for TB2, using methanol instead of acetic anhydride.

After the reaction contents were transferred into sample vials and the solvent allowed to evaporate under the hood for several days. TB2 (39 mg) appeared as a light amber solid, TB5 (46 mg) appeared as a pale, yellow-orange solid.

Analysis:

Each compound was analyzed first using a Shimadzu HPLC equipped with a LG-20AT pump and a SPD-20A UV-VIS detector. The column was used C18 with polar endcapping for the stationary phase, fully porous silica for the solid support, and a central path width of 4 mm. The mobile phase was composed of 70% pH 4 monosodium phosphate 6.25 mM buffer and 30% acetonitrile. A small sample of each compound was dissolved in ethyl acetate and run at 1 mL/minute and the chromatograms compared to that of the original PADK.

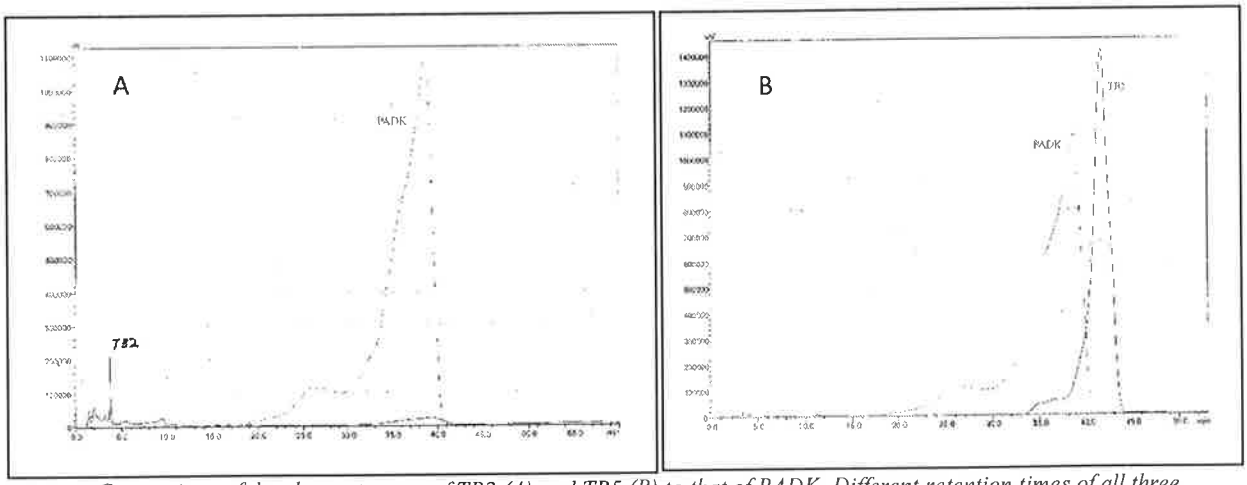


Figure 2: Comparison of the chromatogram of TB2 (A) and TB5 (B) to that of PADK. Different retention times of all three compounds means that all three compounds are different.

The compounds were also analyzed by total attenuated reflectance (ATR) infrared spectrometry. This method was selected because ATR-IR allows for a high quality spectrum to be generated from only a minute quantity of sample. A small was dissolved in ethyl acetate and deposited on the ATR crystal. The spectra were analyzed for differences in their features.

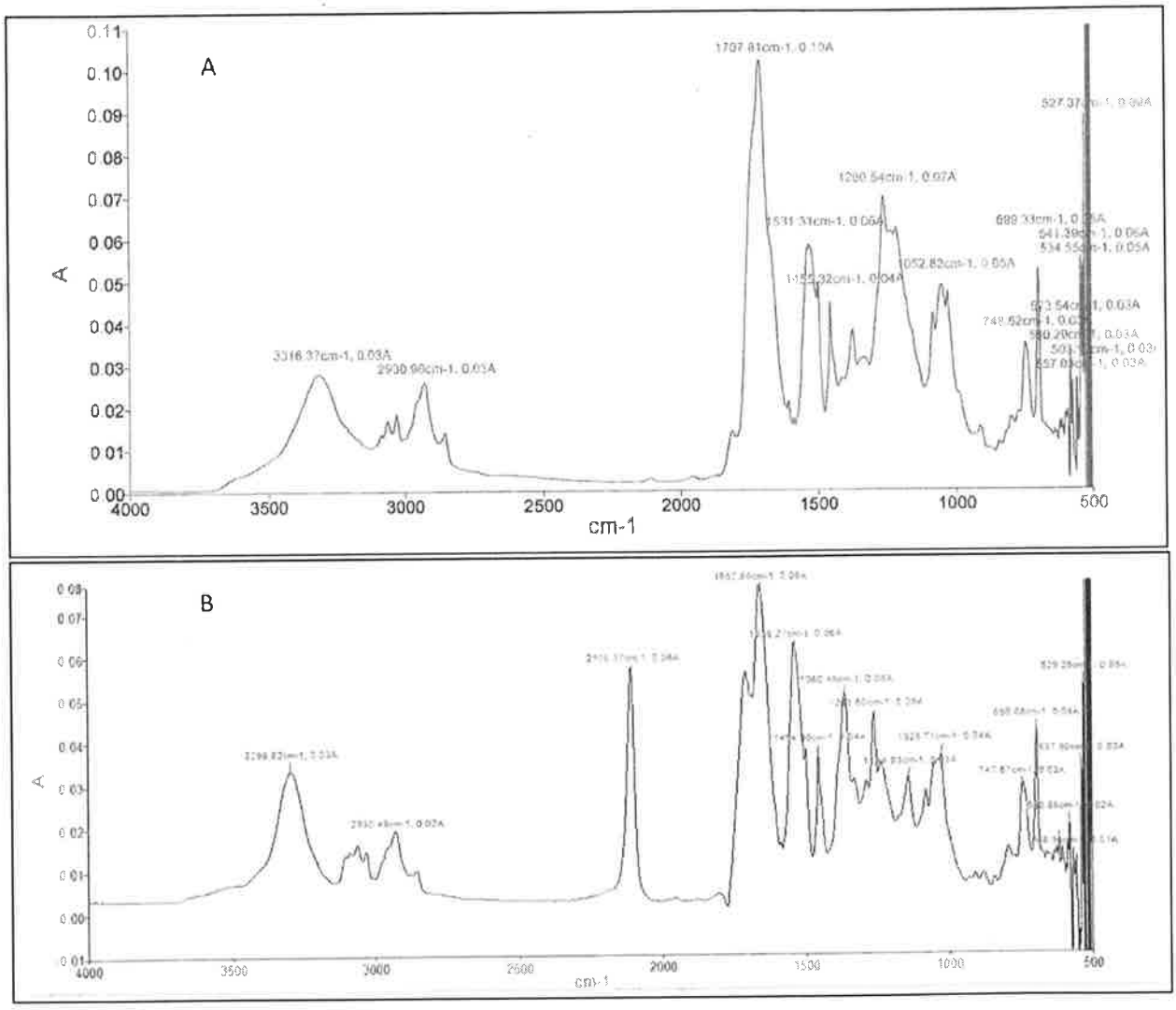
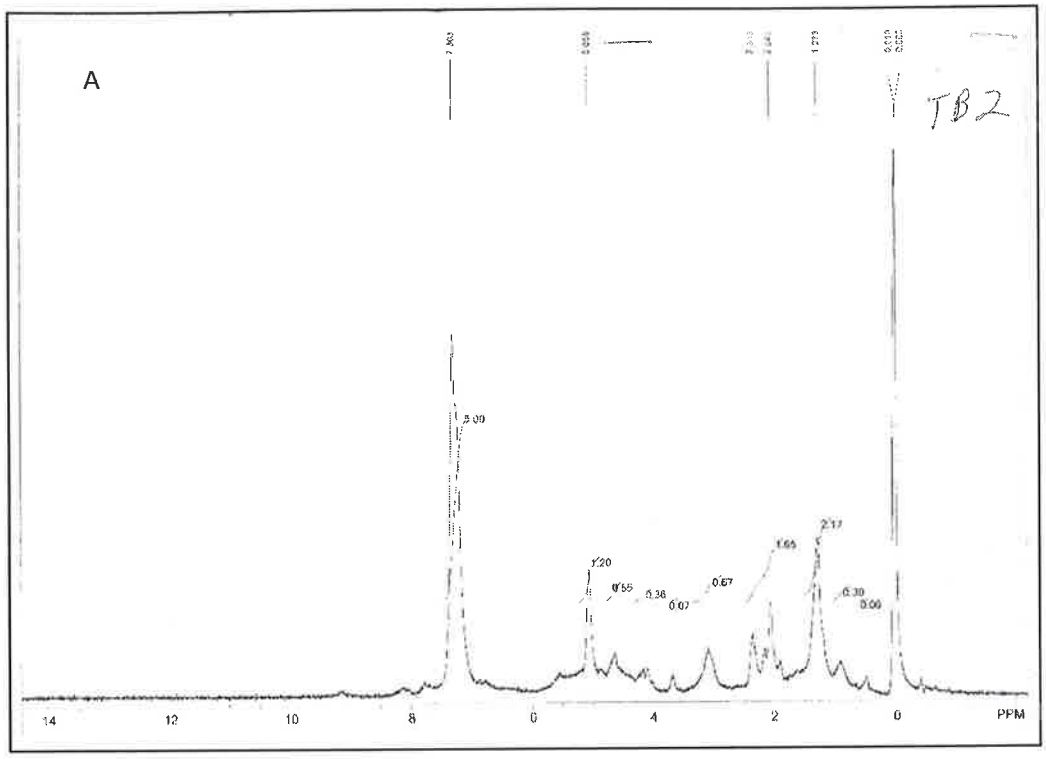


Figure 3: The IR spectra of TB2 (A) and TB5 (B). Most noticeable difference is the presence of the band at 2100 cm^{-1} corresponding to the diazo group of PADK. TB2 has lost the diazo group, TB5 has retained it.

Nuclear magnetic resonance (NMR) spectrometry was also utilized to further elucidate structural differences between the compounds.



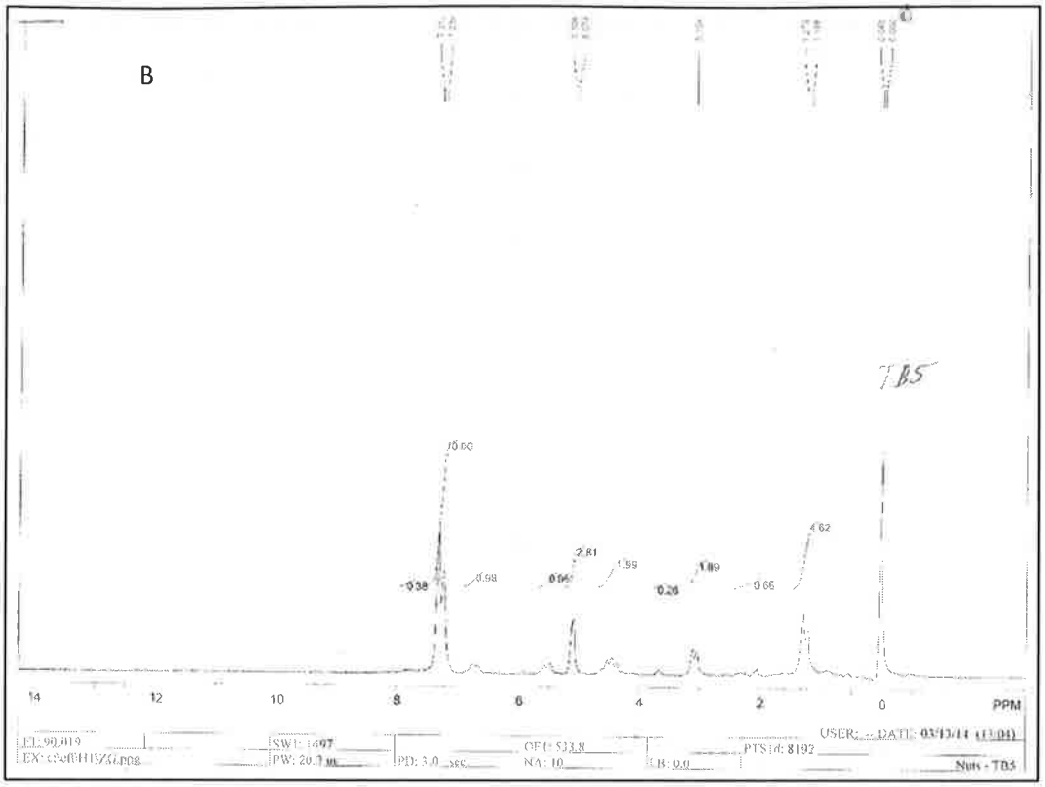


Figure 4: NMR spectra of TB2 (A) and TB5 (B). Spectra are nearly identical except at 2ppm. In TB2 there are additional hydrogens absent from TB5.

Discussion

Beginning with the chromatography results, figures 2a and 2b show that there is a difference in the retention times between all of the compounds. This is a very good sign because it means that we have produced derivatives that are structurally different from PADK as well as from each other. The IR and NMR spectra provide more structural information about the individual compounds, from this we can say exactly what is different. For starters, the IR spectra (figures 3a and 3b) are very similar to one another, which provides more evidence that the two compounds are closely related, but there is also a significant difference between them. The band appearing around 2100 cm^{-1}, corresponding to the diazo group from PADK (Figure 1), is only present in TB5. This means that TB2 has lost the diazo group, and TB5 has retained it. From the ^{1}H NMR

spectra (figures 4a and 4b) the two compounds are very similar in structure having approximately the same number of protons. One major difference is around 2 ppm where TB2 has an additional feature that TB5 does not. This is likely due to the difference in the additional reactants; the acetic anhydride that produced TB2 would likely have added more protons to PADK than TB5's methanol would have, so this result does make sense. NMR is typically a very informative analytical technique, however the baseline in our measurements is very unstable due to the low resolution of the instrument and the high complexity of the molecule. To gain any more meaningful structural information from NMR would require at much more powerful instrument (at least 300 MHz).

Concluding Remarks

From the experimentation described above, we have concluded that: we have synthesized two compounds from PADK; they are structurally similar, and yet different, from PADK and from each other. From here the compounds will be sent to Dr. Ben Bahr for testing of biological activity in tissue samples. If the compounds prove effective, more rigorous efforts will be made to effectively produce and characterize the compounds.

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REFERENCES

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