Disease-Modifying Therapy for Alzheimer's

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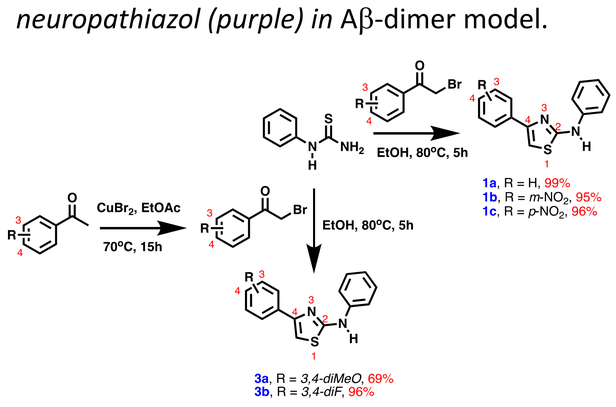
- **Abstract**: The extensive disposition of amyloid-beta (Aβ) plaques cemented between the nerve cells have been known to hold decisive clues to an age-related neurodegenerative disorder – Alzheimer's disease (AD), which is the most prevalent cause of dementia among older people over 65. The progressive memory deficits and other disturbances in Alzheimer's patients' daily activities have often been observed and associated with significant social burden, eventually leading to the increase of morbidity and morality. Because of the modest therapeutic benefit seen with currently available cholinesterase inhibitors and NMDA-receptor antagonist, it is critical to develop novel treatment options for AD. In this regard, our study aims to design, synthesize, and evaluate phenylthiazole based compound libraries with anti-Aβ activity as disease-modifying agents in treating AD. A library of these novel small molecules was synthesized and evaluated for their anti-amyloid aggregation activity in the presence and absence of Aβ using the thioflavin T (ThT)-based fluorescence spectroscopy. The binding interactions were investigated by the computational studies. These investigations have shown that phenylthiazole based derivatives are capable of preventing Aβ aggregation. Future studies such as transmission electron microscopy (TEM) experiments, and the in vitro cell-based assays and in vivo studies will provide more insight on the potential of these novel compounds to treat AD.
- **Keywords**: Alzheimer's disease, disease-modifying therapy, phenylthiazole, anti-aggregation of Aβ, kinetic assay, molecular docking, transmission electron microscopy, in vitro assays.
- **Background**: Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disease, in which the buildup of amyloid-(Aβ) plaques and neurofibrillary tangles (NFTs) in the brain are the prime suspects behind AD. The severity of AD symptoms will gradually lead to progressive decline in memory and cognitive ability, and eventually lead to a disruption in daily activities.[1] AD is the sixth-leading cause of death in U.S., but recent predictions concerning a cause of death for those aged 65 and older may have placed it to the third, just behind heart disease and cancer.[2] As of 2016, approximately 564,000 Canadians have AD. This number is predicted to rise to 937,000 by 2031.[3] To date, there are only two pharmacotherapy options available: acetylcholinesterase (AChE) inhibitors and NMDA-receptor antagonist to help manage AD symptoms and extend life expectancy, but they are unable to cure AD.[4] In order to shed light to the development of new potential amyloid therapy, designing novel small molecule libraries with anti-Aβ aggregation by virtual screening (computational chemistry), synthesis (Figure 1), as well as, *in vitro* and *in vivo* screening of test compound libraries have been used to assess the therapeutic efficacy as anti-AD agents. In this regard, we hypothesize that the designed phenylthiazole-based small molecules bind directly to aggregation prone regions of Aβ (KLVFFA) proteins to effectively block the formation of neurotoxic structures, and thus can halt the disease progression.

Figure 1. *The proposed synthetic scheme and binding modes of N,4-diphenylthiazol-2-amine (**1a**) (A) and neuropathiazol (B), and superimposed binding modes (C) of **1a** (grey) and neuropathiazol (purple) in* Aβ-dimer model.



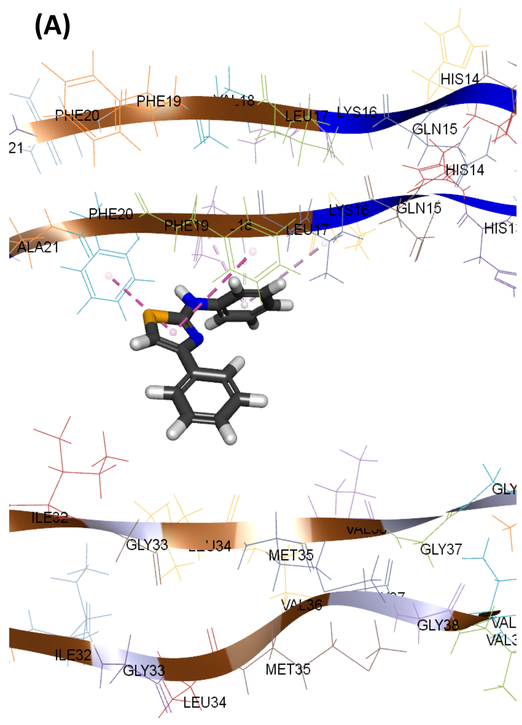


Figure 2. *Pharmacophore model of the structural requirements to prevent various forms of Aβ40 aggregates.*

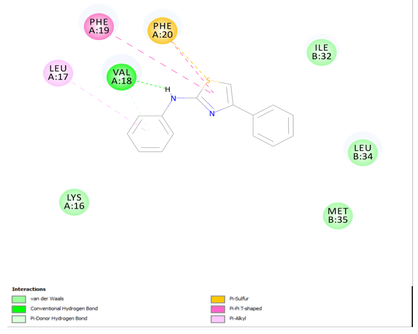


Figure 3. *ThT-monitored 24 h aggregation kinetics of Aβ40 (5 μM) in the presence of 1, 5, 10, and 25 μM of **1a** (top) and methylene blue (bottom) at pH 7.4, 37 °C.*

- *Aggregation kinetics were monitored by ThT-fluorescence spectroscopy (excitation at 440 nm, emission at 490 nm)*

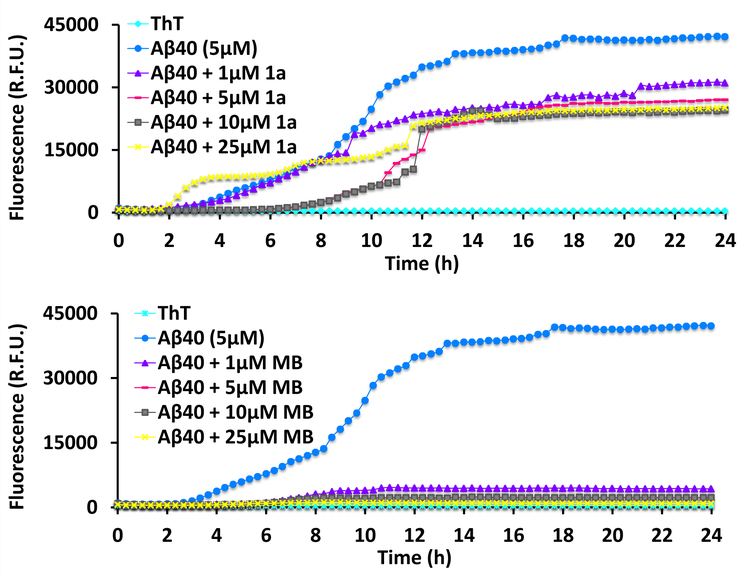
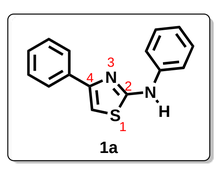


Figure 4. *The aggregation morphology of Aβ40 (25 μM) in the presence and absence of 25 μM of **1a** by TEM at pH 7.4, 37 °C after 24 h incubation.*

- *TEM image resolution is at 100 nm*



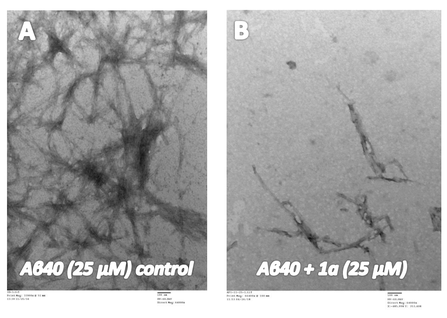


Table 1. *Inhibition data of Aβ40 aggregation*

Compound	% Inhibition (25 μM)
1a	40
1b	70
1c	70
MB	98
OR-G	79

- **Results**: In the absence **1a**, Aβ40 aggregated to form dense tangles (panel A); whereas, in the presence of 25 μM **1a**, a significant inhibition of Aβ40 aggregation was observed (panel B). These results correlated with the data acquired from the ThT-based fluorescence aggregation kinetics of Aβ40 aggregation, where **1a** has a strong ability to suppress the formation of Aβ40 aggregation.

- **Conclusions**: Among the synthesized library evaluated and presented here, **1b** and **1c** were identified as the most potent inhibitor of Aβ40 aggregates (70% inhibition at 25 μM), while **1a** also showed to inhibit the aggregation of Aβ40, which was also validated by the TEM experiments, as they revealed that the disappearance of fibrillar Aβ40 aggregates in the presence of **1a** at 25 μM. Molecular docking studies show that **1a** exhibited its binding modes within the U-shaped conformations in the hydrophobic regions (KLVFFA) of Aβ40-dimer model. These results suggest that **1a** can be a pharmacophore as well as a biomarker for the early treatments of AD.

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