

N,4-Diphenylthiazol-2-amine Derivatives As Amyloid-Beta Aggregation Inhibitors

Praveen Nekkar Rao*, Amy Trinh Pham

School of Pharmacy, University of Waterloo, Health Sciences Campus, Kitchener, Ontario N2L 3G1, Canada

INTRODUCTION: Alzheimer's disease (AD) is a devastating brain disorder that primarily affects the aging population. As life expectancy continues to rise globally, the lack of effective treatments has transformed AD into a major public health challenge, threatening not only individual well-being but also the economic and social stability of societies worldwide. AD has a complex and multifactorial pathophysiology. The current pharmacotherapy options for AD are very limited and provide only symptomatic relief (eg: cholinesterase inhibitors).

One of the leading hypotheses describing AD progression is the amyloid cascade hypothesis, which implicates the formation of neurotoxic amyloid- β aggregates as a central driver of neurodegeneration. The recently approved anti-amyloid therapies underscores the therapeutic relevance of targeting amyloid pathology and supports continued efforts to discover and develop novel small-molecule agents capable of preventing or reducing amyloid aggregation. However, the anti-amyloid antibodies have some disadvantages such i) high cost, ii) requirement for cold-storage and iii) the risk of causing serious adverse events such as brain haemorrhage and bleeding. In this regard, small molecule anti-amyloid therapies represent affordable and accessible alternatives to treat AD. Here, we report the design, synthesis, and biological evaluation of novel small molecules as potential therapeutic agents for the treatment of AD.

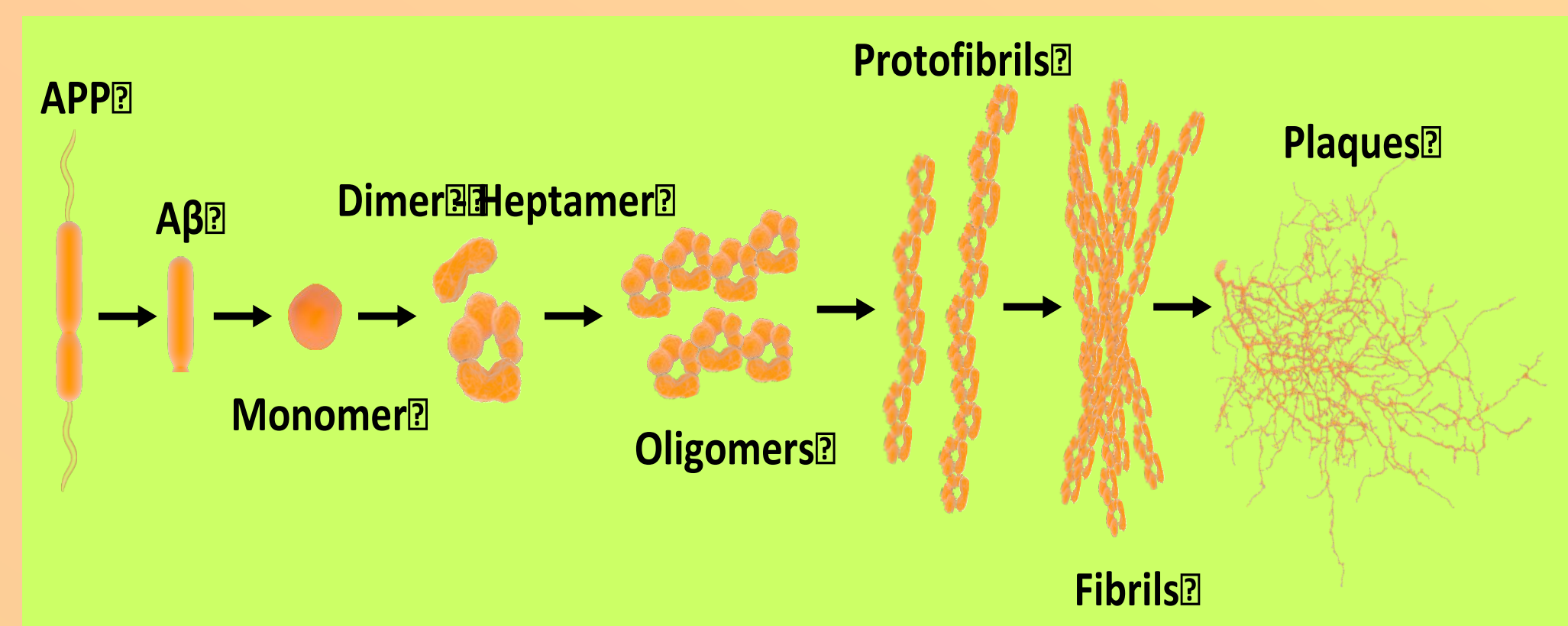
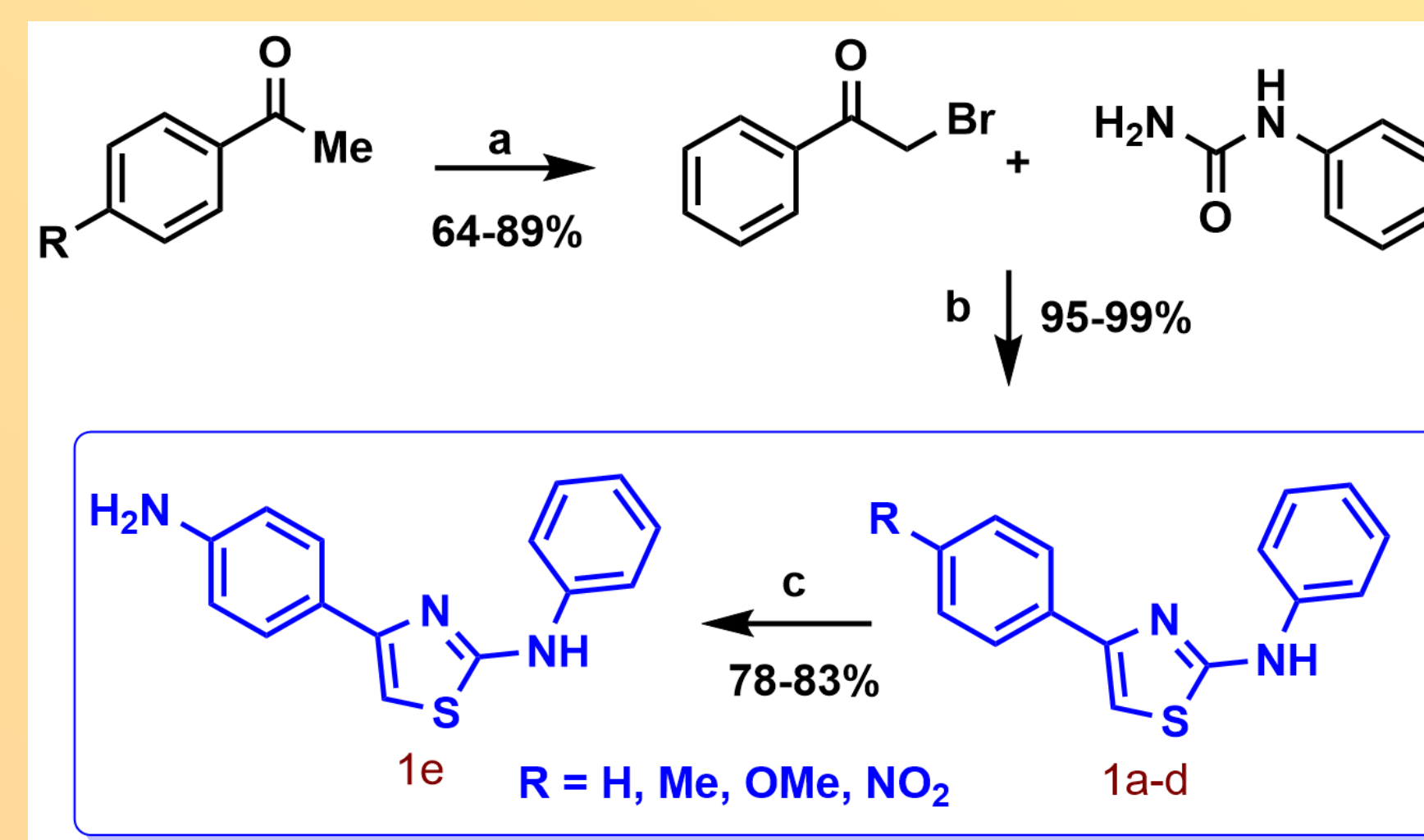


Fig 1: Amyloid cascade in Alzheimer's disease

METHODOLOGY: A novel class of *N*,4-diphenylthiazol-2-amine derivatives were designed, synthesized and evaluated as inhibitors of amyloid-beta ($A\beta_{40}$) aggregation. Computational studies were used to design the small molecule library. Anti- $A\beta_{40}$ was evaluated by carrying out thioflavin-T (ThT) based fluorescence aggregation kinetic studies. The morphology of $A\beta_{40}$ in the presence of test compounds was determined by carrying out electron microscopy experiments. Furthermore, compound toxicities were assessed in mouse hippocampal HT22 neuronal cells and the effect of novel small molecules in preventing $A\beta_{40}$ -mediated cytotoxicity was also determined.



Scheme 1: Reagents and conditions: (a) CuBr_2 , EtOAc, 70 °C, 15 h; (b) EtOH, 80 °C, 5 h; (c) Pd/C, hydrazine hydrate, EtOH, 85 °C, 3-5 h

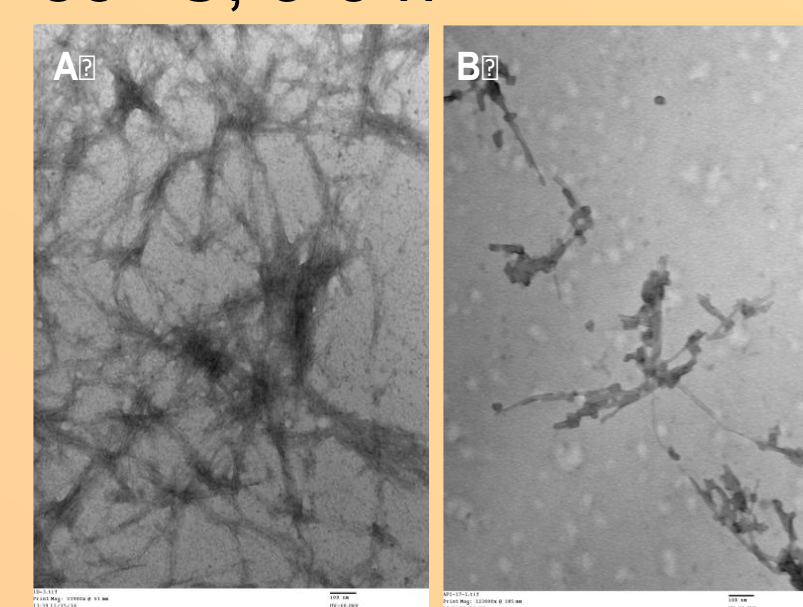


Fig 2: $A\beta_{40}$ alone (5 μM) morphology (A); $A\beta_{40}$ (5 μM) + 1e, 25 μM (B)

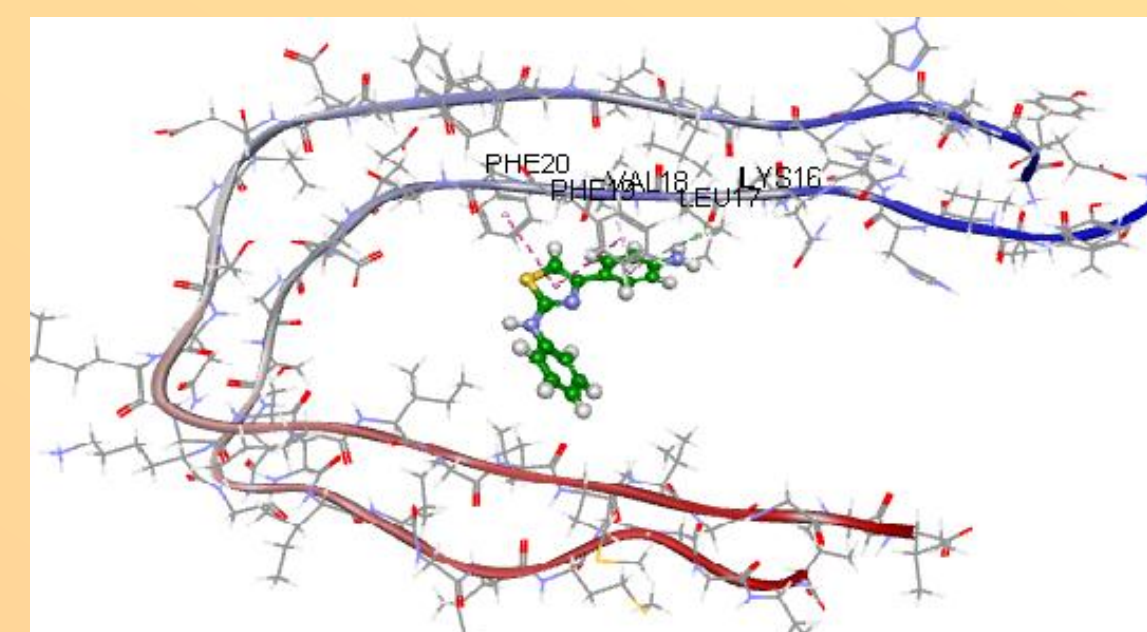


Fig 3: Compd 1e docked in the $A\beta_{40}$ dimer model (PDB id: 2LMN)

RESULTS: Thioflavin-T based fluorescence aggregation studies show that *N*,4-diphenylthiazol-2-amines were able to reduce $A\beta_{40}$ aggregation (inhibition activity 15–75%). Furthermore, electron microscopy studies also confirm the ability of *N*,4-diphenylthiazol-2-amine derivatives to reduce $A\beta_{40}$ aggregation. Cell culture assays demonstrate that the test compound were nontoxic to hippocampal HT22 neuronal cells and were able to rescue HT22 cells from $A\beta_{40}$ -mediated cytotoxicity. Computational studies using the solved 3D structure of $A\beta_{40}$ aggregates helps in understanding the binding interactions of these small molecules.

Table 1: $A\beta_{40}$ Aggregation Inhibition Studies (ThT-Assay)

Compd	R-group	% Inhibition for $A\beta_{40}$ at 25 μM ^a	ClogP ^b
1a	H	37	3.87
1b	Me	56	4.36
1c	OMe	34	3.78
1d	NO_2	70	3.61
1e	NH_2	74	2.64
RVT	-	92	2.83

^aPercent aggregation inhibition values were average $\pm$ SD < 10% (n = 3) for three experiments.

^bClogP values were determined using ChemDraw

RVT - Resveratrol

CONCLUSIONS: Our studies demonstrate that the amyloid cascade in AD can be targeted by designing novel *N*,4-diphenylthiazol-2-amine derivatives using medicinal chemistry principles to develop novel small molecules as potential treatment option for AD.