

## **Chapter 3: Objective, Rationale and Plan of Work**

AD is a complex progressive neurological condition characterized by the gradual loss of cholinergic neurons in both the cerebral cortex and subcortical regions of the brain. Current treatment strategies primarily involve the use of inhibitors targeting acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and NMDA receptors. While these approved treatments offer symptomatic relief and may slow symptom deterioration, they do not halt or prevent the disease's progression. There is a notable absence of drugs that address the fundamental pathological mechanisms underlying AD. Hence, there is an urgent need for therapeutic interventions capable of modifying the disease's course.

There is growing recognition that drugs targeting a single aspect may not suffice in treating intricate conditions like AD, which involve multiple factors. Consequently, the hypothesis suggests that multifunctional drugs, displaying diverse pharmacological effects to address various underlying pathological factors of AD, could serve as effective disease-modifying agents. Guided by this notion, we commenced our drug discovery endeavor to uncover innovative multifunctional compounds with inhibitory activity against AChE/BChE, as well as antioxidant properties, iron chelation ability, and modulation of A $\beta$  aggregation.

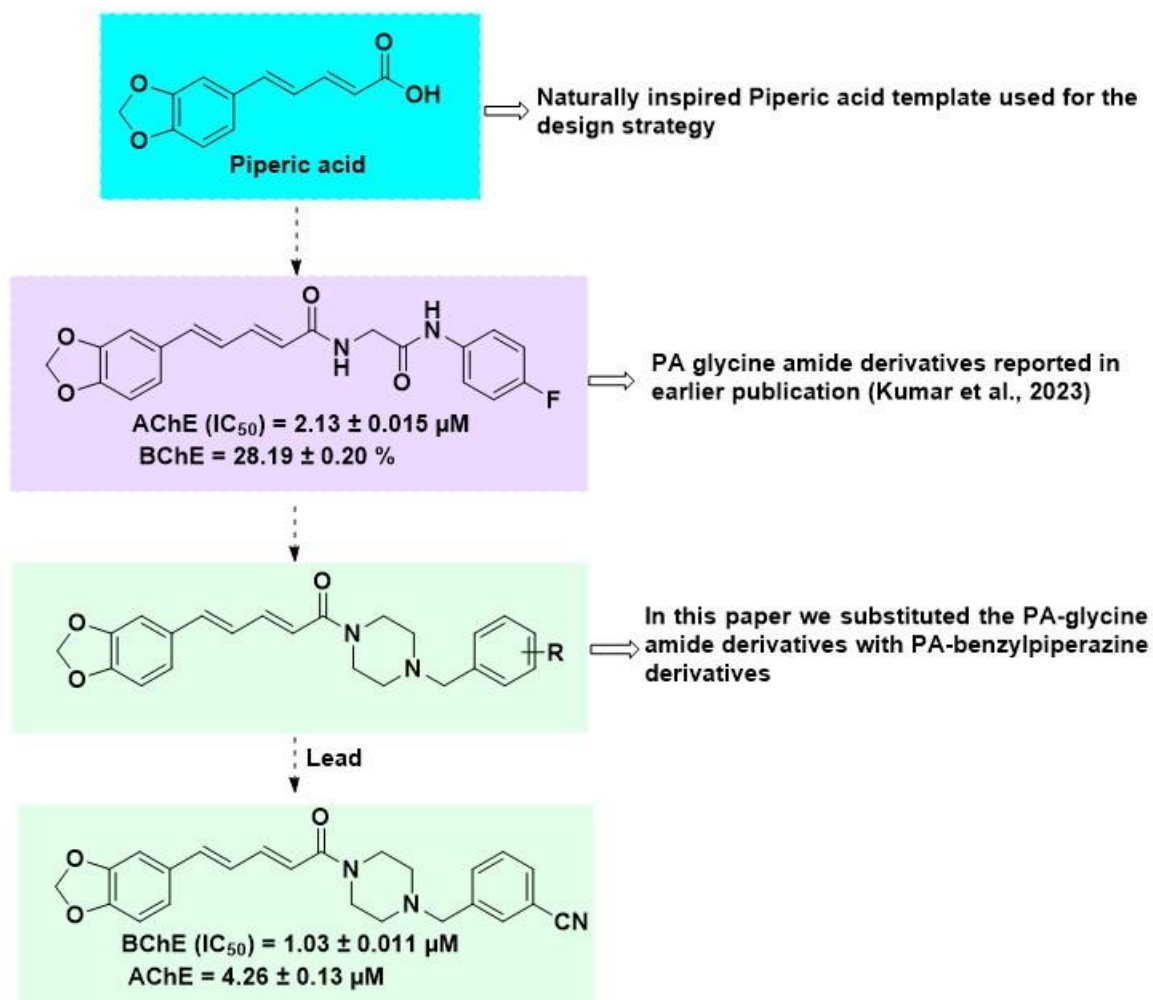
### **3.1. Objective and Rationale**

Currently, there is no known cure for AD. Due to the intricate and multifaceted nature of the disease, it is crucial to discover new therapeutic agents that can target various pathophysiological factors associated with AD. Natural products, such as piperic acid (PA), play a significant role as primary sources of therapeutic agents in the treatment of various diseases, including neurodegenerative disorders.

## Objective, Rationale and Plan of Work

Piperine is a natural compound from black pepper with wide application in Indian kitchens from ancient times (15). From the different literature, it is clear that AChE plays a vital role in the development of AD, and piperic acid (**PA**) is a weak inhibitor of AChE. Therefore, our goal is to improve PA's AChE inhibitory ( $\leq 20\%$  inhibitory activity against the enzyme AChE and BChE at 20  $\mu\text{M}$ ) property through systematic SAR studies. Therefore, we considered developing *in-vivo* efficacious naturally derived PA derivatives to address the issues associated with PAs. Our designed approach is based on our earlier work on ferulic acid, where the coupling of **PA** with various substituted aromatic/heterocyclic moieties via an amide linkage to improve the enzyme activity against AChE and BChE (14, 16) has been effectively carried out. Based on the evaluation of PA's binding analysis to the enzymes, we introduced an appropriate linker as well as a number of hydrophobic or heterocyclic moieties.

As part of our continuous endeavors to create multifunctional therapeutics inspired by natural compounds, we previously disclosed a range of AChE (selective) inhibitors derived from the PA and ferulic acid (FA) templates. However, the developed molecules were found to be weak inhibitors of BChE. Given the fact that BChE plays a key role in different stages of the disease, therefore, we continued our efforts to advance the exploration of the structure-activity relationship (SAR) of PA derivatives in a precisely controlled manner. The objective is to enhance the inhibition of ChEs (especially BChE inhibition) and subsequently identify novel, highly effective multifunctional molecules. This investigation aimed to explore the impact resulting from substituting the substituted phenyl acetamide ring with a substituted benzylpiperazine moiety. The introduction of benzylpiperazine fragment to PA is based on our earlier report.



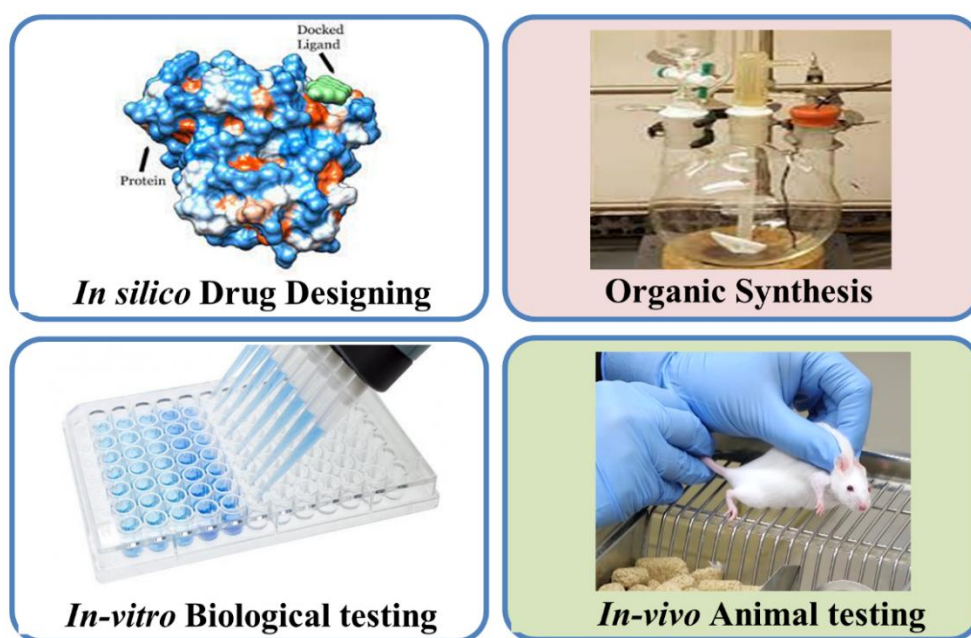
**Figure. 3.1.** Schematic pipeline toward the development of novel piperic acid derivatives with favorable properties.

### 3.2. Plan of Work

It is divided into the following headings:

1. Synthesis of designed molecules.
2. *In-vitro* biological evaluation.
  - ChEs inhibition studies
  - Antioxidant property evaluation
  - Metal chelation study

3. *In-vitro* cell-based experiments.
  - Cytotoxicity studies
  - Neuroprotection studies
4. *In silico* studies.
  - Molecular docking studies
  - Molecular dynamic simulation studies
5. *In-vivo* evaluation of the efficacy in AD models.



**Figure 3.2.** Overview of the designed study.