Objective, Rationale and Plan of Work

3. Objective, Rationale, and Plan of Work

AD is a multifactorial progressive neurological disorder characterized by loss of cholinergic neurons in the brain's cerebral cortex and subcortical regions. The currently used treatment approaches include AChE/BChE and NMDA receptor inhibitors. The presently approved agents for AD management provide symptomatic relief or slow down the worsening of symptoms but do not decelerate or prevent the progression of the disease. There is practically no drug for AD treatment that can address the basic pathophysiological factors responsible for the disease. Therefore, the need for therapeutic agents which will have disease-modifying effects, is of supreme importance.

It is increasingly evident that drugs aiming at a single target may be inadequate for treating complex diseases such as AD, which is multifactorial. Thus, it is hypothesized that multifunctional drugs exhibiting multiple pharmacological activities addressing underlying pathogenic factors of AD should be effective as a disease-modifying agent. With this concept in mind, we initiated our drug discovery approach to identify novel multifunctional agents possessing AChE/BChE inhibitory activity along with antioxidant, iron chelation, and modulation of Aβ aggregation activities.

3.1. Objective and Rationale

We started our journey with ferulic acid (**FA**), a naturally occurring class of polyphenol with potential antioxidant and Aβ modulation properties. In *in-vitro* experiments, **FA** has shown promising neuroprotection. However, **FA** does not effectively interact with AChE and BChE (<20% inhibition of AChE and BChE at 20 μM), evident from the literature and our experiments.

Further, its ability to cross the BBB is limited due to its relatively low lipophilicity (cLog P ~1.5). The low logP value of **FA** is also responsible for its poor brain permeability. One of the major goals behind the novel **FA** analogs design is to improve the AChE inhibition and antioxidant potential of **FA**-derived compounds. We hypothesized that the nature of the extended active site of AChE/BChE and the relatively short structure of the **FA** is probably the reason for poor inhibitory activity of **FA**. Therefore, to develop naturally inspired *in-vivo* active neuroprotective molecules and to overcome the limitations associated with **FA**, we systematically designed and developed novel **FA**-glycine amide derivatives in which **FA** is connected to various aromatic/substituted, aromatic/heterocyclic moieties to impart the cholinergic inhibition property, and to increase the logP of the designed molecules (Schemes 1-2). In our 3nd series of compounds, a piperazine moiety was introduced to impart rigidity and provide a site for protonation in order to improve solubility. The selection of linkers and various hydrophobic or heterocyclic moieties are based on the binding mode analysis of **FA** to the enzymes. The increase in logP of the designed molecules *via* the introduction of hydrophobic features should provide optimal logP values in the range of 2–4 and enable them to cross barriers to reach and interact with the enzymes effectively. Furthermore, potent molecules were evaluated for their multifunctional biological activities, including antioxidant, metal chelation, Aβ modulation studies. We have identified **7a** **(F24)** as one of the lead molecules for the detailed biological evaluation in various cell-based and animal models of AD.

One of the findings from our previous SAR studies on the developed series (1-3) of molecules that we observe a loss in the free radical quenching ability of most developed molecules compared to **FA**. Intriguingly, the phenyl piperazine derivatives (**PPD**, series-2) have shown promising free radical quenching ability ($IC_{50} = 61.98 \pm 0.30 \, \mu M$) for **10b** (Figure 4.8, page # 69) in DPPH assay

with a potency comparable to that of parent natural compound **FA** (IC_{50} = 56.49 ± 0.62 μM). However, the developed **PPD** were found to be moderate to weak inhibitors of the target enzymes (IC_{50}, AChE, 9.91 ± 0.07 to 29.34 ± 0.03 μM, % BChE inhibition, 26.89 ± 0.20 to 44.71 ± 0.05). Given the key role of OS in AD pathogenesis and the potent antioxidant potential of the developed **PPD**, we planned to improve upon the AChE/BChE while maintaining ClogP of the designed molecules in the desired range as per our earlier publication. Hence, in the 4th series of SAR studies, we planned to introduce a spacer between phenyl and piperazine ring in the designed molecules. Therefore, the phenylpiperazine ring was replaced with benzylpiperazine. Despite these structural modifications, none of the tested compounds showed a significant improvement in the enzyme inhibition studies compared to the earlier reported **PPD**. Therefore, to further improve upon the AChE/BChE inhibition, we planned to increase the linker length in our earlier reported potent cholinergic inhibitors glycine amide derivatives. Intriguingly, none of the developed compounds in the 5th series exhibited an improved AChE/BChE inhibitory property (Table 4.9, page # 112) over the earlier reported compounds (**4a, 4f**, and **4j**). One of the major goals of this study is to improve upon the enzyme inhibition and antioxidant properties of our earlier identified molecules. Given the significant role played by the presence of indole moiety in **7a (F24)** in the interaction with the target enzymes and antioxidant property**,** the 6th series of compounds where **FA** and tryptamine moieties were joined through optimized glycine amide to improve upon the AChE/BChE inhibition and antioxidant property.

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

- Aβ aggregation modulation study

3. *In-vitro* cell-based experiments.

- Cytotoxicity studies

- Neuroprotection studies

4. *In silico* studies.

- Molecular docking studies

- Molecular dynamic simulation studies

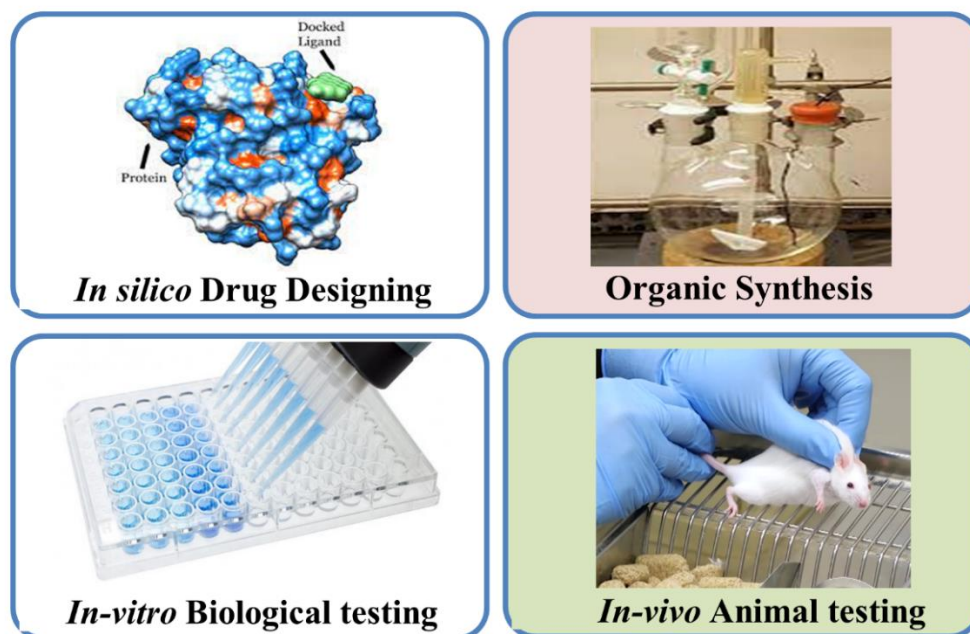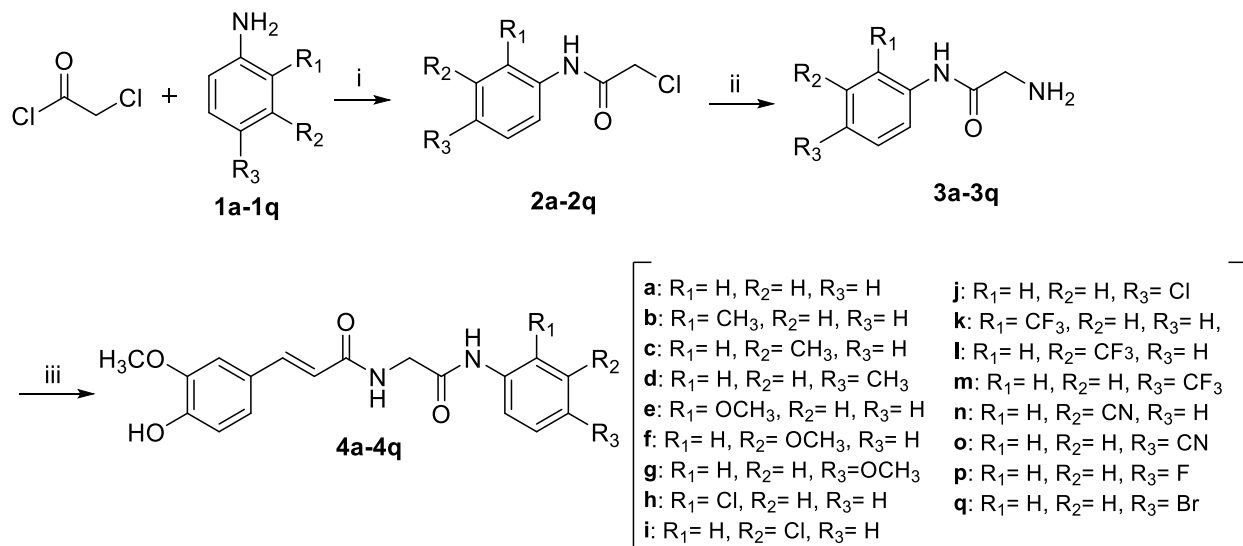5. *In-vivo* evaluation of the efficacy in AD models.

**Figure 3.1.** Overview of the designed study.

3.3. Schemes for the synthesis of FA template based novel compounds

| a: R_1= H, R_2= H, R_3= H	j: R_1= H, R_2= H, R_3= Cl
b: R_1= CH$_3$, R_2= H, R_3= H	k: R_1= CF$_3$, R_2= H, R_3= H,
c: R_1= H, R_2= CH$_3$, R_3= H	l: R_1= H, R_2= CF3, R_3= H
d: R_1= H, R_2= H, R_3= CH$_3$	m: R_1= H, R_2= H, R_3= CF$_3$
e: R_1= OCH$_3$, R_2= H, R_3= H	n: R_1= H, R_2= CN, R_3= H
f: R_1= H, R_2= OCH$_3$, R_3= H	o: R_1= H, R_2= H, R_3= CN
g: R_1= H, R_2= H, R_3=OCH$_3$	p: R_1= H, R_2= H, R_3= F
h: R_1= Cl, R_2= H, R_3= H	q: R_1= H, R_2= H, R_3= Br
i: R_1= H, R_2= Cl, R_3= H	

Scheme 1. Synthesis of FA-acetamide derivatives **4a-4q**. Reagents and Conditions: (i) K$_2$CO$_3$, DCM, 0°C, 2h, 60-70. (ii) NH$_4$OH, 60°C, 6h, 50-60%. (iii) Ferulic acid, EDCI, HOBt, DIPEA, DCM, rt, overnight, 65-75%.
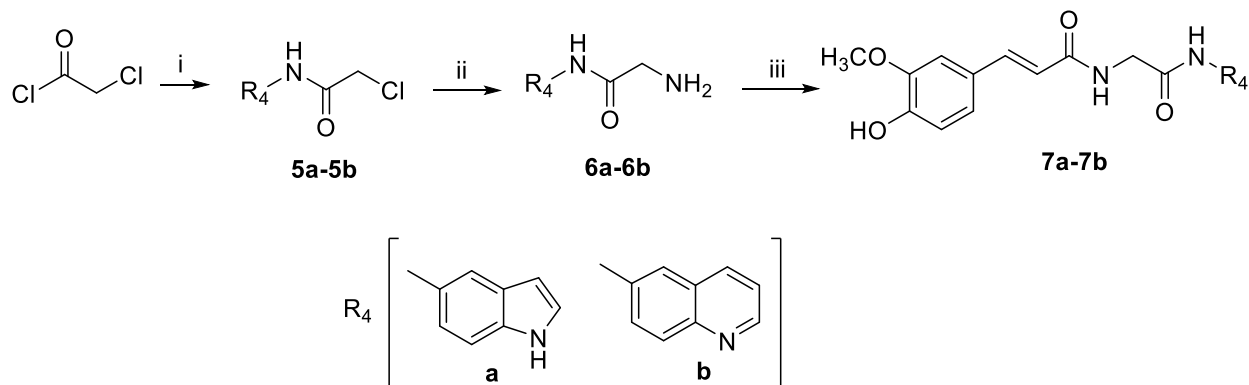

Scheme 2. Synthesis of FA-Indole or quinoline derivatives. Reagents and conditions: (i) 5-aminoindole/6-aminoquinoline, K$_2$CO$_3$, DCM, 0°C, 2h, 75-80%.(ii) NH$_4$OH, 60°C, 6h, 60-70%. (iii) Ferulic acid, EDCI, HOBt, DIPEA, DCM, rt, overnight, 65-70%.
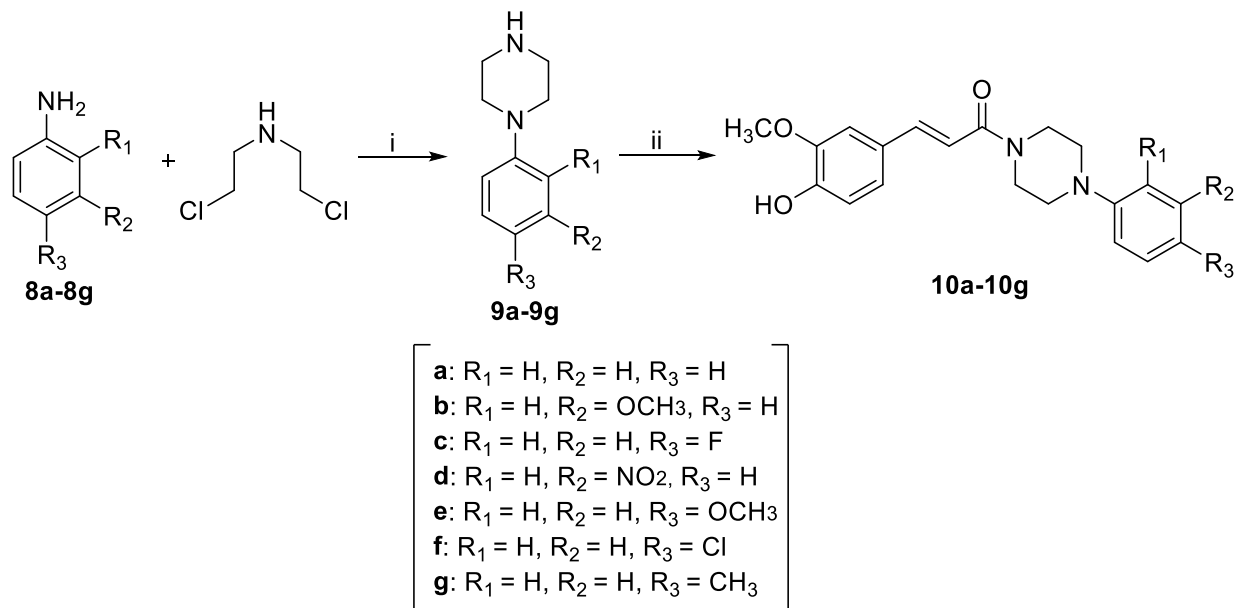
Scheme 3. Synthesis of FA tethered to *N*-phenyl-piperazine scaffolds **10a**-**10g**. Reagents and conditions: (i) Diglyme, 150°C, 8-10 h, 65-70%. (ii) Ferulic acid, EDCI, HOBt,DIPEA, THF, rt, overnight, 80-85%.
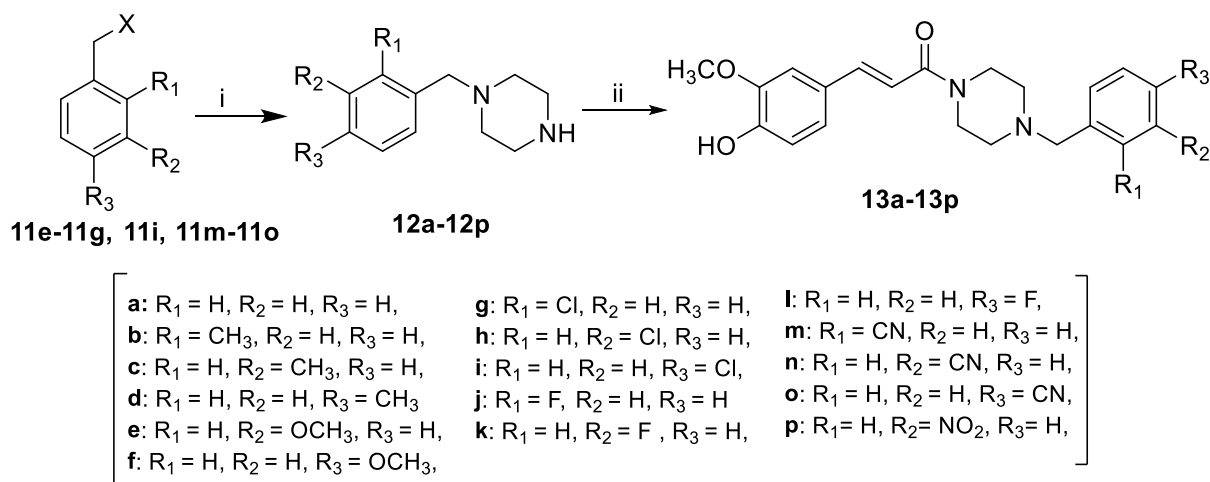
Scheme 4. Synthesis of benzylpiperazine derivatives **13a**-**13p**. Reagents and conditions: (i) Anhydrous piperazine, K_2CO_3, ethanol, reflux, 4-5 h, 75-80%. (ii) Ferulic Acid, EDCI.HCl, HOBT, DIPEA, dry THF, rt, overnight, 70-75%.
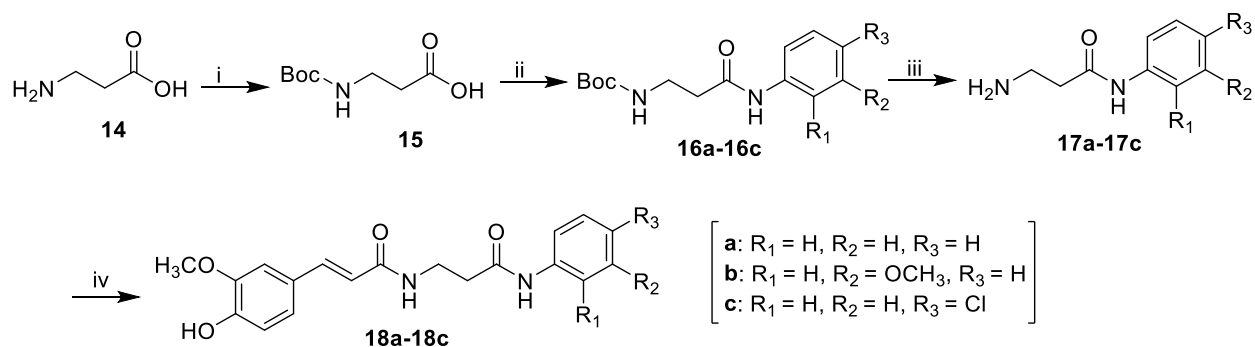
Scheme 5. Synthesis of FA-alanine-amide compounds **18a**-**18c**. Reagents and conditions: (i) Boc-anhydride, DCM, 0°C, overnight, 90%. (ii) aniline/3-methoxyaniline/4-chloroaniline, EDCI.HCl, HOBT, DIPEA, dry THF, rt, overnight, 70-75%. (iii) Ether HCl, methanol, rt, 12h, 80-85%. (iv) Ferulic acid, EDCI.HCl, HOBT, DIPEA, dry THF, rt, overnight, 70-75%.
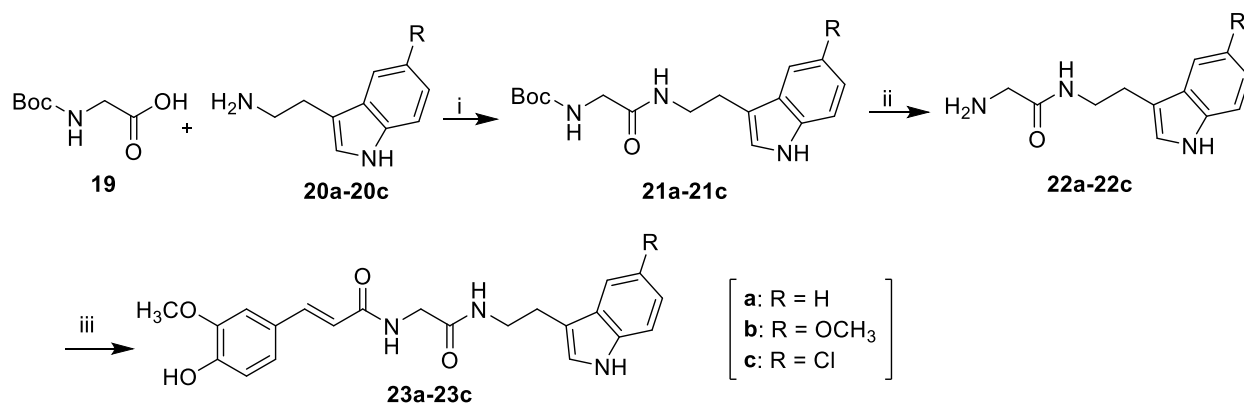
Scheme 6. Synthesis of FA-tryptamine-glycine amide **23a**-**23c**. Reagents and conditions: (i) EDCI.HCl, HOBT, DIPEA, dry THF, rt, overnight, 65-70%; (ii) Ether HCl, methanol, rt ,12h, 80-85%; (iii) Ferulic Acid, EDCI.HCl, HOBT, DIPEA, dry THF, rt, overnight, 65-70%.