Chapter 3

Objective, rationale and plan of work

Chapter 3
Objective, rationale and plan of work

3. Objectives, rationale and plan of work

3.1 Objectives and Rationale

There are two major objectives of the present study. The primary objective is to develop multifunctional fluorescence-emitting novel chemical entities with theranostic i.e., diagnostic and therapeutic potential. The development of a promising theranostic agent requires a rational structural framework combination containing two major functionalities including, a lead structural scaffold from existing therapeutics and biomarker labeling agents. To design the novel series of theranostic agents with the amalgamation of the structural features from the potential anti-AD agents and fluoroprobes with the architecture of electron donor-acceptor. The second objective of the study is to develop multi modal new chemical entities with therapeutic potential to provide modulation of disease progressing factors such as cholinergic pathways, BACE-1 and Aβ aggregation etc. The development of MTDLs is based on rational and holistic view of target combination, ligand selection, and equilibrium of desired activities to maximize efficacy and safety. Modern drug design approaches i.e., scaffold hopping and hybrid drug design considering existing therapeutics of AD. Two different chemical class of ligands viz. chalcone and pyrazoline were identified and derivatized to two series of molecules by utilizing the synthetic suitability.

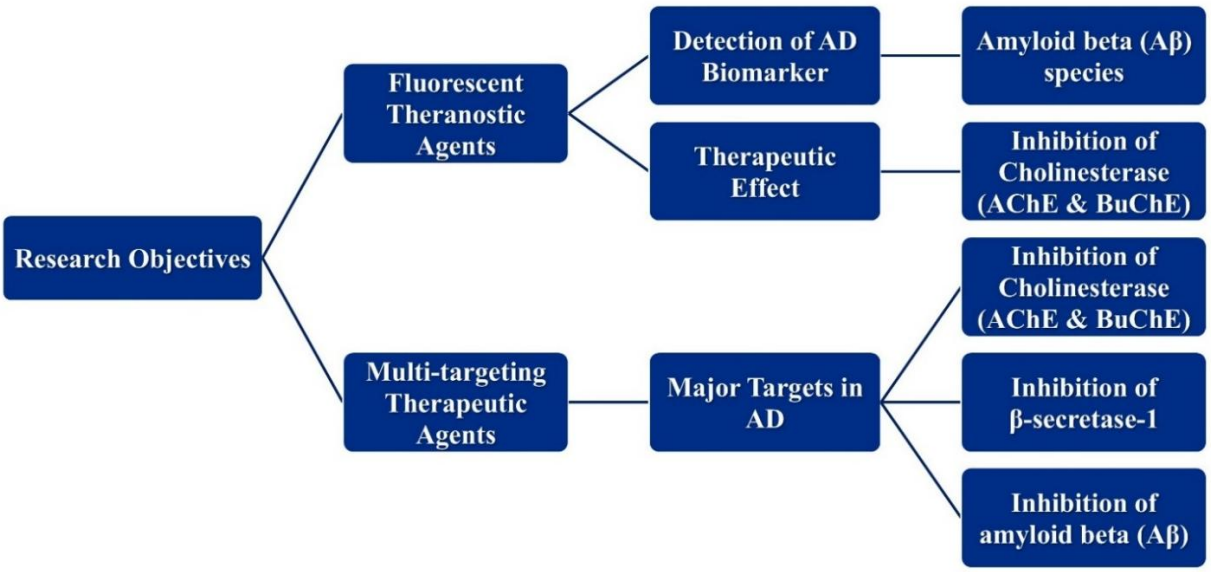

Figure 3.1. Overview of the research objectives.

3.2 Plan of work

3.2.1 Design Strategy

A. Scaffold Hopping

B. Fragment based design

C. Hybrid drug design

D. Lead Optimization

3.2.2 Chemical Synthesis

The synthesis of series of designed derivatives and characterization using physical and spectral methods viz. melting point, TLC, ^{1}H NMR, ^{13}C NMR and High-resolution mass spectrometry. The assessment of purity of synthesized compounds through HPLC method.

3.2.3 Evaluation Methodologies

The diagnostic and therapeutic potential were further evaluated through different *in silico*, *in vitro* and *in vivo* biological studies.

3.2.3.1 Evaluation of physicochemical & spectral properties

3.2.3.2 *In silico* studies

It included the binding mode analysis of synthesized molecules against selected target through molecular docking studies and protein-ligand complex stability by molecular dynamics, density functional theory (DFT) calculation, ADME and toxicity predictions of selected ligands.

3.2.3.3 *In vitro* biological testing

The synthesized theranostic agent and MTDLs were evaluated for the assessment of its potential against respective selected targets. The *in vitro* evaluation studies are including,

- Cholinesterase (AChE, BuChE) inhibition assays.
- Evaluation of selected molecules by PAMPA-BBB assay.
- Evaluation of Theranostics probes for Aβ binding studies and saturation binding assay.
- The lead probe from 1, 3-dimethylbarbituric acid-based derivatives tested for *in situ* fluorescence imaging of Aβ aggregation and fluorescence lifetime measurement.
- MTDLs with potent ChEs inhibition further evaluated for BACE-1 inhibitory potential.
- Lead compound from chalcone and pyrazoline series evaluated against Aβ aggregation inhibition assay.

3.2.3.4 *In vivo* biological evaluation:

- Acute oral toxicity studies
- Scopolamine-induced amnesia model
- Novel Object Recognition Test
- *Ex-vivo* neurochemical expression monitoring
- Aβs deposit fluorescence imaging in the *Drosophila* AD model.