3.1. RATIONALE & OBJECTIVE

Taking cognizance of the structure-activity relationship and reported biological profile as potential anticonvulsants, nipecotic acid and nafimidone were initially selected as the framework for designing the new hybrids in the first series. Moreover, accumulating lines of evidence suggested that hybridization of two or more diverse bioactive molecules with corresponding pharmacophoric features or different mechanisms of action rendered synergistic effects contributing to the overall activity profile of the molecule. The molecular hybridization approach was used in an attempt to design novel derivatives of piperidine-3-carboxylic acid as potential anticonvulsants. With a rationale of synthesizing novel compounds, which can effectively permeate through the BBB, a lipophilic scaffold such as naphthyl ketone has been introduced at the ring nitrogen of nipecotic acid, to obtain novel hybrids with improved efficacy compared to the parent scaffolds. A methylene bridge was introduced for connecting the parent scaffolds to afford the necessary flexibility to the overall structure **(Fig. 3.1)**.

In the second series, we have hypothesized to construct a new molecular framework by synthesizing novel Schiff bases of nipecotic acid connected with ethylene-bridge as potential anticonvulsants, with the same objective of increasing lipophilicity and BBB permeability as compared to parent scaffold. The synthesized compounds were hypothesized to possess structural similarities with tiagabine having nipecotic acid pharmacophore and bioisosterically replaced methanimine group instead of vinyl functionality. One methyl substituted thiophene nucleus of tiagabine was also replaced bioisosterically with substituted phenyl groups **(Fig. 3.1)**.

It was proposed to investigate the *in vivo* anticonvulsant activity and *in vitro* blood–brain barrier (BBB) permeability of novel designed and synthesized molecules

and the results to be affirmed by *in silico* computational studies. Owing to the reported side effects of antiepileptic drugs, it was also proposed to evaluate the effects of the leads on motor coordination, cell viability, renal, hepatic and other haematological parameters.

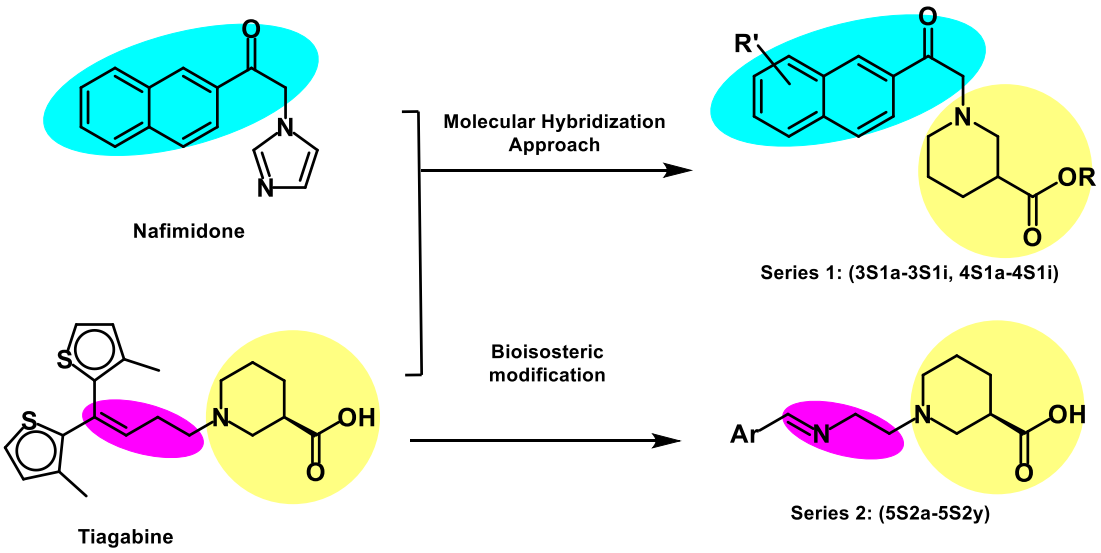


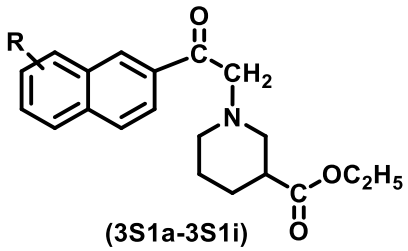
Figure 3.1. The design strategy for the proposed compounds under Series 1 and Series 2.

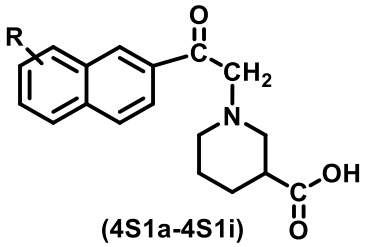
3.2. PLAN OF WORK

A brief outline of the research work carried out is summarized as follows:

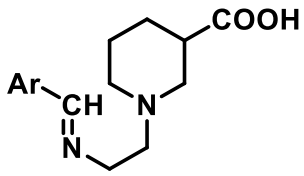
3.2.1. SYNTHESIS

SERIES 1: Synthesis of a series of acetonaphthone tethered piperidine-3-carboxylic acid derivatives.





SERIES 2: Synthesis of a series of novel Schiff bases of 1-(2-aminoethyl)piperidine-3-carboxylic acid.



(5S2a-5S2y)

3.2.2. Characterization of the Synthesized Compounds (Series 1 and 2)

- Physicochemical characterization including melting point, TLC analysis (R_f value) and partition coefficient (Log P).
- Structural confirmation by FT-IR, ^{1}H NMR, ^{13}C NMR and Elemental (CHN) analysis.

3.2.3. Biological Evaluation of Series 1

A) Evaluation of *in vivo* anticonvulsant activity:

- sc-PTZ induced seizures in mice
- Pilocarpine-induced seizures in mice
- DMCM induced seizures in mice

B) Rota-rod performance test in mice

C) MTT assay on neuroblastoma cell line (SH-SY5Y)

D) Repeated dose toxicity studies

E) *In vitro* evaluation of blood-brain barrier permeability (PAMPA-BBB Assay)

3.2.4. Biological Evaluation of Series 2

A) *In vitro* evaluation of blood-brain barrier permeability (PAMPA-BBB Assay)

B) Evaluation of *in vivo* anticonvulsant activity:

- s.c.-PTZ induced seizures in mice
- Pilocarpine-induced seizures in mice
- DMCM induced seizures in mice

C) Rota-rod performance test in mice

D) MTT assay on neuroblastoma cell line (SH-SY5Y)

E) Repeated dose toxicity studies

3.2.5. Computational Studies of the Selected Leads From Series 1 & 2

- Homology modeling of GAT-1
- Molecular docking studies
- Molecular dynamics (MD) studies
- Prediction of drug likeliness and *in silico* ADME properties