In **Series 1,** a total of eighteen acetonaphthones tethered piperidine-3-carboxylic acid (nipecotic acid) derivatives **(3S1a-3S1i & 4S1a-4S1i)** embedded within the same pharmacophore matrix were designed and synthesized. Herein, the substituted naphthalene nucleus at the second position was hybridized with ethyl piperidine-3-carboxylate with an aim to assist the molecule in permeating through the BBB owing to their increased lipophilic nature. The study envisioned to explore the benefits of hybrid pharmacophore approach on the anticipated anticonvulsant activity devoid of neurological, renal, hepatic and haematological side effects associated with the currently available conventional antiepileptic drugs. Compounds **3S1a-3S1i & 4S1a-4S1i** were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental (CHN) analysis.

Post-synthesis and structural characterization, these derivatives were subjected to an initial anti-convulsant *in vivo* screening against sc-PTZ induced convulsions in mice. Based on its outcome, compounds **3S1a, 3S1b, 3S1i, 4S1a, 4S1b,** and **4S1i** exhibiting increased latency of seizures against scPTZ induced seizures in mice were further advanced to *in vivo* Pilocarpine and DMCM induced seizure models.

In vivo results demonstrated that compounds **4S1a, 4S1b, 4S1i** were comparatively more effective to their nipecotic acid ester counterparts **3S1a, 3S1b** and **3S1i** against s.c.-PTZ, pilocarpine, and DMCM induced seizures in mice thereby implying the significance of the free carboxyl group on the overall antiepileptic effect. Also the results of s.c.-PTZ model revealed that substitution at the R6 position of the acetonaphthone ring with an election withdrawing substituent rendered diminished antiepileptic activity as observed for compounds **3S1c, 3S1d, 3S1e** and **4S1c, 4S1d, 4S1e**. Also, substitution of hydrogen with any other group at the **R7** position also affected the antiepileptic effect **(3S1f** and **4S1f).**

The potential hybrids attained after the *in vivo* screening were further evaluated for their effects on motor coordination by rota-rod test on rodents. Findings of this test revealed that the test compounds (**4S1a, 4S1b** and **4S1i**) did not cause any alteration in "fall-off" time on rota rod apparatus as compared to the control, tiagabine and standard (diazepam) indicating their inability to induce any observable signs of impairment in muscle co-ordination.

Neurological side effects of antiepileptic drugs have been reported in several literatures, therefore, the effects of the most active compounds (**4S1a, 4S1b** and **4S1i**) on cell viability was determined in neuroblastoma cell line (SH-SY5Y) by MTT assay. Outcomes of this investigation indicated that the test compounds (**4S1a, 4S1b** and **4S1i**) were not found to alter the cell viability considerably, thus corresponds to the insignificant cell death in the concentrations of the test compounds ranging from 1 µM to 80 µM. The results of the estimation of various haematological and biochemical parameters also confirms the safety of the compound **4S1i** at an equimolar dose relative to 10 mg/kg Tiagabine.

In continuation, the BBB permeability of the active compounds (**4S1a, 4S1b and 4S1i)** was determined by an *in vitro* PAMPA-BBB assay. The outcome of this exercise confirmed the considerable permeability of the test compounds **4a, 4b** and **4i.** The compound **4S1i** was found to be comparatively more permeable (P_e= 8.89) across BBB than the standard tiagabine (P_e= 7.86).

In silico docking study of the potential compounds, **4a, 4b and 4i** contributed to shed light on their binding pose with different essential amino acids within the active site of homology modelled protein structure of GAT-1 GABA transporter. Further, the molecular dynamics (MD) simulation of the most potent compound **4i** (Glide Score: -7.3) helped to adequately understand the role of active site hydration

that prevails under normal physiological conditions which helped in better interpretation of its biological profile.

Finally, *in silico* estimation of drug-like properties was calculated using the QikProp module of Schrödinger Maestro 10.5.014. Predicted values for **QPlogBB** and **CNS activity**, indicates that the selected compounds (**4S1a, 4S1b** and **4S1i**) were found to be active for CNS and might be permeable across BBB. The *in silico* results were further supported by the findings of *in vitro* parallel artificial BBB permeability assay which suggested that the evaluated derivatives exhibited considerable permeability to cross BBB. Results also indicated that none of the evaluated compounds violated the Lipinski's rule of five. Altogether the predicted parameters revealed that the compounds **4S1a, 4S1b,** and **4S1i** exhibit drug-like properties.

Further, In pursuance to develop novel and safe anticonvulsant compounds, a new molecular framework was constructed in **Series 2** by synthesizing novel Schiff bases of 1-(2-aminoethyl)piperidine-3-carboxylic acid. The synthesized compounds also possess structural similarities with tiagabine having nipecotic acid pharmacophore. By invoking the concept of bio-isosterism, the vinyl functionality in tiagabine was swapped with its bioisosteric replacement of methanimine group. Also one methyl substituted thiophene ring of tiagabine was bio-isosterically replaced with the substituted phenyl groups. A total of twenty five target compounds of Series 2 **(5S2a-5S2y)** were successfully synthesized with their structures supported by FT-IR, ^{1}H-NMR, ^{13}C-NMR and elemental (C, H, N) analysis.

The derivatives of **Series 2** were initially screened for *in vitro* permeability across BBB by PAMPA-BBB assay. The assay was performed in the first stage of screening due to ethical constraints and alternatives to minimize animal experimentation. The results of this study demonstrated that the compounds **5S2d,**

5S2f, 5S2j, 5S2l, 5S2m, 5S2n, 5S2w, 5S2x, and **5S2y** showed substantial permeability across artificial BBB. Also the compound **5S2w** exhibited more permeability (*Pe* = 8.93) than the standard drug tiagabine (*Pe* = 7.96).

The potential leads exhibiting *in vitro* BBB permeability were further evaluated for anticonvulsant activity in sc-PTZ and DMCM induced seizure models. Of the twenty five synthesized derivatives, five compounds namely **5S2d, 5S2l, 5S2w, 5S2x** and **5S2y** considerably delayed the onset of seizures and its frequency in a sc-PTZ induced seizure model. The outcome of this investigation also suggested that **5d, 5w,** and **5y** were most potent amongst the synthesized compounds.

Evaluation of the active compounds **(5S2d, 5S2l, 5S2w, 5S2x** and **5S2y)** against *in vivo* DMCM induced seizure model in mice re-affirmed their anticonvulsant potential. The results also showed that amongst the test compounds **5S2d, 5S2w,** and **5S2y** significantly delayed the onset of convulsion as compared to control and other compounds (**5S2l** and **5S2x)** indicating their potency.

Leads from **Series 2 (5S2d, 5S2w,** and **5S2y)** were subjected to rota-rod test to evaluate the effect of the test compounds on motor coordination. Outcomes of this test indicated that all the test compounds were found to be devoid of any adverse effect on motor coordination.

Further assessment of the active compounds **(5S2d, 5S2w,** and **5S2y)** by MTT assay on neuroblastoma cell lines (SHSY-5Y) revealed that MTT reduction was not effected significantly by test compounds **(5S2d, 5S2w,** & **5S2y).** In the given concentration range (1µM to 80 µM) of test compounds, considerable cell death was not observed. The most promising compound **5S2w** was also found safe, as revealed from the outcome of the estimation of different haematological and biochemical parameters.

The binding modes of the compounds **(5S2d, 5S2w,** and **5S2y)** into the active binding site of homology modeled GAT-1 GABA transporter protein through docking studies followed by molecular dynamics (MD) simulation of the most potent compound **5S2w** (Glide Score: -6.2) exemplified their consensual interaction similar to tiagabine, which may explain their underlying mechanism of action. The *in silico* studies also helped to explain the geometric requisites of the ‘‘hybrid-pharmacophore” conducive for GAT-1 inhibition. However, the most active compound 5w can be further quantified in the future by *in vitro* GAT1 inhibitory/binding assay to provide a mechanistic pathway for anticonvulsant activity. Prediction of ‘‘drug-likeliness” for the most active compounds was carried out using QikProp module of Schrödinger Maestro 10.5.014 wherein based on the evaluation of various *in silico* predicted parameters indicated that the compounds **(5S2d, 5S2w,** and **5S2y)** did elicit ‘‘drug-like” characteristics and could be a potential candidate for future development.

In conclusion, the efficacy and safety of the potential leads justifies the rationale behind the study and provide a valuable insight towards the development and optimization more promising compounds with superior anticonvulsant effects. Given the crucial role played by GABA transporters especially GAT-1 in the etiology and management of epilepsy, further advancement in the design and development of selective GAT-1 inhibitors with superior safety profile remains a viable avenue for future investigation.