DESIGN, SYNTHESIS, PHARMACOLOGICAL EVALUATION AND MOLECULAR MODELLING STUDIES OF N-SUBSTITUTED PIPERIDINE-3-CARBOXYLIC ACID DERIVATIVES AS POTENTIAL ANTICONVULSANTS

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ABSTRACT:

Epilepsy is a neurological disorder with a focal origin in the brain and is characterized by paroxysmal cerebral dysrhythmia, recurrent seizures, and disturbance of consciousness. Despite the availability of numerous antiepileptic agents in the drug market, nearly 20–30% of the 70 million epilepsy patients worldwide have insufficient control over seizures and are resistant to the currently available pharmacotherapy. Moreover, the poor tolerability and reported side effects of the antiepileptic drugs have affected the quality of life of the epilepsy patients. Thus, to address these massive challenges, development of novel antiepileptic drugs with improved efficacy, considerable tolerability, and lower toxicity is a paramount necessity.

GABA is the main inhibitory neurotransmitter in the brain of mammals that plays a considerable role in the pathogenesis of epilepsy. Low brain GABA concentration and diminution in GABA-ergic neurotransmission have been observed in a range of epileptic syndromes. The transport of GABA from synaptic cleft to the glial cells as well as presynaptic neurons is mediated by GABA transporters (GATs), which results in the termination of GABA-ergic neurotransmission. GATs are the member of sodium symporters, which belong to solute carrier 6 (SLC6) transporter gene family in humans and mediates neurotransmitter transport. Elevation in GABA concentration within the synaptic cleft is proved to be advantageous in the management of epilepsy. One such strategy for the up-regulation of GABA would be the blockade of specific subtypes of GATs, which are responsible for neuronal and glial uptake of GABA from the synaptic cleft. Inhibitors of GABA uptake are of utmost significance as regulators of neurotransmitter levels that increase the duration of inhibitory postsynaptic potentials to elicit antiepileptic activity.

The discovery of piperidine-3-carboxylic acid (nipecotic acid) as a high-affinity substrate for GABA transporter remarkably added new prospects of targeting GABA uptake systems. It has been reported to exhibit significant *in vitro* activity as an inhibitor of uptake of GABA into neuronal and glial cells. However, it is unable to cross blood brain barrier (BBB) owing to its zwitterionic and polar nature. The synthetic versatility of piperidine-3-carboxylic acid led to the discovery of lipophilic derivatives which have been explored as useful inhibitors of GABA uptake. Despite the synthesis of these diverse derivatives, BBB permeation remains the key bottleneck for brain drug delivery. The single marketed derivative of the piperidine-3-carboxylic acid, i.e., tiagabine is a GAT-1 selective GABA

uptake inhibitor with considerable antiepileptic potential. Reported GABA uptake inhibition triggered by tiagabine has been attributed to the presence of GABA mimetic moiety in the form of piperidine-3-carboxylic acid and a lipophilic di-aromatic region connected by a linker. Tiagabine is effectively used as supportive therapy for the management of complex partial seizures in epilepsy patients. However, its long-term use is associated with adverse effects like asthenia, tremor, concentration difficulties, lethargy, nervousness, and depression. Therefore, scope to develop novel compounds with improved BBB permeation and lesser side effects is still a challenge for medicinal chemists in the discovery of new drugs to treat epilepsy.

Compounds inhibiting GABA uptake have been extensively used as a pharmacological tool to explicate several aspects of neurological processes which involve GABA. Nipecotic acid, which is a reported GABA uptake inhibitor, has gained considerable attention for the design and synthesis of lipophilic analogs with marked antiepileptic activity. Based on the SAR of the reported N-substituted derivatives of nipecotic acid endowed with antiepileptic potential and taking cognizance of known GABA uptake inhibitors especially tiagabine as a benchmark, an attempt was made to synthesize two series of novel derivatives of nipecotic acid.

In search of new chemical entities (NCE's) for the mitigation of epilepsy, naphthyl ketones are considered to be attractive scaffolds owing to their lipophilic nature and reported applications in the design and synthesis of some derivatives with marked antiepileptic activity. With a rationale of synthesizing novel analogs which can effectively permeate through the BBB, a lipophilic scaffold such as naphthyl ketone has been introduced at the ring nitrogen of nipecotic acid, thereby utilizing the concept of molecular hybridization to obtain novel hybrids in the first series. Herein, the substituted naphthalene nucleus at the second position was hybridized with nipecotic acid with an aim to assist the molecule in permeating through the BBB owing to their increased lipophilic nature. A methylene bridge connecting the parent scaffolds afforded the necessary flexibility to the overall structure. The synthesized compounds were evaluated in subcutaneous pentylenetetrazol (sc-PTZ), Pilocarpine and Methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) induced seizure models to obtain potential leads with antiepileptic activity. The potential hybrids attained after the *in vivo* screening were further evaluated for their BBB permeability by an *in vitro* parallel artificial membrane permeability BBB assay

(PAMPA-BBB). Neurological side effects of antiepileptic drugs have been reported in several literatures. The effects of the leads on motor coordination were evaluated by rota-rod test on rodents. The effects of the active compounds on cell viability was determined in neuroblastoma cell line (SH-SY5Y) by 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Also, the the safety profile of the most promising compound and the standard drug (tiagabine) was evaluated with respect to various hematological, hepatic, and renal parameters. Further the homology modelling, *in silico* docking studies and molecular dynamics simulation of the most active compound were performed at the GAT1 active site. QikProp module of Schrödinger Maestro 10.5.014 Release 2016-1 was used to predict the "drug likeliness" of the active compounds (**4S1a, 4S1b** and **4S1i**).

Schiff bases are also reported to be attractive scaffolds in the exploration of NCEs as antiepileptics, owing to their lipophilic nature and reported applications in the design and synthesis of derivatives with marked antiepileptic activity. Based on the philosophy of bio-isosterism, we have hypothesized to construct a new molecular framework by synthesizing another series of novel Schiff bases of 1-(2-aminoethyl)piperidine-3-carboxylic acid as potential anticonvulsants, with the same objective of increasing lipophilicity and BBB permeability with respect to parent scaffold. In this series, the synthesized compounds have been initially screened for their ability to permeate the blood–brain barrier (BBB) by an *in vitro* parallel artificial membrane permeability assay (PAMPA-BBB). PAMPA-BBB was performed in the first step owing ethical considerations and to limit the animal experimentation. The potential leads exhibiting *in vitro* BBB permeability **(5S2d, 5S2f, 5S2j, 5S2l, 5S2m, 5S2n, 5S2w, 5S2x** and **5S2y)** were further evaluated for anticonvulsant activity in sc-PTZ and DMCM induced seizure models. Rota-rod test on rodents, MTT assay and safety profiling was performed with the same objective as in the first series. The leads **(5S2d, 5S2w,** and **5S2y)** were then subjected to molecular docking on the same homology modeled protein to identify and compare the complementary interactions of the compounds with the amino acid residues of the active pocket. The binding modes of the most active compound **(5S2w)** were further probed using molecular dynamics simulation at the GAT1.

Amongst the synthesized derivatives of series 1, compounds **3S1a, 3S1b, 3S1i, 4S1a, 4S1b**, and **4S1i** exhibited increased latency of seizures against subcutaneous

pentylenetetrazole (scPTZ) induced seizures in mice. Derivatives **4S1a, 4S1b, 4S1i** were more effective compared to the ester counterparts **3S1a, 3S1b** and **3S1i** placing the importance of the presence of free carboxyl group in the centre. Predicted values for **QPlogBB** and **CNS activity**, indicates that the selected compounds 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. The compound **4i** was comparatively more permeable (Pe= 8.89) across BBB than the standard tiagabine (Pe= 7.86). *In silico* studies proved the consensual interactions of compound **4i** with the active binding pocket. From Series 2, compounds **5S2d, 5S2f, 5S2j, 5S2l, 5S2m, 5S2n, 5S2w, 5S2x** and **5S2y** elicited considerable *in vitro* permeability across BBB. The outcome of the *in vivo* models suggested that **5S2d, 5S2w,** and **5S2y** were most potent amongst the synthesized compounds. Leads from both the series **(4S1a, 4S1b, 4S1i, 5S2d, 5S2w,** and **5S2y)** 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. The MTT assay revealed that the test compounds **(4S1a, 4S1b, 4S1i, 5S2d, 5S2w,** and **5S2y)** 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 evaluation of various haematological and biochemical parameters revealed the safety of the most promising compounds **(4S1i** & **5S2w)** from both the series at an equimolar dose relative to 10 mg/kg tiagabine. Based on the outcome of the *in silico* studies, we can reasonably infer that the potential derivatives exhibits consensual interactions with the active binding pocket of GAT 1 similar to tiagabine. All together 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.

Dedicated to the Almighty,

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Date:

Place: *Varanasi* *(**Ankit Seth**)*

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% – Percentage

°C – Degree centigrade

µl – Microlitre

µM – Micromolar

ALP – Alkaline phosphatase

ALT – Alanine transaminase

ANOVA – Analysis of variance

AST – Aspartate transaminase

ATP – Adenosine triphosphate

BUN – Blood urea nitrogen

CADD – Computer aided drug design

$CDCl_3$ – Deuterated chloroform

cm – Centimeter

CNS – Central nervous system

CoMFA – Comparative molecular field analysis

CoMSIA – Comparative molecular similarity indices analysis

DMCM – Methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate

ED_{50} – Median effective dose

FDA – Food and drug administration

FTIR – Fourier transform infrared spectroscopy

g – Gram(s)

GABA – γ–aminobutyric acid

GAT – GABA transporters

h – Hour

i.p. – Intraperitoneal

IC_{50} – Half maximal inhibitory concentration

TLC – Thin layer chromatography

TMS – Tetramethylsilane

UV – Ultra violet

XRD – X-ray diffraction

Kg – Kilogram **LBDD** – Ligand based drug design **m.p.** – Melting point **MD** – Molecular dynamics **MES** – Maximal electroshock **MET** – Metrazole **MTT** – 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide **mg** – Milligram **min** – Minutes **ml** – Milliliter **mm** – Millimeter **mmol** – Millimole **Mol. Eq.** – Molar equivalent **NCE** – New chemical entity **NMDA** – N–methyl–D–aspartate **NMR** – Nuclear magnetic resonance **ns** – Nanosecond **OECD** – Organization for economic co–operation and development **OPLS** – Optimised potentials for liquid simulations **P** – Partition coefficient PAMPA – Parallel artificial membrane permeability assay **p.o.** – Per oral **PDB** – Protein data bank **ps** – Picosecond PTZ **QSAR** – Quantitative structure–activity relationship	

R_f – Retention factor **RMSD** – Root mean square deviations **rpm** – Revolutions per minute **s** – Seconds **SBDD** – Structure-based drug design **sc** – Subcutaneous ***sc*PTZ** – Subcutaneous pentylenetetrazole **SD** – Standard deviation **SBDD** – Structure based drug design	

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