4.1. CHEMISTRY

4.1.1. Chemicals and Reagents

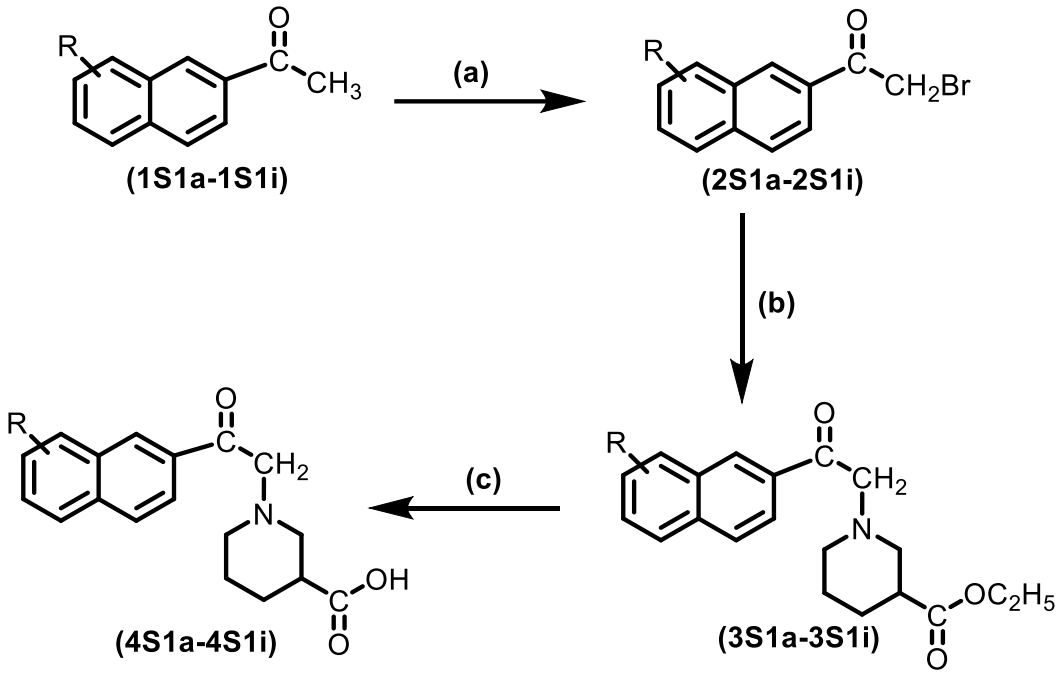
All the solvents and chemicals (analytical grade) were procured from Merck (Germany), Sigma–Aldrich (India) and SD Fine Chemicals (India).

4.1.2. Series 1

Synthesis of acetonaphthone tethered piperidine-3-carboxylic acid derivatives.

4.1.2.1. Method of Preparation

The acetonaphthone tethered piperidine-3-carboxylic acid derivatives were prepared by selective mono-bromination on acetonaphthones using $CuBr_2$ followed by nucleophilic substitution reaction on nipecotic acid (**Scheme 4.1)**.



Scheme 4.1. The general scheme for the synthesis of target compounds **(3S1a-3S1i & 4S1a-4S1i)**: Reagents and conditions: (a) $CuBr_2$ (1.98 mmol), $CHCl_3$: EtOAc (1:1), Reflux overnight; (b) Ethyl nipecotate (3 mmol), K_2CO_3 (6 mmol), THF, RT, 30 h; (c) (i) 3N NaOH, EtOH, RT, (ii) 1N HCl.

4.1.2.1.1. General Procedure for the Synthesis of 2-Bromo-2-acetonaphthones (2S1a-2S1i):

Several 2-Bromo-2-acetonaphthones **(2S1a-2S1i)** were prepared as per the reported procedure [King and Ostrum, 1964]. To a solution of respective 2-aceto

naphthones **(1S1a-1S1i)** (1.0 mmol) in the mixture of chloroform and ethyl acetate (1:1) (60 mL), copper (II) bromide (1.98 mmol) was added with continuous stirring and refluxed overnight. The progress of the reaction was monitored by TLC with DCM/methanol (9.5:0.5). Subsequently, with the completion of the reaction, the solvent was evaporated under vacuum and ether (5 ml) was added. The crude product was filtered and dried to yield the compounds **2S1a-2S1i**, which were utilized directly in the next step without further purification.

*4.1.2.1.2. General Procedure for the Synthesis of Compounds (3S1a-3S1i**):***

The synthesized 2-Bromo-2-acetonaphthones **(2S1a-2S1i)** (2.0 mmol) was mixed with ethyl nipecotate (3.0 mmol) and K_2CO_3 (6.0 mmol) in dry THF (25 mL) and stirred initially for two hours in an ice bath and then stirred for 30 h at room temperature. The progress of the reaction was monitored by TLC using hexane/EtOAc (6:4). After completion of the reaction, the sticky precipitate was filtered and washed with water. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 6:4) to yield the title compounds (**3S1a-3S1i**) as a viscous oil.

4.1.2.1.3. General Procedure for the Synthesis of Compounds (4S1a-4S1i):

The synthesized derivatives **($3S_1$a-$3S_1$i)** were subjected to hydrolysis as per the method reported by Andersen et al. with slight modification to get the title compounds **(4S1a-4S1i).** In this method, the corresponding ester derivatives **(3S1a-3S1i)** (1.0 mmol) and 4N NaOH solution (3.0 mmol) were taken in ethanol (3 mL) and stirred at room temperature until the completion of the reaction. The hydrolysis was monitored by TLC [Mobile phase: hexane/EtOAc (6:4)]. After the completion of reaction (3-6 h), the reaction mixture was kept on an ice bath followed by the workup with 1N HCl [Andersen *et al*., 2001]. The solvent was then evaporated, and the obtained oily product was purified by column chromatography using silica gel (hexane/EtOAc = 6:4) to yield the title compounds **(4S1a-4S1i).**

4.1.2.2. Reaction Mechanism for Series 1

The highly selective mono-bromination reaction on the α-methylene group of acetonaphthones **(1S1a-1S1i)** in the presence of copper (II) bromide gives α-bromoacetonaphthones **(2S1a-2S1i)** [King and Ostrum, 1964]. The nucleophilic substitution reaction (SN_2) takes place at α-carbon of bromoacetonaphthones **(2S1a-2S1i)**, in which the bromide group (leaving group) is substituted with the -NH (nucleophile) of ethyl nipecotate to give the **(3S1a-3S1i)**. The intermediate **(3S1a-3S1i)** was hydrolysed in ethanol under basic condition (NaOH) to yield the free N-substituted piperidine-3-carboxylic acid derivatives **(4S1a-4S1i).**

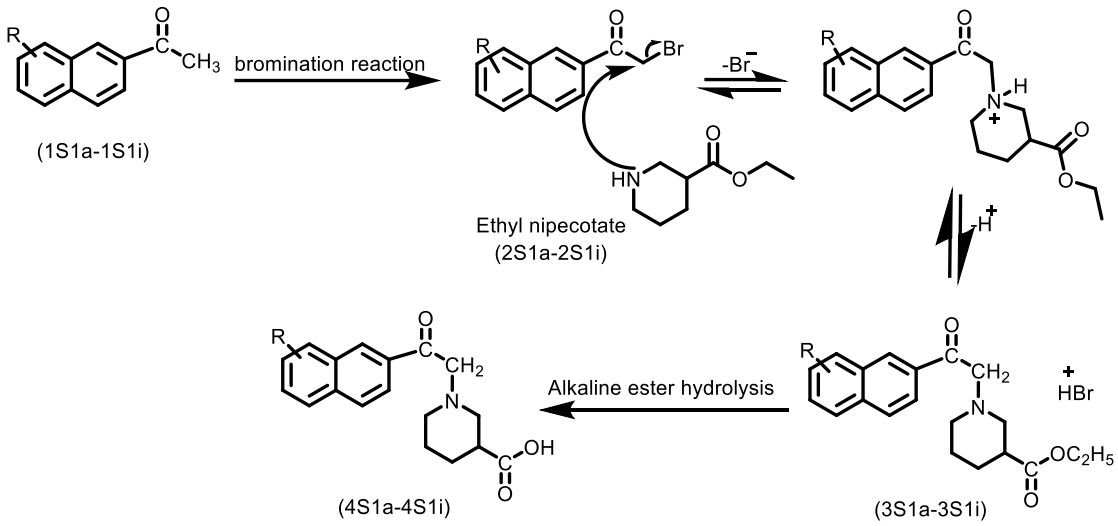


Figure 4.1. Possible mechanism of reaction for the synthesis of compounds **3S1a-3S1i & 4S1a-4S1i.**

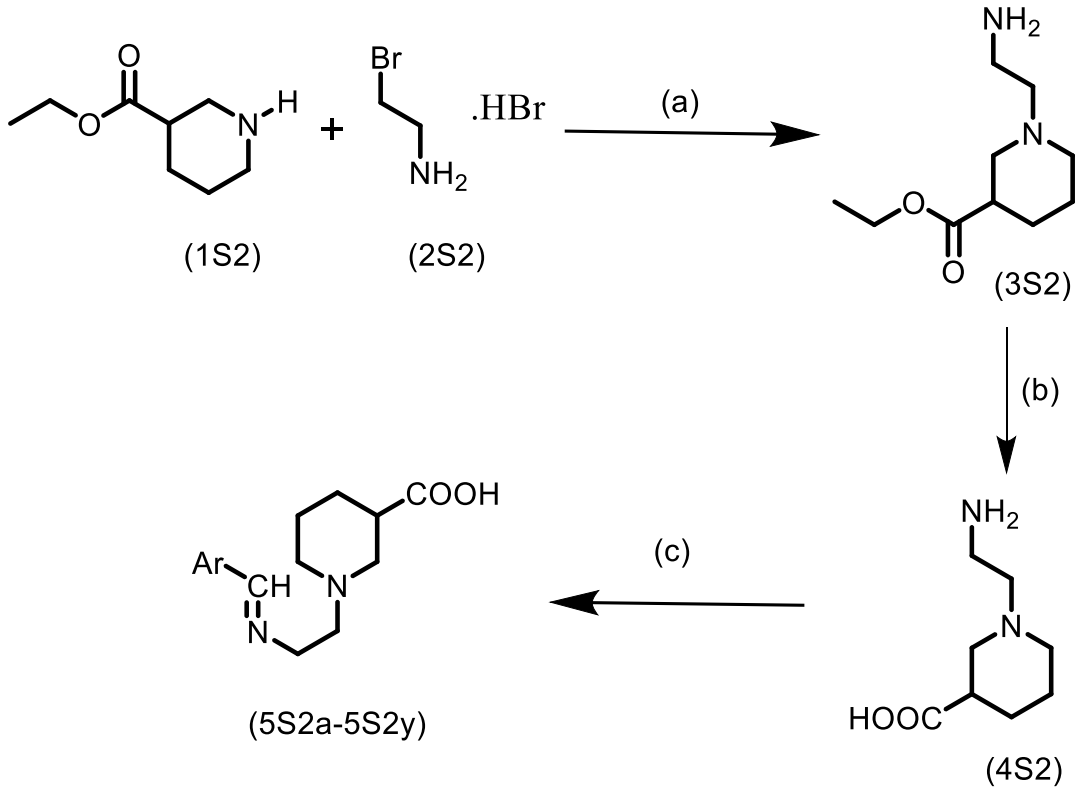
4.1.3. Series 2

Synthesis of a series of novel Schiff bases of N- substituted piperidine-3-carboxylic acid.

4.1.3.1. Method of Preparation

The novel Schiff bases of N-substituted piperidine-3-carboxylic acid were synthesized by nucleophilic substitution reaction takes place at 2-C of 2-bromoethylamine **(2S2)** with the -NH (nucleophile) of ethyl nipecotate **(1S2)** to give

Ethyl 1-(2-aminoethyl) piperidine-3-carboxylate **(3S2)** which was subsequently hydrolyzed to give 1-(2-aminoethyl)piperidine-3-carboxylic acid **(4S2)** followed by the formation of novel Schiff bases **(5S2a-5S2y)** by reacting with the corresponding aromatic aldehyde as depicted in **Scheme 4.2**.



Scheme 4.2. Synthesis of compounds **5S2a-5S2y**; Reagents and conditions: (a) K_2CO_3, KI, 1,4-dioxane, reflux, 30 h; (b) (i) 3N NaOH, EtOH, RT, (ii) 1N HCl; (c) Corresponding aromatic aldehydes, glacial acetic acid, EtOH, reflux, 3-6 h.

4.1.3.1.1. Procedure for the synthesis of ethyl 1-(2-aminoethyl)piperidine-3-carboxylate (3S2):

Compound **3S2** was synthesized as per the reported procedure with slight modification [Murali Dhar et al. 1999]. A mixture of ethyl nepicotate (7.76 mmol), K_2C0_3 (19.5 mmol) and KI (3.9 mmol) was dissolved in 1,4-dioxane with gentle heating. 2-bromo ethylamine hydrobromide (16 mmol) was then added, and the reaction mixture was refluxed for 30 hours [Murali Dhar et al. 1999. The progress of the reaction was monitored by TLC [Mobile phase: DCM/methanol (9.5:0.5)]. The

reaction mixture was cooled to room temperature after completion of the reaction, and dioxane was evaporated under vacuum. The obtained residue was treated with ice-cold 3 N NaOH and further extracted with EtOAc (4 X 120ml). The extracted organic layer was dried over (Na_2SO_4) and concentrated. The crude product was further purified by column chromatography on silica gel using DCM/methanol (9.5:0.5) as the mobile phase to afford the compound **3S2** as viscous brown oil.

4.1.3.1.2. Procedure for the synthesis of 1-(2-aminoethyl)piperidine-3-carboxylic acid (4S2):

The synthesized compound **3S2** was subjected to hydrolysis as per the method reported by Andersen et al. with slight modification to get the title compound. In this method the corresponding ester derivative **3S2** (1.0 mmol) and 4N NaOH solution (3.0 mmol) were taken in ethanol in ethanol (3 mL) and stirred at room temperature until the completion of reaction. The hydrolysis was monitored by TLC using DCM/methanol (9.5:0.5). The reaction mixture was kept on an ice bath followed by the workup with 1N HCl and dried under vacuum to give the compound **4S2**.

4.1.3.1.3. General procedure for the synthesis of compounds (5S2a-5S2y):

Compound **4S2** (1 mmol) was dissolved in absolute ethanol (20 ml) with gentle heating, followed by the addition of glacial acetic acid (2-3 drops). Corresponding aromatic aldehydes (1.2 mmol) were added, and the reaction mixture was refluxed for 3-5 h. The progress of the reaction was monitored by TLC using DCM/methanol (9.5:0.5) as the mobile phase. The obtained precipitate was filtered, dried and purified by column chromatography on silica gel with few drops of triethylamine using DCM/methanol as the mobile phase to yield the target compounds **(5S2a-5S2y)**.

4.1.3.2. Reaction Mechanism for Series 2

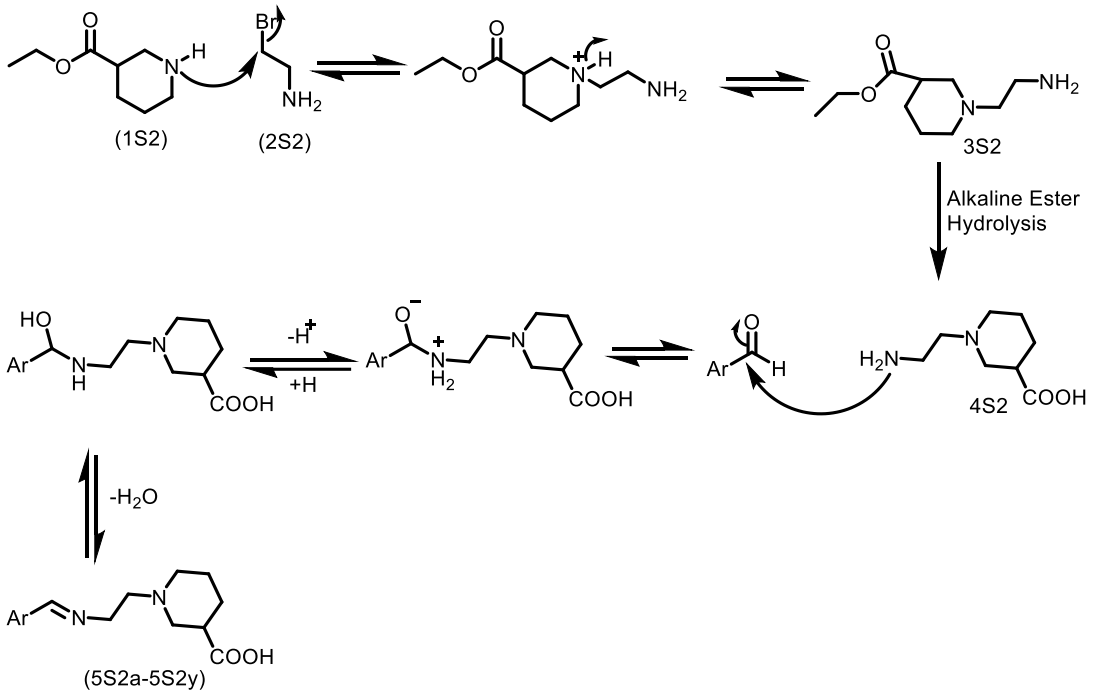


Figure 4.2. Possible mechanism of reaction for the synthesis of compounds **5S2a-5S2y**

The nucleophilic substitution reaction (SN2) takes place at 2-C of 2-bromoethylamine **(2S2)**. The bromide group is substituted with the -NH of ethyl nipecotate **(1S2)** to form the intermediate **(3S2)**. The -NH moiety of ethyl nipecotate, acts as a nucleophile and react with the positively charged carbon atom (2-C) attached to the bromide leaving group to generate compound **3S2**. The intermediate **(3S2)** was hydrolysed in ethanol under basic condition (NaOH) to yield 1-(2-aminoethyl)piperidine-3-carboxylic acid **(4S2)**. The -NH_2 group of the derivative **(4S2)** acts as a nucleophile and attacks onto the carbonyl group of respective aromatic aldehyde to form an unstable aminomethanol intermediate by nucleophilic addition reaction followed by dehydration to give the title compounds **(5S2a-5S2y)**.

4.1.4. Characterization of Synthesized Compounds of Series 1 and Series 2

The following procedures were employed to ascertain the structures of the synthesized compounds.

4.1.4.1. Physicochemical Characterization

All the synthesized compounds were subjected to R_f value, melting point and Log P determination.

4.1.4.1.1. Thin layer chromatography (TLC) analysis (R_f value):

The TLC is a commonly used technique in synthetic chemistry to determine the purity of the compounds and qualitatively monitor the progress of a reaction. It also allows the optimization of solvent system for a particular separation task. The R_f value is a distinctive property of a given compound in a particular solvent system and calculated as follows:

$$\mathbf{R_f} = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent}}$$

Thin layer chromatography was done on a pre-coated Merck silica gel 60F254 aluminum sheets (Merck, Germany) to observe the progress of the reaction. Ascending technique was employed to develop the chromatograms, and the TLC visualization was accomplished by using the ultraviolet cabinet, iodine chamber, and other reagents.

4.1.4.1.2. Melting point determination:

The synthesized compounds of series **1** are oily in nature; therefore melting points were determined for the compounds of series **2** only. Melting points were determined in the open capillaries using Stuart melting point apparatus SMP10® (Barloworld Scientific Ltd., UK), and was uncorrected.

4.1.4.1.2. Log P value determination:

Partition coefficient quantifies the partitioning of a neutral (unionized) substance between water and *n*-octanol. The logarithm of the concentration of the unionized solute in the solvents is called Log P. The Log P was considered to be the measure of lipophilicity. Log P value of all the synthesized compounds was determined experimentally by shake flask method in n–octanol and buffer (pH 7.4).

At different concentrations of the synthesized compounds in water using methanol as a cosolvent, a calibration curve was plotted. The known quantity compounds were dissolved separately in n-octanol and shaken with the buffer on a mechanical shaker for 30 min. To accomplish complete phase separation, the mixture was centrifuged for 20 min, and the n-octanol phase was separated. The absorbance of the buffer phase was then measured, and the Log P was calculated by correlating the absorbance with the concentration in the standard plot [Ghadimi *et al*., 2008]. Partition coefficient (K_{ow}) was calculated using the equation:

$$\mathbf{K_{ow} = C_o/C_w}$$

Where,

C_o = Concentration in octanol phases

C_w = Concentration in water phases

4.1.4.2. Spectral Characterization and Elemental Analysis

All the synthesized compounds were characterized by state of art spectroscopic techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

4.1.4.2.1. FT-IR spectroscopy

IR spectroscopy was performed using FT-IR spectrophotometer Shimadzu 8400S at the scanning range of 4000-400 cm^{-1} at the Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi. Oily products were analyzed in the form of films, and solid compounds were analyzed as KBr pellets. The results of FT-IR spectroscopy were recorded as % Transmittance vs. Wavenumber (cm^{-1}).

4.1.4.2.2. Nuclear magnetic resonance spectroscopy

^{1}H-NMR spectra (500 MHz) and ^{13}C-NMR (125 MHz) was recorded using Bruker Advance spectrophotometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard.

4.1.4.2.3. Elemental analysis

Elemental (C, H, and N) analysis was performed using Exeter CE-440 elemental analyzer at the Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi.

4.2. BIOLOGICAL ACTIVITY OF SERIES 1

4.2.1. Animals

Adult Wistar mice (20 ± 5 g) of either sex were procured from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1ºC and 45-55 % relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water ad libitum unless stated otherwise. Animals were acclimatized to laboratory conditions for at least one week before using them for experiments. Body weight of animals was measured periodically. Principles of laboratory animal care guidelines (NIH publication number 85-23, revised 1985) were followed. Protocols of the study were approved by Animal Ethical Committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Letter No. Dean/12-13/ CAEC/17).

4.2.2. Drug Administration

All the test compounds were administered intraperitoneally at an equimolar dose relative to 10 mg/kg Tiagabine (standard). Control group was administered with physiological saline (0.9%) containing 2.5% tween 80 ip.

4.2.3. *In Vivo* Anti-convulsant Activity

4.2.3.1. s.c.-PTZ Induced Seizures in Mice

Compounds of Series 1 were assessed for their antiepileptic activity in s.c.-PTZ induced seizures model in mice with tiagabine as a standard. Test compounds were administered intraperitoneally, one hour before PTZ challenge at an equimolar

dose relative to 10 mg/kg tiagabine. The PTZ was administered to mice subcutaneously at the dose of 100 mg/kg. Post-administration of PTZ, mice were observed during the next 30 min for the occurrence of clonic seizures. Loss of righting reflex preceded by clonus of the whole body lasting more than 3 s was considered as clonic seizures. Delay in the onset of seizures and reduced frequency by test and the standard drug is considered as anti-epileptic activity. Latency to first seizure and number of seizures were noted and compared to control group [physiological saline (0.9%) containing 2.5% tween 80]. Only those compounds eliciting significant protection against s.c.-PTZ induced seizures were selected for further evaluation [Kowalczyk *et al*., 2014].

4.2.3.2. Pilocarpine Induced Seizures in Mice

Based on the outcome of the initial *in vivo* screening in the s.c.-PTZ model, selected derivatives of Series 1 were further evaluated against pilocarpine-induced seizures in mice. Pilocarpine (400 mg/kg, i.p) was administered one hour after mice were administered with test compounds, tiagabine as standard and vehicle [physiological saline (0.9%) containing 2.5% tween 80] as a control in respective groups. Scopolamine butylbromide (1 mg/kg, i.p.) was administered 45 min before pilocarpine administration to avoid peripheral toxicity and diarrhea, masticatory and stereotyped movements. The latency to the first tonic-clonic seizure episode and latency to the death was recorded [Nielsen *et al*., 1991].

4.2.3.3. DMCM Induced Seizures in Mice

The compounds were tested for their antiepileptic activity following the standard method described by Nielsen *et. al*. The selected compounds of Series 1 and standard drug tiagabine were injected to mice 30 min prior to the administration of DMCM (15 mg/kg i.p.). The observations were made for next 30 min for the presence

of tonic and clonic seizures. The latency to the first convulsion was noted [Nielsen *et al*., 1991].

4.2.4. Rota-rod Performance Test in Mice

The test is used to observe any motor incoordination caused by the test compounds. The test involves measurement of fall off time of mice on a rotating rod (10 rpm). The test was conducted in two sessions- before drug treatment and one hour after drug treatment. Only those animals that stay more than 3 minutes on rotating rods were selected for the test. Each mouse was placed on rotating rod and "fall-off" time was noted. The animals were then treated with the selected compounds of Series 1, tiagabine, diazepam and the vehicle in case of control. One hour after the drug treatment, animals were again placed on rotating rod and "fall-off" time was noted [Kulkarni and Joseph, 1998].

4.2.5. MTT Assay on Neuroblastoma Cell Line (SH-SY5Y)

The effect of the most active compounds from Series 1 on neuroblastoma cell line SH-SY5Y (procured from National Center for Cell Science, Pune) was evaluated by MTT assay. Initially, 96-well plates were seeded with SH-SY5Y cells at a density of 1x 10^5/well in 100 µL of the medium, followed by 24 hours of incubation at 37°C in 5% CO_2. Different concentrations of the test compounds (ranging from 1µM to 160 µM) were then incubated with the cells for further 24 hours. After the exposure of cells to the different concentrations of the test drugs, 20 µL of 5 mg/ml MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was added to each well and incubated for 3 hours (at 37°C, 5% CO_2). After incubation, the formation of a purple colored precipitate was observed under a microscope and solubilized by adding 1 ml of dimethylsulfoxide (DMSO). Cell viability was measured by determining absorbance at 570 nm in a microplate-reader (Synergy HTX, BioTek, USA). The outcomes were expressed as a growth percentage in each well relative to

the control cells incubated in the absence of test compounds [Regulska *et al*., 2010, Shidore *et al*., 2016]. Percentage cell viability was measured by using the following formula:

Percentage Cell Viability = Absorption of sample/ absorption of control x 100

4.2.6. Repeated dose toxicity studies

Adult Wistar mice (20 ± 5 g) of either sex were distributed into 4 groups **(Control, 4S1i, 5S2w** and **Tiagabine)** of 06 animals each. OECD guideline (Organization for Economic Co-operation and Development, Guideline-407) was followed during the study with slight modifications. Test compounds (**4S1i & 5S2)** were administered i.p. for 28 consecutive days (once daily at 24 hour intervals) at an equimolar dose relative to 10 mg/kg Tiagabine. Control group was administered with physiological saline (0.9%) containing 2.5% tween 80 intraperitoneally. Body weights of animals, as well as their food and water consumptions were monitored daily throughout the study period. They were fasted overnight prior to blood collection by retro-orbital technique on the day 29th of the study. The blood samples were analysed for hematological [hemoglobin, total leukocyte count, differential leukocyte count (DLC)] and biochemical (glucose, cholesterol, alkaline phosphatase, aspartate transaminase (AST), alanine aminotransferase (ALT), blood urea nitrogen, creatinine and total protein) parameters [Nakache *et al*., 2017].

4.2.7. *In Vitro* Parallel Artificial Membrane BBB Permeability Assay (PAMPA)

BBB permeability of the selected leads from Series 1 was determined by the PAMPA-BBB assay. Dodecane was purchased from the Avra Synthesis and Porcine Brain Lipid (PBL) was purchased from the Avanti polar lipids. Acceptor microplates with PVDF membrane, pore size 0.45 μm and donor microplates were procured from the Merck Millipore. The primary stock solutions were prepared by dissolving 5 mg of selected compounds in the 1 ml of DMSO. Secondary stock solutions were

prepared by diluting 10 µL of these solutions to 200 fold using a buffer of pH 7.5 to obtain a final concentration of 25 µg/ml. Secondary stock solutions 200 µL were added to the donor well plate. The filter membrane of the acceptor plate was coated with 4 µL of 20 mg/ml PBL in dodecane. Acceptor plate was filled with 200 µL of buffer (pH 7.4). Acceptor plate was kept carefully over the donor plate like a sandwich and incubated for 18 h. The concentration of drug in the acceptor, donor, and reference well was determined by UV spectroscopy using microplate reader. Each of the samples was scanned for at least five different wavelengths and in three independent runs. PAMPA model was validated by using nine commercial drugs with known BBB permeability. Linear correlation was established by using the experimentally obtained permeability [$P_{e\ (Exp)}$] and reference permeability [$P_{e\ (Ref)}$] [Di *et al*., 2003].

4.3. BIOLOGICAL ACTIVITY OF SERIES 2

In the second series, all the procedures and protocols are kept same with some changes in the sequence of studies and number of *in vivo* models. Following ethical considerations and alternatives to minimize the laboratory animal experimentation, PAMPA studies were initially performed to screen the synthesized compounds for their ability to permeate the blood-brain barrier (BBB). The potential leads exhibiting *in vitro* BBB permeability were further evaluated for anticonvulsant activity in sc-PTZ and DMCM induced seizure models following the same procedures mentioned above. Pilocarpine model of epilepsy was excluded from the biological evaluation protocol of the second series due to the high mortality rate of the animals, as indicated by the results of first series. Rota-rod test on rodents and MTT assay were performed with the same objective and procedures as in the first series.

4.4. Statistical Analysis

The experimental results are expressed as the mean ± S.D (n = 6) followed by a one-way analysis of variance (ANOVA). Tukey's multiple comparisons test were applied for determining the statistical significance between different groups. InStat Graph Pad Software (San Diego, CA, USA) was used for all statistical analyses and a p-value < 0.05 was considered significant.

4.5. COMPUTATIONAL STUDIES

4.5.1. Homology Modelling of GAT-1

Homology modeling was performed as per the method of Singh and coworkers [Singh *et al*., 2018]. It was carried out in three principle steps. The first step involves the alignment of the amino acid sequence of Drosophila dopamine transporter (dDAT) with that of the target sequence (GAT-1). The second step includes model building from the alignment, followed by validation.

From UniProt website (http://www.uniprot.org/uniprot/P30531) the complete sequence of human sodium- and chloride-dependent GABA transporter 1(GAT1) (UniProtKB: P30531) was downloaded. To find the suitable template psi-BLAST was performed against Protein Data Bank (PDB). A template of Drosophila melanogaster dopamine transporter (dDAT) (PDB ID: 4XP4_A) [Wang et al. 2015] was chosen as the template for building multiple homology models. The sequences of the template (4XP4_A) GAT1 were aligned using multiple sequence alignment programs (Clustal W). In the model generation, a known disulfide bridge between C164 and C173 located in the extracellular-loop 2 of GAT1 was included [Jurik *et al*., 2015]. A total of 10 models were prepared using the knowledge-based method implemented in the Schrödinger Suite 2016 [Kim and Cho, 2016]. The generated models were then subjected to loop refinement using the Prime module implemented in the Schrödinger suite. During model preparation, the Na+ and Cl- ions were retained. The homology

model was evaluated through computations of molecular interactions fields and sequence identities. The quality of the models was assessed by using Ramachandran plot. The best model was validated by docking tiagabine (a known inhibitor) which matches with the previous studies and mutagenesis data.

4.5.2. Molecular Docking Studies

In silico docking simulation protocols were performed using Schrödinger Glide module in Schrödinger Suite 10.5.014 MM Share Version 3.3.014 Release 2016-1 with workstation 4x Intel(R) Xeon(R) CPU E5-1607 v3 @ 3.10 GHz on Kernel Linux operating environment. The potential ligands of series 1 **(4S1a, 4S1b & 4S1i)** and series 2 **(5S2d, 5S2w & 5S2y)** were selected and prepared using LigPrep module. The minimum energy conformers of selected ligands were generated using OPLS2005 force field. Homology modeled protein structure of GAT1 GABA transporter was refined and corrected using Protein Preparation Wizard module. The structure of the protein was further optimized using PropKa method at default pH value 7.0 and minimization was performed at restrained minimization converge heavy atoms to RMSD 0.30 Å. Receptor Grid was generated surrounding the active binding pocket of the protein. The grid box of 10 Å was created by supplying the x, y and z coordinates covered with active site amino acids residues. The prepared grid and docking simulation protocols of Glide (Grid-Based Ligand Docking with Energetics) extra precision (XP) mode were validated by docking the tiagabine ligand. During the docking simulations, the protein was kept rigid, and ligands were kept flexible. All other parameters of Glide module were maintained at their default values. The docking results were studied using the Glide XP visualizer module to gain insights of the interactions of ligands with the amino acid residues. The results of score and interactions were analyzed in comparison to tiagabine.

4.5.3. Molecular Dynamics (MD) Simulation Protocol for Compound 4S1i

To confirm the stability of binding mode interactions of the most promising compounds of series 1 & 2 (**4S1i & 5S2w)**, their docked complex was further utilized for molecular dynamics simulation run using the Desmond module of Schrödinger Maestro 10.5.014 program with an OPLS-AA force field in an explicit solvent with the TIP3P water model. The docked complex of **4S1i & 5S2w** with GABA GAT1 transporter protein was soaked adequately in 14,594 TIP3P water molecules, and the system was neutralized by adding 4Na+ ions to balance the overall charge of the system.

The generated system with water molecules consisted of total 52,420 atoms. The system was further minimized to maximum 7000 steps. The recording interval energy was kept at 1.2 ps and the trajectory was set at 9.6. A constant number of atoms (N), pressure (P), and temperature (T) (NVT) molecular dynamics was performed for the first 100 ps, during which the temperature of the system was raised from 0-300 K. For further simulations, the system was maintained at constant temperature (300 K) and pressure (1.0132 bar) till the complete cycle of 20 ns and 50 ns simulation run for compound **4S1i** and **5S2w** respectively.

4.5.4. Prediction of drug likeliness and *in silico* ADME properties

The *in silico* ADME of the selected compounds of series 1 & 2 was predicted by using QikProp 3.1 (Schrödinger; LLC, USA) program. Maestro 8.5 build panel was used to build the ligands. Ligprep 2.2 version v22208 (Schrödinger, LLC, USA) application was used for ligand preparation. The best-fit ligands were neutralized before being subjected to QikProp analysis. Various pharmacokinetic properties consisting of principal descriptors have been estimated such as log P (octanol/water), molecular weight, polar surface area, brain/blood partition coefficient, CNS activity, binding to human serum albumin and Lipinski's rule of five.