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DESIGN AND SYNTHESIS OF NOVEL N-(4-(PYRIDIN-4-YLOXY) BENZYLIDENE)-4-[4-(SUBSTITUTED)PHENYL] SEMICARBAZIDES AS POTENTIAL ANTICONVULSANT AGENTS

Prem Singh[1],* and Laxmi Tripathi[2]

[1]Research Scholar , IFTM University, Moradabad, Uttar Pradesh, India.
[2]Moradabad Educational Trust, Group of Institution, Faculty of Pharmacy, Moradabad, Uttar Pradesh, India.

***Corresponding Author: Prem Singh**
Research Scholar, IFTM University, Moradabad, Uttar Pradesh, India.

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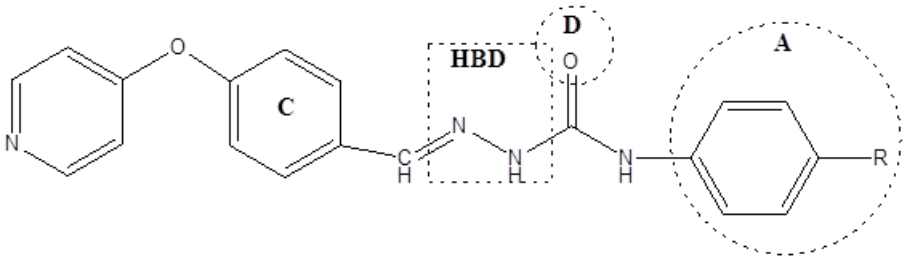
ABSTRACT
A new series of N-(4-(pyridin-4-yloxy) benzylidene)-4-[4-(substituted)phenyl] semicarbazides (PSSD 17-24) were designed and synthesized keeping in view the structural requirement of pharmacophore and evaluated for their possible anticonvulsant activity. All derivatives were synthesized by the given scheme and reaction process was monitored by thin layer chromatography. The structure of synthesized derivatives was confirmed by FT-IR, ^{1}H NMR, mass spectroscopy and elemental analysis methods. The anticonvulsant activity was established after intraperitoneal administration in MES, and $_{SC}$MET seizure models. The most active compound of the series was 1-(4-(pyridin-4-yloxy)benzylidene)-4-p-tolylsemicarbazide (PSSD21). A molecular docking study was carried out in order to assess the interaction and binding modes with target receptor/enzyme. Titled compounds were found to strongle bind to human gamma-aminobutyric acid receptor ($GABA_AR$-β3). A Computational study was also carried to predict the pharmacokinetic properties of the synthesized compounds.

KEYWORDS: N-(4-(pyridin-4-yloxy) benzylidene)-4-[4-(substituted)phenyl] semicarbazides, Synthesis & Characterization, Anticonvulsant Activity, Neurotoxicity, Molecular docking Computational Study.

INTRODUCTION
Anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure. The use of current antiepileptic drugs has been questioned due to the non selectivity of the drugs and the undesirable side effects posed by them. This lead to the search of antiepileptic compounds with selective activity and lower toxicity.[1] Semicarbazones are synthesized by the condensation of semicarbazide and aldehyde/ketone. The literature survey revealed that semicarbazones had emerged as compounds with diverse biological activities including anticonvulsant, antitubercular, anticancer, and antimicrobial.[2] Semicarbazones have been developed as versatile anticonvulsant pharmacophore. It has displayed potent anticonvulsant effect in a wide variety of preclinical anticonvulsant models. Till date various semicarbazone derivatives containing 1,3,4-oxadiazole, 1,3,4- thiadiazole, pyrimidine, benzothiazole and substituted phenyl/aryl ring have been synthesized and evaluated for anticonvulsant activity. The semicarbazone based pharmacophore model with four binding sites is essential for anticonvulsant activity. This model comprises of an aryl hydrophobic binding site, hydrogen bonding domain, an electron donor group and another hydrophobic-hydrophilic site regulating the pharmacokinetic properties of the anticonvulsant.[3]

This research work reports the synthesis and anticonvulsant activities of N- (4-(pyridin-4-yloxy) benzylidene) – 4 - [4-(substituted) phenyl] semicarbazides. Their chemical structures were characterized using IR, ^{1}H NMR, MS and elemental analysis techniques. All the synthesized compounds comprised of the essential pharmacophoric elements (Fig.1) that are necessary for good anticonvulsant activity as suggested by Pandeya et al.[4] ie. an aryl hydrophobic binding site (A), hydrogen bonding domain (HBD), an electron donor group (D) and another hydrophobic-hydrophilic site (C). The anticonvulsant activity was evaluated by using experimental epilepsy models, i.e., maximal electroshock (MES) and subcutaneous metrazole (scMET) test in mice. The rotorod assay was performed in mice to evaluate the neurotoxicity of the compounds. The molecular docking study of designed molecules was carried out in order to assess the interaction and binding modes of synthesized compounds with target receptor/enzyme using Glide extra precision (XP) Maestro 10.1 Schrodinger software.

A computational study was also carried out to predict the pharmacokinetic parameters and Log P values.



N- (4-(pyridin-4-yloxy) benzylidene) -4- [4-(substituted) phenyl]semicarbazides

Fig. 1: Essential pharmacophoric elements for anticonvulsant activity.

EXPERIMENTAL

All chemicals & solvents were purchased from Sigma and Himedia Chemicals, supplied by S.K. Traders Indore (M.P.). Melting points were determined on a Tempo capillary melting point apparatus, Mumbai and are uncorrected. The FT-IR (Fourier transform infrared) spectrum was recorded (νmax in cm^{-1}) on Bruker Tensor 27 FT-IR spectrometer. ^{1}H-NMR (proton nuclear magnetic resonance) spectrum were recorded at 300 MHz, after dissolving in a suitable solvent (DMSO or D2O) on Bruker Avance II 400 MHz, USA FT-NMR spectrometer using tetramethylsilane as internal standard and chemical shifts (δ) are reported in parts per million (ppm). The spin multiplicities are indicated by symbols, s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). The purity of the compounds was ascertained by thin layer chromatography (TLC) and elemental analysis. Plates for TLC were prepared with silica gel G and activated at 110^0C for 30 min. Iodine vapors was used to develop the TLC plates. The mass spectra were recorded on a Waters Micromass ZQ 2000 mass spectrometer. Elemental analyses were performed on a Vario EL-III analyzer.

Synthesis of 4-(Pyridin-4-yloxy)-benzaldehyde (3): A mixture of o-chloropyridine (1) (0.002 mol), p-hyroxybenzaldehyde (2) (0.002 mol), potassium carbonate (0.002 mol) and 10 mL of dry dimethyl formamide was taken in a round bottom flask and heated at 70 ^{0}C for 3-4 hr under reduced pressure. Reaction mixture was poured into water and extracted with ethyl acetate. The combined extracts were washed with water and dried over anhydrous sodium sulfate. Molecular Weight: 199.21; Yield: 72.5%; M.P.: 127-129 ^{0}C. The purity of compound was checked by TLC using: Silica gel G, Solvent system; Ethyl acetate: ethanol (3:2) and detecting agent; iodine vapours. Only one sport was obtained (Rf, 0.71).[5]

Synthesis of 4-(substituted) phenyl urea (5): Aniline and/or p-substituted aniline (4) (0.1 mol) was dissolved in glacial acetic acid (10 mL) and diluted with water (upto 100 mL). To this solution an equimolar (0.1 mol) quantity of sodium cyanate in warm water (50 mL) was added with stirring. The reaction mixture was allowed to stand for 30 min, and then compounds were filtered, washed with water and dried after recrystallization from boiling water.[6]

Synthesis of 4-(substituted) phenyl semicarbazides (6): To an aqueous solution of 4-(substituted) phenyl urea (5) (0.1 mol); an equimolar quantity of hydrazine hydrate and ethanol (2 mL) was added. The reaction mixture was refluxed for 30 min and cooled in ice. Sodium hydroxide (4 g) was added to make the reaction mixture alkaline, before refluxing. The product was filtered under suction and recrystallized from ethanol.[6]

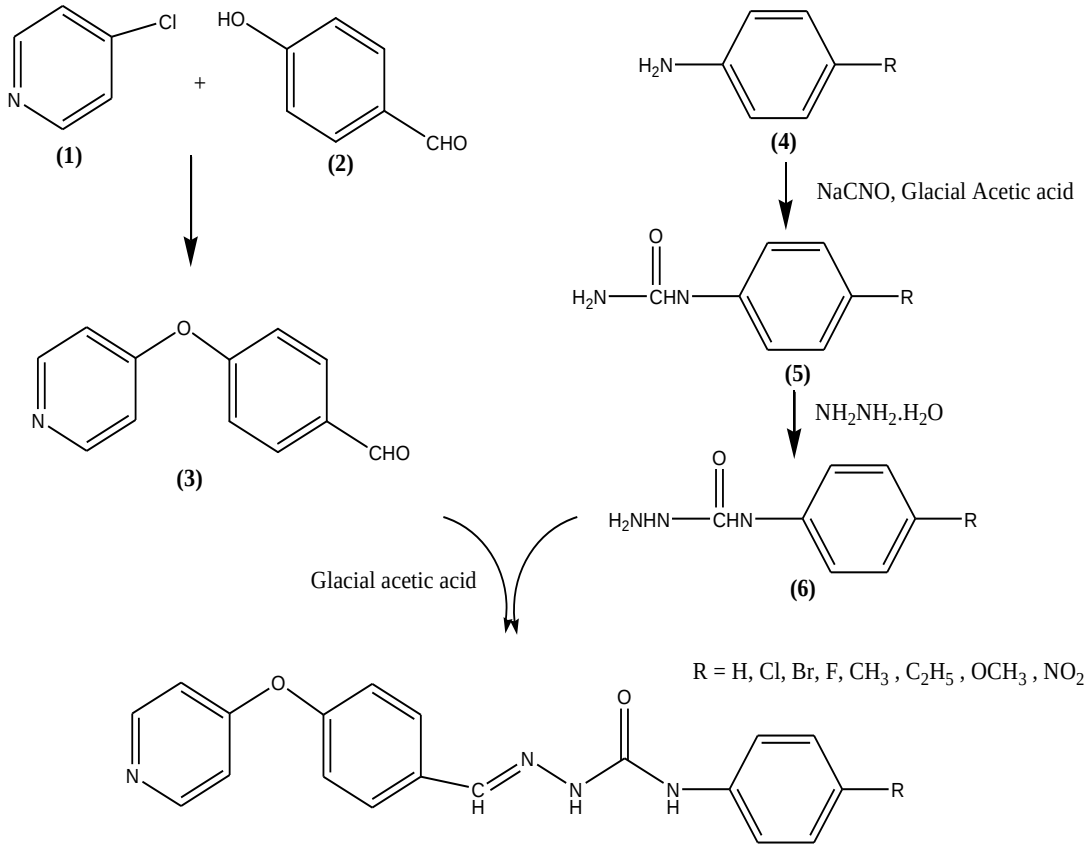
Synthesis of N- (4-(pyridin-4-yloxy) benzylidene) -4- [4-(substituted) phenyl]semicarbazide (PSSD17-PSSD24) : A mixture of 4-(pyridin-4-yloxy)-benzaldehyde (3) (0.002 mol), 4-(substituted) phenylsemicarbazides (6) (0.002 mol) and 10 ml of ethanol were taken in a round bottom flask. Few drops of glacial acetic acid was added and refluxed for 2-3 hr. The reaction was monitored by TLC and the reaction mixture was poured into ice. The precipitate was filtered and washed with sodium acetate (0.005mol). The crude solid was dried and recrystallized with hot ethanol to obtain compounds (PSSD17-PSSD24)[7]. The scheme for synthesis of the titled compounds is given in figure 2. The physical data of the titled compounds are given in Table 1 and the spectral and elemental analysis data are given below.

N-(4-(pyridin-4-yloxy)benzylidene)-4-phenylsemicarbazide (PSSD17): FT-IR (KBr, cm^{-1}) ν: 3455, 3315 (N-Hstr.), 3034 (C-Hstr.), 2886 (=C-Hstr.), 2000-1670 (overtone for mono substitution on aromatic ring), 1659 (C=Ostr.), 1638 (Azomethine C=Nstr.), 1599 (Phenyl ring Strech.), 1465 (Phenyl C-H in plane bending), 1282 (C-Nstr. coupled with N-H bending), 1255 (C-Ostr.), 1047 (N-Nstr.), 787 & 707 (C-H out of plane bending for mono substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.48 (s, 1H, Ph-C<u>H</u>=N at N_1), 8.36-8.33 (d, 2H, pyridine ring protons at C_2 & C_6), 7.72-7.70 (d, 2H, Ortho phenyl ring protons at N_4), 7.49-7.47 (d, 2H, Ortho benzyl ring protons at), 7.33-7.29 (t, 2H, meta phenyl ring protons at N_4), 7.12-7.10 (d, 2H, pyridine ring protons at C_3 & C_5), 7.08-7.04 (t, 1H, para phenyl ring protons at N_4), 6.89-6.87 (d, 2H, meta benzyl ring

protons at N_1), 6.09 (s, 2H, semicarbazide NH protons). MS (m/z, %): M^+ : 319.38, (M^+ + 1: 331.14), M^+ - C_6H_6 : 251.85, M^+ - $C_7H_8N_2O$: 205.75, M^+ - C_5H_5N : 255.17. Anal. Calcd: C, 68.66; H, 4.85; N, 16.86; O, 9.63. Found: C, 68.66; H, 4.85; N, 16.86; O, 9.63. Found: C, 68.67; H, 4.87; N, 16.81; O, 9.65.

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-chlorophenyl)semicarbazide (PSSD18): FT-IR (KBr, cm^{-1}) ν: 3445, 3316 (N-Hstr.), 3037 (C-Hstr.), 2876 (=C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1658 (C=Ostr.), 1642 (Azomethine C=Nstr.), 1596 (Phenyl ring Strech.), 1475 (Phenyl C-H in plane bending), 1288 (C-Nstr. coupled with N-H bending), 1252 (C-Ostr.), 768 (C-Clstr.), 1048 (N-Nstr.), 848 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.54 (s, 1H, Ph-CH=N at N_1), 8.40-8.38 (d, 2H, pyridine ring protons at C_2 & C_6), 7.62-7.60 (d, 2H, Ortho phenyl ring protons at N_4), 7.51-7.49 (d, 2H, Ortho benzyl ring protons at N_1), 7.35-7.31 (t, 2H, meta phenyl ring protons at N_4), 7.09-7.07 (d, 2H, pyridine ring protons at C_3 & C_5), 6.85-6.83 (d, 2H, meta benzyl ring protons at N_1), 6.06 (s, 2H, semicarbazide NH protons). MS (m/z, %): M^+ : 365.18, (M^+ + 1 for ^{35}Cl : 366.24, M^+ + 2 for ^{37}Cl : 366.23), M^+ - C_6H_5Cl : 254.66, M^+ - $C_7H_7ClN_2O$: 199.79, M^+ -C_5H_5N : 290.48. Anal. Calcd: C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72. Found: C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72. Found: C, 62.24; H, 4.14; Cl, 9.63; N, 15.23; O, 8.74.

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-bromophenyl)semicarbazide (PSSD19): FT-IR (KBr, cm^{-1}) ν: 3441, 3322 (N-Hstr.), 3028 (C-Hstr.), 2883 (=C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1688 (C=Ostr.), 1636 (Azomethine C=Nstr.), 1589 (Phenyl ring Strech.), 1469 (Phenyl C-H in plane bending), 1282 (C-Nstr. coupled with N-H bending), 1261 (C-Ostr.), 546 (C-Br str.), 1043 (N-Nstr.), 842 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.52 (s, 1H, Ph-CH=N at N_1), 8.44-8.42 (d, 2H, pyridine ring protons at C_2 & C_6), 7.60-7.58 (d, 2H, Ortho phenyl ring protons at N_4), 7.50-7.44 (m, 2H, meta phenyl ring protons at N_4 & 2H Ortho benzyl ring protons at N_1), 7.11-7.09 (d, 2H, pyridine ring protons at C_3 & C_5), 6.89-6.87 (d, 2H, meta benzyl ring protons at N_1), 6.09 (s, 2H, semicarbazide NH protons). MS (m/z, %): M^+ : 410.04, (M^+ + 1 for ^{79}Br: 412.11, M^+ + 2 for ^{81}Br : 411.06), M^+ - C_6H_5Br : 257.10, M^+ - $C_7H_7BrN_2O$: 199.08, M^+ -C_5H_5N : 334.01. Anal. Calcd: C, 55.49; H, 3.68; Br, 19.43; N, 13.62; O, 7.78. Found: C, 55.52; H, 3.74; Br, 19.46; N, 13.68; O, 7.84.



N- (4-(pyridin-4-yloxy) benzylidene) -4- [4-(substituted) phenyl]semicarbazide

Fig. 2: Synthetic scheme for synthesis of N- (4-(pyridin-4-yloxy) benzylidene) -4- [4-(substituted) phenyl]semicarbazide (PSSD17- PSSD24)

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-fluorophenyl)semicarbazide (PSSD20): FT-IR (KBr, cm^{-1}) ν: 3452, 3323 (N-Hstr.), 3041 (C-Hstr.), 2879 (=C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1689 (C=Ostr.), 1647 (Azomethine C=Nstr.), 1588 (Phenyl ring Strech.), 1471 (Phenyl C-H in plane bending), 1276 (C-Nstr. coupled with N-H bending), 1250 (C-Ostr.), 1239 (C-Fstr.), 1041 (N-Nstr.), 844 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.49 (s, 1H, Ph-CH=N at N_1), 8.39-8.37 (d, 2H, pyridine ring protons at C_2 & C_6), 7.69-7.67 (d, 2H, Ortho phenyl ring protons at N_4), 7.50-7.48 (d, 2H Ortho benzyl ring protons at N_1), 7.13-7.11 (d, 2H, pyridine ring protons at C_3 & C_5), 7.01-6.99 (d, 2H, meta phenyl ring protons at N_4), 6.92-7.90 (d, 2H, meta benzyl ring protons at N_1), 6.05 (s, 2H, semicarbazide NH protons). MS (m/z, %): M^+ : 351.72, (M^+ + 1 for ^{19}F : 352.33), M^+ - C_6H_5F : 256.47, M^+ - C_7H_7 FN_2O : 199.67, M^+ - C_5H_5N : 272.19. Anal. Calcd: C, 65.14; H, 4.32; F, 5.42; N, 15.99; O, 9.13. Found: C, 65.20; H, 4.32; F, 5.47; N, 15.94; O, 9.18.

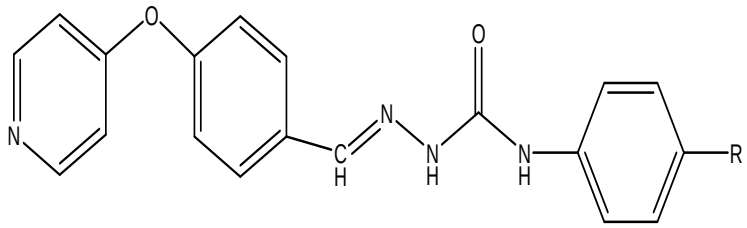
N-(4-(pyridin-4-yloxy)benzylidene)-4-p-tolylsemicarbazide (PSSD21): FT-IR (KBr, cm^{-1}) ν: 3448, 3313 (N-Hstr.), 3032 (C-Hstr.), 2972, 2876 (C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1660 (C=Ostr.), 1642 (Azomethine C=Nstr.), 1598 (Phenyl ring Strech.), 1469 (Phenyl C-H in plane bending), 1455 (Asym. methyl C-H bending), 1375 (Sym. methyl C-H bending), 1284 (C-Nstr. coupled with N-H bending), 1254 (C-Ostr.), 1045 (N-Nstr.), 846 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.64 (s, 1H, Ph-CH=N at N_1), 8.48-8.46 (d, 2H, pyridine ring protons at C_2 & C_6), 7.61-7.59 (d, 2H, Ortho phenyl ring protons at N_4), 7.53-7.51 (d, 2H, Ortho benzyl ring protons at N_1), 7.14-7.08 (m, 2H, meta phenyl ring protons at N_4 & 2H, pyridine ring protons at C_3 & C_5), 6.73-6.71 (d, 2H, meta benzyl ring protons at N_1), 6.08 (s, 2H, semicarbazide NH protons), 2.39 (s, 3H, CH_3 protons at phenyl ring). MS (m/z, %): M^+ : 348.27, (M^+ + 1: 350.22), M^+ - C_7H_8 : 245.47, M^+ - $C_8H_{10}N_2O$: 200.22, M^+ - C_5H_5N : 270.12. Anal. Calcd: C, 69.35; H, 5.24; N, 16.17; O, 9.24. Found: C, 69.38; H, 5.25; N, 16.15; O, 9.22.

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-ethylphenyl)semicarbazide (PSSD22): FT-IR (KBr, cm^{-1}) ν: 3418, 3389 (N-Hstr.), 3062, 2957, 2866 (C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1682 (C=Ostr.), 1622 (Azomethine C=Nstr.), 1579 (Phenyl ring Strech.), 1462 (Phenyl C-H in plane bending), 1451 (Asym. methyl C-H bending), 1382 (Sym. methyl C-H bending), 1278 (C-Nstr. coupled with N-H bending), 1271 (C-Ostr..), 1038 (N-N str.), 844 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.61 (s, 1H, Ph-CH=N at N_1), 8.45-8.43 (d, 2H, pyridine ring protons at C_2 & C_6), 7.60-7.58 (d, 2H, Ortho phenyl ring protons at N_4), 7.49-7.47 (d, 2H, Ortho benzyl ring protons at N_1), 7.18-7.16 (d, 2H, meta phenyl ring protons at N_4), 7.08-7.06 (d, 2H, pyridine ring protons at C_3 & C_5), 6.88-6.86 (d, 2H, meta benzyl ring protons at N_1), 6.12 (s, 2H, semicarbazide NH protons), 2.67 (s, 2H, CH_2 protons at phenyl ring), 1.37 (s, 3H, CH_3 protons at phenyl ring). MS (m/z, %): M^+: 361.26, (M^+ + 1: 362.17), M^+ - C_3H_5 : 334.23, M^+ - C_8H_{10} : 259.20, M^+ - $C_9H_{12}N_2O$: 199.18, M^+ - C_5H_5N : 285.24. Anal. Calcd: C, 69.98; H, 5.59; N, 15.55; O, 8.88. Found: C, 69.90; H, 5.66; N, 15.50; O, 8.94.

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-methoxyphenyl)semicarbazide (PSSD23): FT-IR (KBr, cm^{-1}) ν: 3451, 3326 (N-Hstr.), 3054, 2970, 2882 (C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1658 (C=Ostr.), 1648 (Azomethine C=Nstr.), 1586 (Phenyl ring Strech.), 1453 (Phenyl C-H in plane bending), 1462 (Asym. methyl C-H bending), 1372 (Sym. methyl C-H bending), 1279 (C-Nstr. coupled with N-H bending), 1264 (C-Ostr.), 1047 (N-N str.), 846 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.57 (s, 1H, Ph-CH=N at N_1), 8.40-8.38 (d, 2H, pyridine ring protons at C_2 & C_6), 7.59-7.57 (d, 2H, Ortho phenyl ring protons at N_4), 7.51-7.49 (d, 2H, Ortho benzyl ring protons at N_1), 7.09-7.07 (d, 2H, pyridine ring protons at C_3 & C_5), 6.87-6.85 (d, 2H, meta benzyl ring protons at N_1), 6.791-6.77 (d, 2H, meta phenyl ring protons at N_4), 6.11 (s, 2H, semicarbazide NH protons), 3.81 (s, 3H, CH_3 protons at phenyl ring). MS (m/z, %): M^+ : 361.04, (M^+ + 1: 362.03), M^+ - CH_4O : 332.08, M^+ - C_7H_8O : 256.00, M^+ - $C_8H_{10}N_2O$: 197.05, M^+ - C_5H_5N : 285.01. Anal. Calcd: C, 66.29; H, 5.01; N, 15.46; O, 13.25. Found: C, 66.24; H, 5.09; N, 15.53; O, 13.29.

N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-nitrophenyl)semicarbazide (PSSD24): FT-IR (KBr, cm^{-1}) ν: 3437, 3347 (N-Hstr.), 3077, 2988, 2879 (C-Hstr.), 2000-1670 (overtone for di substitution on aromatic ring), 1683 (C=Ostr.), 1637 (Azomethine C=Nstr.), 1581 (Phenyl ring Strech.), 1463 (Phenyl C-H in plane bending), 1452 (Asym. methyl C-H bending), 1382 (Sym. methyl C-H bending), 1276 (C-Nstr. coupled with N-H bending), 1262 (C-Ostr.), 1052 (N-N str.), 845 (C-H out of plane bending for di substitution). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.67 (s, 1H, Ph-CH=N at N_1), 8.50-8.48 (d, 2H, pyridine ring proton at C_2 & C_6), 8.23-8.21 (d, 2H, meta phenyl ring protons at N_4), 7.97-7.95 (d, 2H, Ortho phenyl ring protons at N_4), 7.49-7.47 (d, 2H, Ortho benzyl ring protons at N_1), 7.10-7.08 (d, 2H, pyridine ring protons at C_3 & C_5), 6.89-6.87 (d, 2H, meta benzyl ring protons at N_1), 6.13 (s, 2H, semicarbazide NH protons). MS (m/z, %): M^+: 376.01, (M^+ + 1: 377.02), M^+ - HNO_2 : 332.04, M^+ - $C_6H_5NO_2$: 256.10, M^+ - $C_7H_7N_3O_3$: 199.28, M^+ - C_5H_5N : 301.19. Anal.Calcd: C, 60.47; H, 4.01; N, 18.56; O, 16.96. Found: C, 60.42; H, 4.03; N, 18.51; O, 16.99.

Table-1: Physical Data of N- (4-(Pyridin-4-Yloxy) Benzylidene) -4- [4-(Substituted) Phenyl]Semicarbazides (Pssd17- Pssd24).



Properties / Titled Compounds	R	Mol. Formula	Mol. Wt.	M.P. in ^{0}C	% Yield	Rf
PSSD17	-H	$C_{19}H_{16}N_4O_2$	332.36	225-227	68.29	0.61
PSSD18	-Cl	$C_{19}H_{15}ClN_4O_2$	366.80	202-204	72.63	0.65
PSSD19	-Br	$C_{19}H_{15}BrN_4O_2$	411.25	194-196	64.51	0.61
PSSD20	-F	$C_{19}H_{15}FN_4O_2$	350.35	148-150	68.35	0.53
PSSD21	-CH_3	$C_{20}H_{18}N_4O_2$	346.38	189-192	76.95	0.62
PSSD22	-C_2H_5	$C_{21}H_{20}N_4O_2$	360.41	130-132	58.90	0.57
PSSD23	-OCH_3	$C_{20}H_{18}N_4O_3$	362.38	140-142	64.55	0.59
PSSD24	-NO_2	$C_{19}H_{15}N_5O_4$	377.35	228-230	58.32	0.53

Pharmacological Evaluation

The newly synthesized N-(4-(pyridin-4-yloxy)benzylidene)-4-[4-(substituted) phenyl] semicarbazides PSSD 17-24 were subjected to anticonvulsant screening according to the protocols of anticonvulsant drug development (ADD) program of National Institute of Health, Bethesda, USA. Albino mice of either sex weighing between 25-30 g were used in this study. All albino mice employed in this study were approved by the Institutional Animal Ethics Committee of Sapience Bioanalytical Research Laboratory, Bhopal (1413/PO/E/S/11/cpcsea) and experiment was carried out as per CPCSEA guidelines. The animals were kept in large spacious hygienic cages during the course of the experimental period. The animals had free access to standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22±1°C with 12 hrs light-dark cycle. The animals were divided into three groups of six animals each: Group I: Control group (distilled water treated). Group II: Test group (test compounds were dissolved in polyethylene glycol-400 and administered at a dose of 30, 100, 300 mg/kg i.p. doses), Group III: Standard group, on reference drug (phenytoin 30 mg/kg i.p.). Anticonvulsant activity was established by maximal electroshock (MES) and subcutaneous metrazole (scMET) models at drug doses of 30, 100 and 300 mg/kg at three different time intervals. Neurotoxicity of the compound was determined by Rotorod method. Results of anticonvulsant activity and neurotoxicity are shown in table 2.

Maximum Electroshock (MES) Seizure Test: Each treatment group and vehicle control group consisted of six animals; 60 Hz of alternating current (50 mA in mice) was applied via ear-clip electrodes for 0.2 s. Mice were tested at 30 minutes and 4 h following doses of 30, 100 and 300 mg/kg of test compound. The test compounds were dissolved polyethylene glycol (PEG) and injected intraperitoneally (*i.p.*) at dose of 30, 100 and 300 mg/kg 30 minutes before seizures induction. Phenytoin was used as reference drugs. Abolition of hind limb tonic extensor component indicated the test compound's ability to inhibit MES induced seizure spread and was defined as protection.[8]

Subcutaneous Pentylenetetrazole (scPTZ) Seizure Test: Animals were divided into three groups each comprising of six animals. One group was used for studying the effect of PTZ, the second group for control and the third group to study effect with reference to the standard. The subcutaneous dose of pentylenetetrazole (85mg/kg) at which 95% of the animals showed convulsive reaction was determined by a dose-percent effect curve. The synthesized compounds were administered at the three graded doses, namely, 30, 100, and 300mg/Kg, intraperitoneally. At the anticipated time, PTZ was then administered subcutaneously in the posterior midline of mice. The absence of clonic spasm in half or more of the animals in the observed time periods indicates the compounds capacity to terminate the effect of pentylenetetrazole on seizure threshold. The results of test compound were compared with control and standard compounds.[9]

Neurotoxicity screening: The activity of the drugs interfering with motor coordination was checked by the rotarod test. The mice were trained to stay on an accelerating rotarod that rotates at 6 revolutions per minute. Trained animals were given *i.p.* injection of the test compounds in doses of 30, 100, 300 mg/kg. The rod diameter was 3.2cm. Neurotoxicity indicated by the inability of the animal to maintain equilibrium on the rotarod for at least 1 min in each of three trials. The dose, at which the animals were unable to grasp the rotarod, was determined. The number of animals in each group (N) =6; solvent used polyethylene glycol; dosed with 30,100 and 300 mg/kg were administered i.p. The figures

indicate the minimum dose whereby bioactivity was demonstrated in half or more mice. The (-) indicates an absence of activity at maximum dose administered (300mg/kg). In neurotoxicity screen, the figure indicates the dose at which no neurotoxicity was observed and the dash (-) indicates compounds are not tested.[10]

TABLE-2: Anticonvulsant Activity And Neurotoxicity Of N- (4-(Pyridin-4-Yloxy) Benzylidene) -4- [4-(Substituted) Phenyl]Semicarbazides (Pssd17-24).

Compounds	Intraperitoneal injection in rat[a]							
	MES screen (h)			scMET screen (h)			Neurotoxicity screen (h)	
	0.5	2.0	4.0	0.5	2.0	4.0	0.5	4.0
PSSD 17	--	--	--	--	--	--	--	--
PSSD 18	--	--	--	--	--	--	--	--
PSSD 19	--	--	--	--	--	--	--	--
PSSD 20	--	100[b]	--	--	--	--	--	300[c]
PSSD 21	--	--	300[b]	--	--	300[b]	--	--
PSSD 22	--	--	--	--	--	--	--	--
PSSD 23	--	--	--	--	--	--	--	--
PSSD 24	--	100[b]	300[b]	--	--	--	--	--
Phenytoin	30	30	30	--	--	--	100	100
Normal Saline	--	--	--	--	--	--	--	--

[a]Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the rat. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg).

[b] In the MES screen, compound PSSD 20 & 24 showed protection (2/3, 2 h) at a dose of 100 mg/kg. compound PSSD 21 & 24 showed protection (1/1, 4 h) at a dose of 300 mg/kg and scPTZ screen, compound PSSD 21 showed protection (1/1, 4 h) at a dose of 300 mg/kg.

[c] Minor toxicity (1/2, 4 h) at a dose of 300 mg/kg was observed with compound PSSD 20.

Computational Study

Molecular docking: The molecular docking study of designed molecules was carried out in order to assess the interaction and binding modes with target receptor/enzyme using Glide extra precision (XP) Maestro 10.1 Schrodinger, running on Windows i5 (intel core processor) operating system [Schrodinger, Version 10.1, 2016]. The 2D structure for synthesized compounds was generated and then converted to their respective 3D structures with use of Ligplot. The PDB file of the X-ray crystal structure of the human gamma-aminobutyric acid receptor, **$GABA_AR$-β3 homopentamer** (**PDB ID: 4COF**) was downloaded from RCSB Protein Data Bank. The protein was prepared using the protein preparation wizard and grid was generated for co-crystal ligand, **benzamidine** using receptor grid generation. The water residues beyond 5 Å were eliminated. The protein was optimized by assigning H-bonds and minimization at OPLS 2005 force field. The docked pose of ligands and their interactions were analyzed after the end of molecular docking. The docking scores of PSSD 17-24 and reference compound VPA (Valproic acid) are presented in Table 3.

TABLE 3: Docking Scores of Pssd 17-24 And Vpa.

Ligand	GScore	Dock Score	Lipophilic EvdW	Phob En	Phob EnHB	H-Bond	Electro	PiCat	Expos Penal	Rot Penal
PSSD 18	-5.60	-5.41	-3.80	-0.95	0.00	-0.61	-0.37	0.00	0.42	0.37
PSSD 24	-5.36	-5.18	-2.77	-0.97	-0.75	-0.16	-0.45	0.00	0.08	0.41
PSSD 17	-5.10	-4.91	-3.13	-0.99	0.00	-0.64	-0.46	0.00	0.48	0.44
PSSD 20	-5.07	-4.89	-3.00	-1.60	0.00	-0.12	-0.13	0.00	0.28	0.40
PSSD 21	-4.86	-4.49	-2.89	-0.80	-0.75	-0.16	-0.42	0.00	0.72	0.48
PSSD 22	-5.07	-4.29	-3.16	-0.13	0.00	0.00	-0.68	-1.50	1.55	0.44
PSSD 19	-5.03	-4.25	-2.65	0.00	0.00	0.00	-0.66	-1.67	1.11	0.30
PSSD 23	-4.35	-4.16	-2.19	-0.80	-0.75	-0.44	-0.69	0.00	0.37	0.44
VPA	-4.03	-4.03	-1.29	-1.40	-1.00	0.00	-0.35	0.00	0.00	0.60

*Note: Results are obtained in XP visualizer (application which shows results obtained by Extra precision docking) and the short forms used stands for

G-score : Glide Sore
PhobEnHB : Reward for hydrophobically packed H-bond,
PhobEn : Hydrophobic enclosure reward
Penalties : Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts
Expos Penal : Penalty for solvent-exposed ligand groups; cancels van der Waals terms.
Rot Penal : Rotatable bond penalty

Determination of ADME Properties: A computational study for determination of ADME properties of synthesized compounds was performed. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestine and blood-brain barrier (BBB) crossing.[11] TPSA, miLog P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five[12] were calculated using Molinspiration online property calculation toolkit.[13] The percentage of absorption (%ABS) was calculated using TPSA.[14] Results for various parameters are given in table 4.

Table 4: Pharmacokinetic Parameters Important For Good Oral Bioavailability Of N- (4-(Pyridin-4-Yloxy) Benzylidene) -4- [4-(Substituted) Phenyl]Semicarbazides (Pssd17-24)

Compounds	% ABS	TPSA (A^2)	n-ROTB	MW	MV	n-OHNH donors	n-ON acceptors	Lipinski's violations
Rule	--	--	--	<500	--	<5	<10	≤1
PSSD 17	82.9	75.61	5	232.36	298.75	2	6	0
PSSD18	82.9	75.61	5	366.81	312.27	2	6	0
PSSD 19	82.9	75.61	5	411.26	316.62	2	6	0
PSSD 20	82.9	75.61	5	350.35	303.67	2	6	0
PSSD 21	82.9	75.61	5	346.39	315.30	2	6	0
PSSD 22	82.9	75.61	6	360.42	298.75	2	6	0
PSSD 23	79.7	84.85	6	362.39	324.28	2	7	0
PSSD 24	67.1	121.44	6	377.36	322.07	2	9	0

Determination of Log P Value: Synthesized compounds showed dependence of biological activity on lipophillic character in a congeneric series. In particular, for drugs acting on central nervous system to be potent, they have to cross blood-brain barrier (BBB), thus potency has been correlated with optimum lipophilicity (Log P) near 2. In this study, we attempted to correlate the anticonvulsant activity of congeners with their calculated Log P value. The experimental Log P values were determined using the octanol-phosphate buffer method[15]. The data is showed in Table 5.

Table 5: Log P Value For N- (4-(Pyridin-4-Yloxy) Benzylidene) -4- [4-(Substituted) Phenyl] Semicarbazides (Pssd17-24).

Compounds	Experimental Log P	Experimental Log P (miLog Pa)
Rule	--	**≤5**
PSSD 17	3.24	3.88
PSSD18	3.80	4.56
PSSD 19	4.07	4.96
PSSD 20	3.40	4.04
PSSD 21	3.72	4.33
PSSD 22	4.14	4.79
PSSD 23	3.11	3.94
PSSD 24	3.44	3.84

RESULTS AND DISCUSSION

A new series of N-(4-(pyridin-4-yloxy) benzylidene)-4-[4-(substituted) phenyl]semicarbazides **PSSD 17-24** was synthesized in an effort to obtain the drugs with improved anticonvulsant activity. The target compounds **PSSD 17-24** have been synthesized by reaction of 4-(pyridinyl-4-oxy)–benzaldehyde and 4-(substituted) phenylsemicarbazides. All the synthesised compounds were characterized by analytical and spectroscopic methods. The compounds were evaluated for their anticonvulsant activity in MES and scMET models. In the MES screen, compound PSSD 20 & 24 showed protection (2/3, 2 h) at a dose of 100 mg/kg. compound PSSD 21 & 24 showed protection (1/1, 4 h) at a dose of 300 mg/kg and scMET screen, compound PSSD 21 showed protection (1/1, 4 h) at a dose of 300 mg/kg. A test used to identify compounds that elevate seizure threshold. Minor toxicity (1/2, 4 h) as motor impairment was observed at a dose of 300 mg/kg with compound **PSSD 20**. None of the other compounds showed neurotoxicity in the highest administered dose (300 mg/kg). The present study revealed that compounds **PSSD21** and **PSSD24** attenuated MES induced tonic seizures indicating anticonvulsant activity. The MES test activity of compounds showed their potential to prevent seizure spread and identified agents with profound activity against generalized tonic clonic seizures.

The molecular docking study of the synthesized molecules was carried out in order to assess the interaction and binding modes with target receptor/enzyme. All the compounds were found to

strongly bind by completely occupying the active sites (at the site of benzamine, co-crystal ligand of the protein as predicted by SiteMap) in the target protein ($GABA_AR$-β3). Only 01 compound found to have less docking score compared to valproic acid (VPA), the standard anti-convulsant drug and others outscored it. Almost all (08 compounds) compounds showed high docking scores than VPA, the standard anti-convulsant drug.

Among all the titled compound PSSD**18** was found to be most potent and have high docking score of (**-5.41**). The binding mode of compound **PSSD 17** assumes favorable orientation due to two hydrogen bonds compared with reference **VPA** (**-4.03** as Dock Score) **and** respectively as represented in 2D ligands interaction diagram (**Fig. 3**).

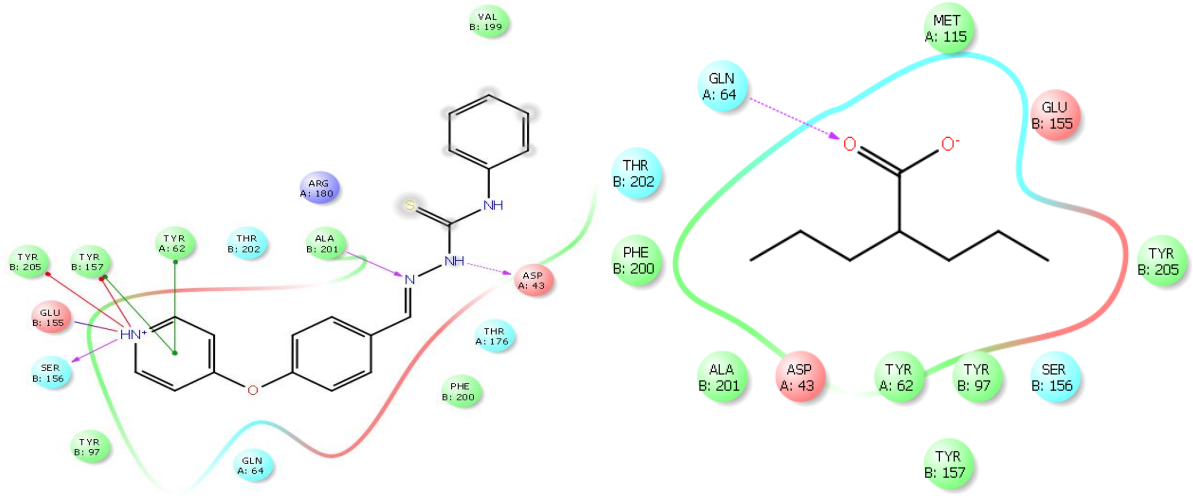


Fig. 3: 2D Ligplot diagrammed of comparative interactions of compound 17 (left) and VPA (right) at the binding site of $GABA_AR$-β3.

The docked pose (**Fig. 4**) of the compound **17** showed the hydrogen bond interaction by ALAB:201 of protein with N of C=N and GLUB:155 with NH of pyrimidine ring in the compound along with two pi-pi staking of pyrimidine ring with TYRA: 62 and TYRB: 157, a salt bridge of NH with GLUB: 155 and negative charges formed with TYRB: 205,157 of the same ring and that of VPA in which one carbonyl group forms H-bonds with GLYA 64. Our lead enjoys very good enthalpy reward (**-2.77**) compared to standard (**-1.29**) over other compounds and is having very less rotatable bond penalty of 0.41 whereas the standard compound VPA suffers with high rotatable bond penalty 0.60.

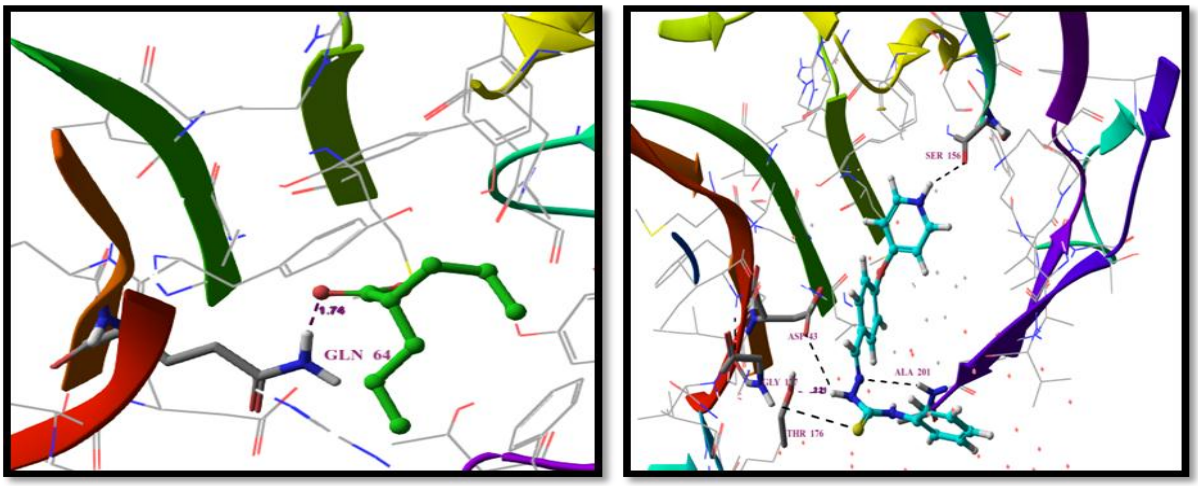


Fig. 4: 3D Docked pose of PSSD 17 (left) and VPA (right).

Whereas incase of surface exposed penalty, it suffers a little bit **(0.34)** which is of less importance in front of rotatable bond penalty as shown in (**Fig. 5**). High rewards and less penalties of ligand Compound **18** is the rationale for high docking score and potency towards the receptor. The docked pose of second most dock ranked compound **24** with dscore -5.18 has no surface penalty but rotatable bond penalty (0.41) which is comparable to the best one and slightly less hydrophobic enthalpy reward -2.77 possesing good hydrogen bond interactions with receptor.

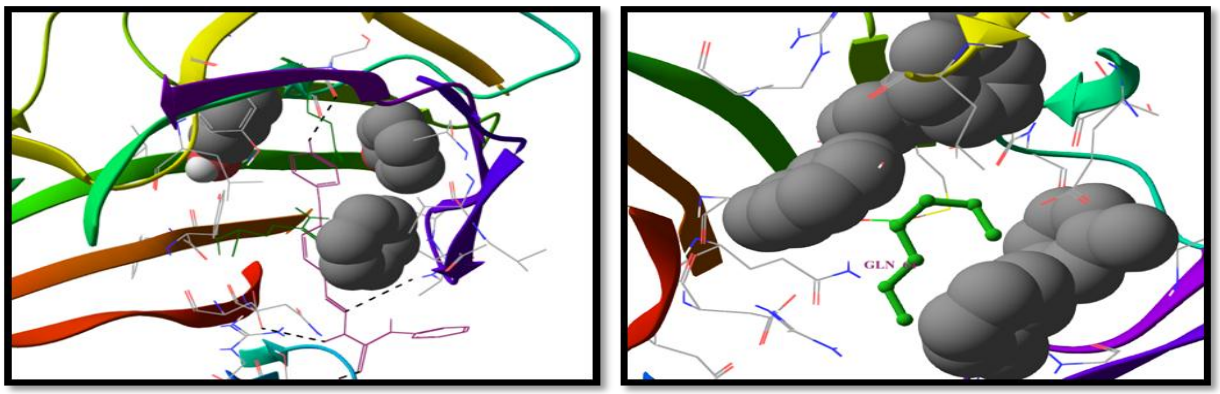


Fig. 4: 3D pose (in left) of PSSD 17 (represented in green) enjoying enthalpy reward (portion of the ligand denoted in ball & socket form). At right, less but completely, the standard VPA is rewarded.

A computational study for determination of ADME properties of the synthesized compounds was performed. All titled compounds exhibited a good %ABS ranging from 67.1 to 82.9%. All compounds violated Lipinski's parameters, making them potentially promising agents for treatment of epilepsy. It was attempted to correlate the anticonvulsant activity of congeners with their calculated Log P value. All compounds showed lipophilic character. As observed some of the experimental values were in good agreement with the theoretical values suggesting that these have an excellent potential for CNS activity.

CONCLUSION

The above study makes us to conclude that compounds N-(4-(pyridin-4-yloxy) benzylidene)-4-p-tolylsemicarbazide **(PSSD21)** and N-(4-(pyridin-4-yloxy)benzylidene)-4-(4-nitrophenyl)semicarbazide **(PSSD24)** showed anticonvulsant activity in MES model and thus have the potential to prevent seizure spread. N-(4-(pyridin-4-yloxy) benzylidene) -4- [4-(substituted) phenyl]semicarbazides (**PSSD17-24**) showed good docking with human gamma-aminobutyric acid receptor ($GABA_AR$-β3) and the total docking score is found to be a comparable to reference drug valproic acid. This shows that the probable mechanism of action of these compounds may be augmentation of GABAergic activity in the brain.

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