5.B. SYNTHESIS OF NOVEL SCHIFF BASES OF 1-(2-AMINOETHYL) PIPERIDINE-3-CARBOXYLIC ACID. [SERIES 2]

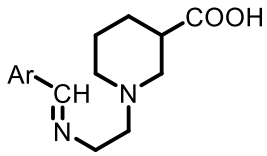
5.B.1. CHEMISTRY

The synthesis of the target compounds **5S2a-5S2y** was performed according to the reaction sequence outlined in **Scheme 4.1**. At the outset, Ethyl 1-(2-aminoethyl)piperidine-3-carboxylate **(3S2)** was synthesized by piperidine-3-carboxylic acid ethyl ester **(1S2)** and 2-bromoethylamine hydrobromide **(2S2)** by nucleophilic substitution (SN_2) reaction. Compound **3** which is an ester derivative of the parent cyclic amine (nipecotic acid) was used for protecting carboxyl group in the reaction. The ethyl ester group of compound **3S2** was further hydrolyzed in alcoholic alkaline solution to generate 1-(2-aminoethyl)piperidine-3-carboxylic acid **(4S2)**. The compounds **5S2a-5S2y** were synthesized by the nucleophilic addition of the amino group of compound **4S2** to the carbonyl group of corresponding aromatic aldehydes forming an unstable aminomethanol intermediate followed by dehydration in an acidic environment to generate an imine. Presence of an ethylene bridge in title compounds provides the necessary flexibility to the overall structure.

5.B.1.1. Physicochemical Characterization

DCM/methanol (9.5:0.5) was used as solvent system to determine the R_f values and to monitor the progress of the reaction. Subsequently, the percentage yield of the title compounds was calculated. To determine the melting points, open capillary tubes were used on a Stuart Melting Point apparatus (SMP10). The experimental Log P value of synthesised compounds was determined by octanol/water “shake-flask” method using Shimadzu UV/Visible spectrophotometer to establish lipophilicity. The general chemical structure and the physicochemical properties of the final compounds **(5S2a-5S2y)** are presented in **Table 5.12**.

Table 5.12. Chemical structures and physicochemical properties of compounds **5S2a-5S2y**



Comp.	Ar-group	R_f[a]	Log P[b]	Melting Point (°C)
5S2a	Phenyl	0.46	2.36	192-194
5S2b	2-Chlorophenyl	0.37	2.62	162-164
5S2c	4-Chlorophenyl	0.37	2.60	167-169
5S2d	2,3-Dichlorophenyl	0.30	3.16	181-183
5S2e	2,4-Dichlorophenyl	0.28	3.31	172-174
5S2f	2,3,6-Trichlorophenyl	0.26	3.48	187-189
5S2g	3-Fluorophenyl	0.43	2.42	158-160
5S2h	4-Fluorophenyl	0.42	2.46	166-168
5S2i	2,4-Difluorophenyl	0.41	2.51	177-179
5S2j	3,4,5-Trifluorophenyl	0.34	2.90	186-188
5S2k	3-Bromophenyl	0.29	3.28	155-157
5S2l	4-Bromophenyl	0.31	3.20	158-160
5S2m	2,6-Dibromophenyl	0.27	3.47	148-150
5S2n	4-Nitrophenyl	0.25	3.41	212-214
5S2o	2-Nitrophenyl	0.26	3.37	222-224
5S2p	2-Hydroxyphenyl	0.52	2.12	198-200
5S2q	4-Hydroxyphenyl	0.56	2.08	213-215
5S2r	3-Methoxyphenyl	0.49	2.27	204-206
5S2s	4-Methoxyphenyl	0.48	2.29	209-211
5S2t	3,4-Dimethoxyphenyl	0.51	2.18	224-226
5S2u	3,4,5-Trimethoxyphenyl	0.54	2.10	217-219
5S2v	4-Methylphenyl	0.35	2.82	177-179
5S2w	Napthyl	0.28	3.42	228-230
5S2x	4-Trifluoromethoxyphenyl	0.24	3.78	163-165
5S2y	3-Trifluoromethylphenyl	0.30	3.27	181-183

[a] R_f values are determined using DCM/methanol (9.5:0.5) as mobile phase. [b]Log P values of all the compounds were determined experimentally using shake flask method.

5.B.1.2. Spectral Characterization and Elemental Analysis

The structures of the synthesized compounds were ascertained by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The amino group in compound **3S2** was confirmed by the presence of asymmetric N-H stretching around 3366 cm^{-1} and symmetric N-H stretching around 3298 cm^{-1} respectively. The characteristic broad O-H stretching peak observed at around 3236 cm^{-1} in compound **4S2** depicted the presence of H-bonded O-H group. The absorption bands in the compounds **5S2a-5S2y** showed characteristic skeletal frequencies for C=O and C=N at 1729-1706 cm^{-1} and 1628-1612 cm^{-1}, respectively. A singlet varying from δ_H 11.6-11.18 ppm in ^{1}H NMR belonged to piperidine-CO-OH**,** and the singlet appeared at δ_H 9.02-8.10 ppm revealed the presence of N=CH- methaneimine protons. The chemical shifts from 181.2-172.9 ppm and 164.3-156.7 ppm in ^{13}C NMR confirmed the existence of C=O and C=N, respectively. Results of the elemental analysis were found within ± 0.4% of the theoretical values and were well within the limit.

Ethyl 1-(2-aminoethyl) piperidine-3-carboxylate (3S2)

FT-IR (KBr, cm^{-1}): 3366 (Asymmetric N-H), 3298 (Symmetric N-H), 2868 (C-H), 1748 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 4.21 (q, *J* = 6.0 Hz, 2H, CH_2 carboxylate), 3.25 (dd, *J* = 12.4, 7.8 Hz, 1H, piperidine), 2.96-2.90 (m, 1H, piperidine), 2.88-2.65 (m, 4H, NCH_2CH_2N), 2.56 (t, *J* = 4.9 Hz, 1H, piperidine), 2.52–2.41 (m, 1H, piperidine), 2.38–2.26 (m, 1H, piperidine), 1.91-1.64 (m, 3H, piperidine), 1.49 (2H, s, NH_2), 1.42 (3H, t, *J* = 6.0 Hz, CH_3 carboxylate). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 173.9, 61.7, 60.8, 56.8, 53.6, 44.6, 39.3, 24.9, 22.6, 14.7. Anal. calcd. for **$C_{10}H_{20}N_2O_2$:** C, 59.97; H, 10.07; N, 13.99. Found: C, 59.82; H, 10.04; N, 13.95.

1-(2-aminoethyl)piperidine-3-carboxylic acid (4S2)

FT-IR (KBr, cm^{-1}): 3363 (Asymmetric N-H), 3304 (Symmetric N-H), 3212 (O-H), 2860 (C-H), 1724 (C=O).^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.46 (s, 1H, COOH), 3.01 (dd, *J* = 12.4, 7.6 Hz, 1H, piperidine), 2.83-2.68 (m, 5H, piperidine H, NCH_2CH_2N), 2.56 (1H, t, *J* = 4.9 Hz, piperidine), 2.53–2.41 (1H, m, piperidine), 2.32–2.23 (1H, m, piperidine), 2.11 (1H, dd, *J* = 14.0, 6.1 Hz, piperidine), 1.77-1.59 (3H, m, piperidine), 1.45 (2H, s, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 182.9, 67.4, 62.0, 60.2, 47.5, 45.9, 31.7, 29.2. Anal. Calcd. for **$C_8H_{16}N_2O_2$:** C, 55.79; H, 9.36; N, 16.27. Found: C, 55.61; H, 9.34; N, 16.31.

1-(2-(benzylideneamino)ethyl)piperidine-3-carboxylic acid (5S2a)

Yield: 125 mg, 48.07%. FT-IR (KBr, cm^{-1}): 3220 (O-H), 3042 (Ar C-H), 2880 (C-H), 1712 (C=O), 1616 (C=N), 1586 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.32 (s, 1H, COOH), 8.42 (s, 1H, imine CH=N), 7.48-7.27 (m, 5H, phenyl), 3.67 (t, *J* = 7.4 Hz, 2H, $C{=}NCH_2$), 3.04 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.84 (t, *J* = 7.2 Hz, 2H, NCH_2), 2.78–2.67 (m, 2H, piperidine), 2.63 (m, 1H, piperidine), 2.32 (m, 1H, piperidine), 2.11 (m, 1H, piperidine), 1.77–1.64 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 181.2, 163.3, 135.7, 132.1, 128.8, 125.5, 63.1, 59.8, 59.5, 56.3, 49.1, 24.4, 20.4. Anal. Calcd. for **$C_{15}H_{20}N_2O_2$:** C, 69.20; H, 7.74; N, 10.76. Found: C, 69.39; H, 7.76; N, 10.74.

1-(2-((2-chlorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2b)

Yield: 153 mg, 52.04 %. FT-IR (KBr, cm^{-1}): 3236 (O-H), 3042 (Ar C-H), 2889 (C-H), 1716 (C=O), 1612 (C=N), 1585 (Ar C=C) 798 (C-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.42 (s, 1H, COOH), 8.32 (s, 1H, imine CH=N), 7.44-7.22 (m, 4H, phenyl), 3.58 (t, *J* = 7.1 Hz, 2H, $C{=}NCH_2$), 3.18 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.71 (t, *J* = 7.1 Hz, 2H, NCH_2), 2.68–2.57 (m, 2H, piperidine), 2.51–2.47 (m, 1H, piperidine), 2.41-2.28 (m, 1H, piperidine), 2.08-2.04 (m, 1H, piperidine), 1.76-1.59 (m, 3H,

piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 179.4, 161.9, 136.4, 134.2, 132.4, 130.7, 128.5, 124.3, 60.1, 57.8, 56.1, 55.7, 48.2, 24.6, 22.1. Anal. Calcd. for **$C_{15}H_{19}ClN_2O_2$**: C, 61.12; H, 6.50; N, 9.50. Found: C, 61.03; H, 6.49; N, 9.52.

1-(2-((4-chlorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2c)

Yield: 133 mg, 45.24 %. FT-IR (KBr, cm^{-1}): 3238 (O-H), 3041 (Ar C-H), 2878 (C-H), 1712 (C=O), 1614 (C=N), 1582 (Ar C=C) 812 (C-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.41 (s, 1H, COOH), 8.28 (s, 1H, imine CH=N), 7.51 (d, *J* = 7.5 Hz, 2H phenyl), 7.34 (d, *J* = 7.5 Hz, 2H, phenyl), 3.66 (t, *J* = 4.9 Hz, 2H, C=NCH_2), 2.98 (dd, *J* = 12.4, 7.8 Hz, 1H, piperidine), 2.76 (t, *J* = 4.9 Hz, 2H, NCH_2), 2.66–2.51 (m, 2H, piperidine), 2.47–2.31 (m, 1H, piperidine), 2.27-2.16 (m, 1H, piperidine), 2.09–1.95 (m, 1H, piperidine), 1.68-1.47 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 177.1, 160.9, 137.7, 135.1, 129.7, 128.9, 60.1, 58.8, 57.5, 56.7, 55.1, 25.4, 21.4. Anal. Calcd. for **$C_{15}H_{19}ClN_2O_2$**: C, 61.12; H, 6.50; N, 9.50. Found: C, 61.29; H, 6.52; N, 9.47.

1-(2-((2,3-dichlorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2d)

Yield: 168 mg, 51.22 %. FT-IR (KBr, cm^{-1}): 3233 (O-H), 3041 (Ar C-H), 2876 (C-H), 1725 (C=O), 1617 (C=N), 1590 (Ar C=C) 797, 788 (C-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.39 (s, 1H, COOH), 8.77 (s, 1H, imine CH=N), 7.58-7.20 (m, 3H, phenyl), 4.00 (t, *J* = 4.8 Hz, 2H, C=NCH_2), 3.23 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 3.05–2.96 (m, 2H, NCH_2), 2.86–2.79 (m, 2H, piperidine), 2.65 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.52–2.47 (m, 1H, piperidine), 2.37-2.31 (m, 1H, piperidine), 1.92-1.83 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 173.68, 160.7, 132.4, 131.5, 131.1, 130.9, 125.5, 123.8, 56.8, 52.7, 52.6, 51.0, 38.2, 22.2, 19.9. Anal. Calcd. for **$C_{15}H_{18}Cl_2N_2O_2$**: C, 54.72; H, 5.51; N, 8.51. Found: C, 54.89; H, 5.52; N, 8.50.

1-(2-((2,4-dichlorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2e)

Yield: 148 mg, 45.12 %. FT-IR (KBr, cm^{-1}): 3230 (O-H), 3040 (Ar C-H), 2880 (C-H), 1718 (C=O), 1619 (C=N), 1591 (Ar C=C) 826, 798 (C-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.51 (s, 1H, COOH), 8.31 (s, 1H, imine CH=N), 7.41-7.21 (m, 3H, phenyl), 3.54 (t, *J* = 7.41Hz, 2H, C=NCH_2), 3.18 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.68 (t, *J* = 7.1 Hz, 2H, NCH_2), 2.66–2.55 (m, 2H, piperidine), 2.49 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.40–2.0 (m, 1H, piperidine), 2.13-2.02 (m, 1H, piperidine), 1.78-1.59 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 178.4, 161.7, 137.8, 135.5, 132.1, 130.9, 130.3, 128.8, 59.5, 55.5, 55.3, 53.7, 40.99, 24.2, 21.3. Anal. Calcd. for **$C_{15}H_{18}Cl_2N_2O_2$**: C, 54.72; H, 5.51; N, 8.51. Found: C, 54.85; H, 5.53; N, 8.53.

1-(2-((2,3,6-trichlorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2f)

Yield: 172 mg, 47.51 %. FT-IR (KBr, cm^{-1}): 3226 (O-H), 3066 (Ar C-H), 2871 (C-H), 1727 (C=O), 1611 (C=N), 1586 (Ar C=C) 811, 787, 778 (C-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.21 (s, 1H, COOH), 8.40 (s, 1H, imine CH=N), 7.23-7.15 (m, 2H, phenyl), 3.50 (t, *J* = 5.1 Hz, 2H, C=NCH_2), 2.97 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.33 (t, *J* = 7.1 Hz, 2H, NCH_2), 2.66–2.55 (m, 2H, piperidine), 2.49 (dd, *J*= 12.3, 7.7 Hz, 1H, piperidine), 2.35–2.30 (m, 1H, piperidine), 2.14-1.95 (m, 1H, piperidine), 1.77-1.47 (m, 3H piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 174.4, 159.7, 136.8, 134.5, 132.8, 131.9, 131.3, 129.9, 59.1, 57.5, 56.2, 54.1, 40.4, 25.2, 21.9. Anal. Calcd. for **$C_{15}H_{17}Cl_3N_2O_2$:** C, 49.54; H, 4.71; N, 7.70. Found: C, 49.40; H, 4.70; N, 7.72.

1-(2-((3-fluorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2g)

Yield: 144 mg, 51.79 %. FT-IR (KBr, cm^{-1}): 3229 (O-H), 3049 (Ar C-H), 2890 (C-H), 1721 (C=O), 1625 (C=N), 1594 (Ar C=C) 1278 (C-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.51 (s, 1H, COOH), 8.30 (s, 1H, imine CH=N), 7.39-7.03 (m, 4H, phenyl),

3.70 (t, J = 5.0 Hz, 2H, C=NCH$_2$), 2.99 (dd, J = 12.4, 7.6 Hz, 1H, piperidine), 2.77 (t, J = 5.0 Hz, 2H, NCH$_2$), 2.68–2.54 (m, 2H, piperidine), 2.46-2.29 (m, 1H, piperidine), 2.23–2.19 (m, 1H, piperidine), 2.04-1.97 (m, 1H, piperidine), 1.71-1.50 (m, 3H, piperidine). ^{13}C NMR (125 MHz, CDCl$_3$) δ ppm: 177.4, 164.3 (d, $J_{C,F}$ = 259.4 Hz, phenyl), 162.1 (d, $J_{C,F}$ = 4.5 Hz, imine CH), 139.8 (d, $J_{C,F}$ = 7.1 Hz, phenyl), 129.5 (d, $J_{C,F}$ = 7.1 Hz, phenyl), 124.8 (d, $J_{C,F}$ = 4.1 Hz, phenyl), 118.4 (d, $J_{C,F}$ = 26.2 Hz, phenyl), 115.5 (d, $J_{C,F}$ = 27.3 Hz, phenyl), 59.4, 55.5, 55.1, 53.4, 40.7, 25.9, 22.9. Anal. Calcd. for **C$_{15}$H$_{19}$FN$_2$O$_2$**: C, 64.73; H, 6.88; N, 10.07. Found: C, 64.94; H, 6.89; N, 10.03.

1-(2-((4-fluorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2h)

Yield: 122 mg, 43.88 %. FT-IR (KBr, cm^{-1}): 3228 (O-H), 3039 (Ar C-H), 2887 (C-H), 1729 (C=O), 1620 (C=N), 1598 (Ar C=C) 1318 (C-F). ^{1}H NMR (500 MHz, CDCl$_3$) δ ppm: 11.18 (s, 1H, COOH), 8.27 (s, 1H, imine CH=N), 7.64 (d, J = 7.5 Hz, 2H, phenyl), 7.06 (d, J = 7.5 Hz, 2H, phenyl), 3.59 (t, J = 5.3 Hz, 2H, C=NCH$_2$), 3.05 (dd, J = 12.3, 7.7 Hz, 1H, piperidine), 2.90 (t, J = 5.3 Hz, 2H, NCH$_2$), 2.83–2.69 (m, 2H, piperidine), 2.68–2.49 (m, 1H, piperidine), 2.33-2.17 (m, 1H, piperidine), 2.16–1.91 (m, 1H, piperidine), 1.86-1.42 (m, 3H, piperidine). ^{13}C NMR (125 MHz, CDCl$_3$) δ ppm: 176.4, 163.3 (d, $J_{C,F}$ = 248.8 Hz, phenyl), 162.6 (imine CH,), 133.4 (d, $J_{C,F}$ = 3.8 Hz, phenyl), 131.7 (d, $J_{C,F}$ = 8.1 Hz, 2×CH, phenyl), 115.6 (d, $J_{C,F}$ = 24.8 Hz, 2×CH, phenyl), 60.1, 55.8, 55.2, 53.7, 40.9, 25.9, 22.6. Anal. Calcd. for **C$_{15}$H$_{19}$FN$_2$O$_2$**: C, 64.73; H, 6.88; N, 10.07. Found: C, 64.89; H, 6.86; N, 10.09.

1-(2-((2,4-difluorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2i)

Yield: 153 mg, 51.69 %. FT-IR (KBr, cm^{-1}): 3242 (O-H), 3036 (Ar C-H), 2872 (C-H), 1716 (C=O), 1612 (C=N), 1588 (Ar C=C) 1319, 1292 (C-F). ^{1}H NMR (500 MHz, CDCl$_3$) δ ppm: 11.21 (s, 1H, COOH), 8.29 (s, 1H, imine CH=N), 7.53-6.80 (m, 3H, phenyl), 3.53 (t, J = 5.3 Hz, 2H, C=NCH$_2$), 3.06–2.81 (m, 1H, piperidine), 2.74 (t, J

= 7.1 Hz, 2H, NCH_2), 2.64–2.57 (m, 2H, piperidine), 2.34–2.29 (m, 1H, piperidine), 2.21–2.08 (m, 1H, piperidine), 2.05–1.99 (m, 1H, piperidine), 1.72-1.61 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 175.8, 165.3 (dd, $J_{C,F}$ = 190.1, 12.7 Hz, phenyl), 163.4 (dd, $J_{C,F}$ = 188.3, 12.8 Hz, phenyl), 160.6 (d, $J_{C,F}$ = 6.7, imine CH), 133.7 (dd, $J_{C,F}$ = 11.1, 3.5 Hz, phenyl), 121.7 (dd, $J_{C,F}$ = 8.3, 3.4 Hz, phenyl), 113.6 (dd, $J_{C,F}$ = 22.0, 3.6 Hz, phenyl), 103.7 (t, $J_{C,F}$ = 24.9 Hz, phenyl), 60.2, 55.7, 54.8, 53.8, 41.3, 24.9, 22.1. Anal. Calcd. for **$C_{15}H_{18}F_2N_2O_2$**: C, 60.80; H, 6.12; N, 9.45. Found: C, 60.66; H, 6.11; N, 9.47.

1-(2-((3,4,5-trifluorobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2j)

Yield: 168 mg, 53.50 %. FT-IR (KBr, cm^{-1}): 3238 (O-H), 3038 (Ar C-H), 2868 (C-H), 1710 (C=O), 1616 (C=N), 1588 (Ar C=C) 1306, 1292, 1286 (C-F). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.24 (s, 1H, COOH), 8.29 (s, 1H, imine CH=N), 7.10 (dd, *J* = 8.0, 4.9 Hz, 2H, phenyl), 3.83 (t, *J* = 4.7 Hz, 2H, $C=NCH_2$), 3.47 (dd, *J* = 12.4, 7.9 Hz, 1H, piperidine), 2.90 (t, *J* = 4.7 Hz, 2H, NCH_2), 2.77–2.54 (m, 2H, piperidine), 2.26 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.18–2.11 (m, 1H, piperidine), 2.14–2.08 (m, 1H, piperidine), 1.73-1.37 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 174.4, 161.4 (t, $J_{C,F}$ = 4.1 Hz, imine CH), 149.8 (ddd, $J_{C,F}$ = 223.8, 24.8, 7.2 Hz, phenyl), 140.5 (dt, $J_{C,F}$ = 49.8, 28.2 Hz, phenyl), 135.8 (td, $J_{C,F}$ = 5.9, 3.9 Hz, phenyl), 111.9 (ddd, $J_{C,F}$ = 27.9, 7.2, 4.1 Hz, phenyl), 59.2, 56.4, 54.6, 40.1, 25.8, 21.4. Anal. Calcd. for **$C_{15}H_{17}F_3N_2O_2$**: C, 57.32; H, 5.45; N, 8.91. Found: C, 57.21; H, 5.47; N, 8.89.

1-(2-((3-bromobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2k)

Yield: 166 mg, 49.11 %. FT-IR (KBr, cm^{-1}): 3242 (O-H), 3028 (Ar C-H), 2868 (C-H), 1719 (C=O), 1622 (C=N), 1588 (Ar C=C), 628 (C-Br). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.52 (s, 1H, COOH), 8.28 (s, 1H, imine CH=N), 7.90-7.19 (m, 4H, phenyl), 3.59 (t, *J* = 6.2 Hz, 2H, $C=NCH_2$), 3.10 (dd, *J* = 12.2, 7.7 Hz, 1H, piperidine), 2.81 (t,

J = 6.2 Hz, 2H, NCH_2), 2.74–2.70 (m, 2H, piperidine), 2.52–2.48 (m, 1H, piperidine), 2.34-2.29 (m, 1H, piperidine), 2.24-2.09 (m, 1H, piperidine), 1.84-1.62 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.5, 162.9, 138.1, 134.3, 131.4, 130.6, 126.5, 121.8, 69.2, 57.9, 56.2, 55.4, 47.3, 24.4, 21.9. Anal. Calcd. for **$C_{15}H_{19}BrN_2O_2$**: C, 53.11; H, 5.65; N, 8.26. Found: C, 53.30; H, 5.65; N, 8.24.

1-(2-((4-bromobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2l)

Yield: 143 mg, 42.31 %. FT-IR (KBr, cm^{-1}): 3258 (O-H), 3053 (Ar C-H), 2898 (C-H), 1724 (C=O), 1619 (C=N), 1602 (Ar C=C), 639 (C-Br). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.42 (s, 1H, COOH), 8.26 (s, 1H, imine CH=N), 7.49 (d, *J* = 7.5 Hz, 2H, phenyl), 7.45 (d, *J* = 7.5 Hz, 2H, phenyl), 3.57 (t, *J* = 5.3 Hz, 2H, $C=NCH_2$), 3.05 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.90 (t, *J* = 5.3 Hz, 2H, NCH_2), 2.66–2.50 (m, 2H, piperidine), 2.37 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.26-2.21 (m, 1H, piperidine), 2.15–1.96 (m, 1H, piperidine), 1.74-1.54 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 177.1, 160.9, 137.7, 135.1, 129.7, 128.9, 60.1, 58.8, 57.5, 56.7, 55.1, 25.4, 21.4. Anal. Calcd. for **$C_{15}H_{19}BrN_2O_2$**: C, 53.11; H, 5.65; N, 8.26. Found: C, 53.02; H, 5.64; N, 8.27.

1-(2-((2,6-dibromobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2m)

Yield: 196 mg, 47.12 %. FT-IR (KBr, cm^{-1}): 3255 (O-H), 3059 (Ar C-H), 2865 (C-H), 1718 (C=O), 1622 (C=N), 1598 (Ar C=C), 616 (C-Br). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.51 (s, 1H, COOH), 8.38 (s, 1H, imine CH=N), 7.44-7.07 (m, 3H, phenyl), 3.51 (t, *J* = 5.1 Hz, 2H, $C=NCH_2$), 3.05 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.96 (t, J = 5.0 Hz, 2H, NCH_2), 2.80–2.55 (m, 2H, piperidine), 2.34 (dd, J = 12.4, 7.8 Hz, 1H, piperidine), 2.31–2.25 (m, 1H, piperidine), 2.10-2.06 (m, 1H, piperidine), 1.80-1.51 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 173.4, 156.7, 137.9, 132.9, 131.3, 124.9, 59.7, 55.9, 55.6, 54.7, 40.1, 24.8, 22.6. Anal. Calcd. for **$C_{15}H_{18}Br_2N_2O_2$**: C, 43.09; H, 4.34; N, 6.70. Found: C, 43.22; H, 4.35; N, 6.68.

1-(2-((4-nitrobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2n)

Yield: 162 mg, 53.11 %. FT-IR (KBr, cm^{-1}): 3233 (O-H), 3062 (Ar C-H), 2879 (C-H), 1706 (C=O), 1612 (C=N), 1596 (Ar C=C), 1562 (N=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.28 (s, 1H, COOH), 8.56 (s, 1H, imine CH=N), 8.28 (d, *J* = 7.5 Hz, 2H, phenyl), 8.11 (d, J = 7.5 Hz, 2H, phenyl), 3.66 (t, *J* =5.3 Hz, 2H, C=NCH_2), 2.95 (dd, *J* = 12.4, 7.9 Hz, 1H, piperidine), 2.71 (t, *J* = 6.2 Hz, 2H, NCH_2), 2.64–2.47 (m, 2H, piperidine), 2.39 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.34-2.18 (m, 1H, piperidine), 2.14–1.92 (m, 1H, piperidine), 1.87-1.32 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.3, 161.2, 151.1, 140.5, 130.7, 124.6, 61.3, 57.2, 55.5, 54.7, 41.1, 26.4, 21.8. Anal. Calcd. for **$C_{15}H_{19}N_3O_4$**: C, 59.01; H, 6.27; N, 13.76. Found: C, 59.21; H, 6.25; N, 13.80.

1-(2-((2-nitrobenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2o)

Yield: 178 mg, 58.36 %. FT-IR (KBr, cm^{-1}): 3242 (O-H), 3055 (Ar C-H), 2869 (C-H), 1721 (C=O), 1619 (C=N), 1585 (Ar C=C), 1545 (N=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.42 (s, 1H, COOH), 8.54 (s, 1H, imine CH=N), 8.09-7.39 (m, 4H, phenyl), 3.65 (t, *J* = 6.2 Hz, 2H, C=NCH_2), 2.98 (dd, *J* = 12.2, 7.7 Hz, 1H, piperidine), 2.79 (t, *J* = 6.2 Hz, 2H, NCH_2), 2.68–2.63 (m, 2H, piperidine), 2.57–2.41 (m, 1H, piperidine), 2.37-2.24 (m, 1H, piperidine), 2.26-2.11 (m, 1H, piperidine), 1.74-1.51 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 172.9, 160.1, 148.2, 133.7, 132.6, 131.7, 128.3, 124.9, 67.2, 58.4, 56.9, 55.2, 40.3, 24.1, 22.5. Anal. Calcd. for **$C_{15}H_{19}N_3O_4$**: C, 59.01; H, 6.27; N, 13.76. Found: C, 59.18; H, 6.28; N, 13.73.

1-(2-((2-hydroxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2p)

Yield: 161 mg, 58.33 %. FT-IR (KBr, cm^{-1}): 3368 (sharp, O-H), 3238 (broad, O-H), 3039 (Ar C-H), 2869 (C-H), 1717 (C=O), 1616 (C=N), 1588 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.37 (s, 1H, COOH), 9.02 (1H, s, Phenyl OH), 8.23 (s, 1H, imine CH=N), 7.45-6.78 (m, 4H, phenyl), 3.62 (t, *J* = 6.8 Hz, 2H, C=NCH_2), 3.22

(dd, *J* = 12.3, 7.6 Hz, 1H, piperidine), 2.81 (t, *J* = 6.8 Hz, 2H, NCH_2), 2.75–2.61 (m, 2H, piperidine), 2.66–2.54 (m, 1H, piperidine), 2.39-2.32 (m, 1H, piperidine), 2.13-2.09 (m, 1H, piperidine), 1.75-1.65 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.4, 164.1, 160.8, 133.1, 129.3, 121.7, 120.3, 117.9, 62.2, 59.4, 57.7, 55.7, 40.9, 24.4, 22.1. Anal. Calcd. for **$C_{15}H_{20}N_2O_3$**: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.01; H, 7.28; N, 10.12.

1-(2-((4-hydroxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2q)

Yield: 146 mg, 52.89 %. FT-IR (KBr, cm^{-1}): 3376 (sharp, O-H), 3239 (broad, O-H), 3068 (Ar C-H), 2862 (C-H), 1724 (C=O), 1628 (C=N), 1592 (Ar C=C). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.28 (s, 1H, COOH), 8.90 (s, 1H, Phenyl OH), 8.25 (s, 1H, imine CH=N), 7.40 (d, *J* = 7.5 Hz, 2H, phenyl), 6.81 (d, *J* = 7.5 Hz, 2H, phenyl), 3.69 (t, *J* = 5.0 Hz, 2H, C=NCH_2), 2.98 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.77 (t, *J* = 5.0 Hz, 2H, NCH_2), 2.49–2.27 (m, 2H, piperidine), 2.29 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.10–1.93 (m, 1H, piperidine), 1.68–1.47 (m, 1H, piperidine), 1.46-1.32 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.4, 161.6, 159.5, 131.3, 127.5, 116.6, 60.5, 57.4, 55.7, 52.9, 40.8, 23.4, 21.5. Anal. Calcd. for **$C_{15}H_{20}N_2O_3$**: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.42; H, 7.31; N, 10.17.

1-(2-((3-methoxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2r)

Yield: 128 mg, 44.14 %. FT-IR (KBr, cm^{-1}): 3237 (O-H), 3049 (Ar C-H), 2885 (C-H), 1723 (C=O), 1618 (C=N), 1590 (Ar C=C). ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.21 (s, 1H, COOH), 8.27 (s, 1H, imine CH=N), 7.34-6.87 (m, 4H, phenyl), 3.81 (s, 3H, OCH_3), 3.72 (t, *J* = 5.4 Hz, 2H, C=NCH_2), 2.98 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.84 (t, *J* = 5.4 Hz, 2H, NCH_2), 2.74–2.70 (m, 2H, piperidine), 2.67–2.48 (m, 1H, piperidine), 2.45-2.28 (m, 1H, piperidine), 2.20-2.08 (m, 1H piperidine), 1.79-1.56 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 175.4, 162.7, 160.1, 139.3, 129.3, 122.6, 117.5, 112.1, 59.2, 56.8, 55.9, 55.2, 53.5, 40.8, 24.1, 22.3.

Anal. Calcd. for **$C_{16}H_{22}N_2O_3$**: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.40; H, 7.64; N, 9.63.

1-(2-((4-methoxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2s)

Yield: 122 mg, 42.06 %. FT-IR (KBr, cm^{-1}): 3229 (O-H), 3040 (Ar C-H), 2875 (C-H), 1716 (C=O), 1622 (C=N), 1590 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.36 (s, 1H, COOH), 8.24 (s, 1H, imine CH=N), 7.53 (d, *J* = 7.5 Hz, 2H, phenyl), 6.93 (d, *J* = 7.5 Hz, 2H, phenyl), 3.83 (s, 3H, OCH_3), 3.68 (t, *J* =4.9 Hz, 2H, C=NCH_2), 2.99 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.76 (t, *J* = 4.9 Hz, 2H, NCH_2), 2.64–2.52 (m, 2H, piperidine), 2.40 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.24–2.15 (m, 1H, piperidine), 2.05–1.93 (m, 1H, piperidine), 1.76-1.46 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 175.4, 162.3, 161.3, 131.7, 128.4, 114.5, 60.2, 57.8, 56.9, 55.2, 52.1, 40.9, 24.4, 22.8. Anal. Calcd. for **$C_{16}H_{22}N_2O_3$**: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.29; H, 7.66; N, 9.68.

1-(2-((3,4-dimethoxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2t)

Yield: 157 mg, 49.66 %. FT-IR (KBr, cm^{-1}): 3228 (O-H), 3034 (Ar C-H), 2865 (C-H), 1716 (C=O), 1618 (C=N), 1585 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.37 (s, 1H, COOH), 8.19 (s, 1H, imine CH=N), 7.26-6.89 (m, 3H, phenyl), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.71 (t, *J* = 4.9 Hz, 2H, C=NCH_2), 2.97 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.74 (t, *J* = 4.9 Hz, 2H, NCH_2), 2.63–2.54 (m, 2H, piperidine), 2.38 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.25–2.16 (m, 1H, piperidine), 2.02–1.95 (m, 1H, piperidine), 1.66-1.46 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 175.7, 162.5, 152.7, 149.5, 131.4, 124.6, 113.4, 112.8, 60.7, 57.8, 56.7, 55.7, 52.5, 40.7, 24.2, 22.1. Anal. Calcd. for **$C_{17}H_{24}N_2O_4$**: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.51; H, 7.53; N, 8.76.

1-(2-((3,4,5-trimethoxybenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2u)

Yield: 175 mg, 50.00 %. FT-IR (KBr, cm^{-1}): 3219 (O-H), 3040 (Ar C-H), 2873 (C-H), 1711 (C=O), 1620 (C=N), 1581 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.39 (s, 1H, COOH), 8.24 (s, 1H, imine CH=N), 6.87 (s, 2H, phenyl), 3.83 (s, 6H, 2 X OCH_3), 3.79 (t, *J* = 4.9 Hz, 2H, C=NCH_2), 3.68 (s, 3H, OCH_3), 3.51 (dd, *J* = 12.5, 7.9 Hz, 1H, piperidine), 2.84 (t, *J* = 4.9 Hz, 2H, NCH_2), 2.69–2.53 (m, 2H, piperidine), 2.28 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.25–2.09 (m, 1H, piperidine), 1.76–1.58 (m, 1H, piperidine), 1.52-1.39 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 177.4, 161.6, 154.3, 140.6, 135.7, 108.9, 59.8, 56.4, 57.4, 56.7, 54.2, 40.8, 25.2, 21.1. Anal. Calcd. for **$C_{18}H_{26}N_2O_5$**: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.85; H, 7.46; N, 7.80.

1-(2-((4-methylbenzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2v)

Yield: 123 mg, 44.89 %. FT-IR (KBr, cm^{-1}): 3229 (O-H), 3049 (Ar C-H), 2872 (C-H), 1718 (C=O), 1622 (C=N), 1591 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.47 (s, 1H, COOH), 8.18 (s, 1H, imine CH=N), 7.51 (d, *J* = 7.5 Hz, 2H, phenyl), 7.19 (d, *J* = 7.5 Hz, 2H, phenyl), 3.69 (t, *J* = 4.9 Hz, 2H, C=NCH_2), 2.99 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.76 (t, *J* = 4.9 Hz, 2H, NCH_2), 2.70–2.52 (m, 2H, piperidine), 2.42 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.36 (s, 3H, CH_3), 2.22–2.17 (m, 1H, piperidine), 2.03–1.96 (m, 1H, piperidine), 1.67-1.49 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 174.4, 161.24, 142.5, 132.4, 129.5, 128.4, 59.6, 55.7, 55.5, 53.78, 41.1, 24.9, 23.6, 20.8. Anal. Calcd. for **$C_{16}H_{22}N_2O_2$:** C, 70.04; H, 8.08; N, 10.21. Found: 69.79; H, 8.10; N, 10.18.

1-(2-((naphthalen-2-ylmethylene)amino)ethyl)piperidine-3-carboxylic acid (5S2w)

Yield: 149 mg, 48.06 %. FT-IR (KBr, cm^{-1}): 3239 (O-H), 3039 (Ar C-H), 2881 (C-H), 1710 (C=O), 1618 (C=N), 1588 (Ar C=C). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.60 (s, 1H, COOH), 8.10 (s, 1H, imine CH=N), 8.08-7.26 (m, 7H, naphthyl H), 3.89 (t, *J* = 4.8 Hz, 2H, C=NCH_2), 3.08 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.86 (t, 2H, NCH_2), 2.74–2.57 (m, 2H, piperidine), 2.47 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.38–2.30 (m, 1H, piperidine), 2.25–2.12 (m, 1H, piperidine), 1.87-1.60 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.4, 162.4, 134.9, 133.8, 133.5, 129.7, 129.3, 129.0, 128.4, 126.9, 126.4, 59.5, 55.4, 55.3, 53.7, 40.9, 24.9, 22.6. Anal. Calcd. for **$C_{19}H_{22}N_2O_2$**: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.77; H, 7.13; N, 9.05.

1-(2-((4-(trifluoromethoxy)benzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2x)

Yield: 187 mg, 54.36 %. FT-IR (KBr, cm^{-1}): 3237 (O-H), 3046 (Ar C-H), 2881 (C-H), 1712 (C=O), 1619 (C=N), 1579 (Ar C=C), 1356 (C-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 11.55 (s, 1H, COOH), 8.27 (s, 1H, imine CH=N), 7.55 (d, *J* = 7.5 Hz, 2H, phenyl), 6.97 (d, *J* = 7.5 Hz, 2H, phenyl), 3.60 (t, *J* = 5.3 Hz, 2H, C=NCH_2), 3.05 (dd, *J* = 12.3, 7.7 Hz, 1H, piperidine), 2.89 (t, *J* = 5.3 Hz, 2H, NCH_2), 2.77-2.67 (m, 2H, piperidine), 2.66 (dd, *J* = 12.5, 7.7 Hz, 1H, piperidine), 2.28–2.20 (m, 1H, piperidine), 2.11–1.98 (m, 1H, piperidine), 1.79-1.57 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 173.4, 161.8, 152.6, 134.8, 129.4, 121.8 (q, $J_{C,F}$ = 325.1, 193.1 Hz, OCF_3), 121.2, 60.1, 57.5, 56.4, 55.8, 52.6, 40.4, 23.4, 21.5. Anal. Calcd. for **$C_{16}H_{19}F_3N_2O_3$**: C, 55.81; H, 5.56; N, 8.14. Found: C, 55.63; H, 5.57; N, 8.11.

1-(2-((3-(trifluoromethyl)benzylidene)amino)ethyl)piperidine-3-carboxylic acid (5S2y)

Yield: 176 mg, 53.66 %. FT-IR (KBr, cm^{-1}): 3243 (O-H), 3068 (Ar C-H), 2878 (C-H), 1719 (C=O), 1616 (C=N), 1588 (Ar C=C), 1346 (C-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ

ppm: 11.33 (s, 1H, COOH), 8.46 (s, 1H, imine CH=N), 7.89-7.25 (m, 4H, phenyl), 3.77 (t, J = 5.1 Hz, 2H, C=NCH_2), 3.06 (dd, J = 12.5, 7.7 Hz, 1H, piperidine), 2.87 (t, J = 5.0 Hz, 1H, NCH_2), 2.81–2.65 (m, 3H, NCH_2, piperidine), 2.53-2.29 (m, 2H, piperidine), 2.23-2.07 (m, 1H, piperidine), 1.88-1.64 (m, 3H, piperidine). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 176.4, 162.1, 137.0, 129.75, 129.73, 129.6, 129.0 (q, $J_{C,F}$ = 33.2 Hz, phenyl C-3), 124.1, 123.6 (q, $J_{C,F}$ = 272.5 Hz, CF_3), 59.5, 55.4, 55.3, 53.7, 40.9, 24.9, 22.6. Anal. Calcd. for **$C_{16}H_{19}F_3N_2O_2$**: C, 58.53; H, 5.83; N, 8.53. Found: C, 58.69; H, 5.81; N, 8.51.

5.B.2. BIOLOGICAL ACTIVITY

5.B.2.1. *In vitro* PAMPA-BBB assay

Determination of brain permeability is very crucial for antiepileptic drugs to reach the target site and elicit its effect. PAMPA-BBB is a technique to observe the BBB permeation of drug molecules. In the current work, the permeability of the synthesized compounds was evaluated by PAMPA-BBB as per the reported procedure of Di et al. [Di *et al*., 2003]. This system is a prototype of BBB that measures the effective permeability (P_e, cm/s) of an artificial lipid membrane and thereby predicts the rate of trans-cellular passive diffusion of drugs across the BBB. A plot of experimentally obtained permeability [$P_{e(Exp)}$] versus permeability reported in the literature [$P_{e(Ref)}$] provided a good linear correlation $P_{e(Exp)} = 1.308\ P_{e(Ref)} - 0.8394$ (R^2=0.9317). Using this equation, we have calculated the cut-off limits for determining the BBB permeability of the test compounds. The values of $P_{e(Ref)}$ were taken from the limits established by Di *et al*. The findings suggested that the compounds **5S2d, 5S2f, 5S2j, 5S2l, 5S2m, 5S2n, 5S2w, 5S2x,** and **5S2y** exhibited considerable permeability across BBB. **5w** was more permeable (P_e = 8.93) than the standard tiagabine (P_e = 7.96) **(Table 5.13).**

Table 5.13. Permeability analysis using PAMPA-BBB assay (**Series 2**)

Comp.	PAMPA- BBB permeability[a] $P_{e(exp)}$ (10^{-6} cm s^{-1})	PAMPA- BBB Prediction (CNS+[b], CNS-[c], CNS±[d])
Validation of the model by nine commercial drugs		
Verapamil	16.00	CNS+
Diazepam	16.00	CNS+
Progesterone	9.30	CNS+
Atenolol	0.80	CNS-
Dopamine	0.20	CNS-
Lomefloxacin	1.10	CNS-
Alprazolam	5.40	CNS+
Chlorpromazine	6.50	CNS+
Oxazepam	10.00	CNS+
Evaluation of *Pe* (10^{-6} cm s^{-1}) for the test compounds and standard		
Tiagabine (standard)	7.86	CNS+
5S2a	3.32	CNS±
5S2b	3.69	CNS±
5S2c	4.18	CNS±
5S2d	6.52	CNS+
5S2e	4.23	CNS±
5S2f	5.69	CNS+
5S2g	1.66	CNS-
5S2h	1.98	CNS±
5S2i	4.18	CNS±
5S2j	5.53	CNS+
5S2k	4.14	CNS±
5S2l	7.24	CNS+
5S2m	4.76	CNS+
5S2n	4.84	CNS+
5S2o	3.12	CNS±
5S2p	3.15	CNS±
5S2q	2.75	CNS±
5S2r	2.43	CNS±
5S2s	3.02	CNS±

5S2t	1.19	CNS-
5S2u	1.56	CNS-
5S2v	2.85	CNS±
5S2w	8.93	CNS+
5S2x	7.51	CNS+
5S2y	6.98	CNS+

[a]Data represented are the mean of assay for commercial drugs (n = 2);
[b]'CNS+' (prediction of high BBB permeation); P_e (10^{-6} cm s^{-1}) > 4.3926.
[c]''CNS-' (prediction of low BBB permeation); P_e (10^{-6} cm s^{-1}) < 1.7766.
[d]'CNS±' (prediction of uncertain BBB permeation); P_e (10^{-6} cm s^{-1}) 4.3926 to 1.7766

5.B.2.2. *In Vivo* Anti-convulsant Activity

5.B.2.2.1. s.c.-PTZ Induced Seizures in Mice

Manipulation of GABA metabolism, synaptic uptake mechanism and its receptor complex along with neuronal ion channels has been a central theme for research to discover safe and effective novel drugs for the treatment of epilepsy. Tiagabine is a recent entrant in the category of anti-epileptic drug that has a distinct mechanism of reuptake inhibition of GABA at the synapse. Tiagabine augments the level and neuro-inhibitory activity of GABA by interfering the function of GABA transporters specifically GAT1.

Subcutaneous injection of PTZ is validated and most commonly used rodent model of epilepsy. In this test inhibitory potential of test drugs to suppress or delay the seizures induced by PTZ is measured. It was observed that the test compounds **5S2d, 5S2l, 5S2w, 5S2x** and **5S2y** significantly delayed the onset of seizures and its frequency**.** However, compounds **5S2n, 5S2f, 5S2m, and 5S2j** failed to exhibit anti-seizure activity in this model **(Table 2)**. Tiagabine also significantly delayed the onset of seizures and frequency of seizures. **5S2d, 5S2w,** and **5S2y** were most potent amongst synthesized compounds.

Table 5.14. Effect of the test compounds (**Series 2**) on s.c. PTZ induced seizures

Comp.	Latency of seizures (seconds)*	Frequency of seizures (numbers)*
Control	554.16 ± 18.84	4.33 ± 0.81
5S2d	1036.50 ± 20.56[a]	1.66 ± 0.81 [a]
5S2f	555.33 ± 12.82	3.66 ± 0.81
5S2j	564.16 ± 25.46	3.83 ± 0.75
5S2l	843.16 ± 21.94 [a]	1.83 ± 0.75 [a]
5S2m	560.83 ± 19.45	4.33 ± 0.81
5S2n	565.16 ± 46.82	4.16 ± 0.75
5S2w	1181.66 ± 19.16 [a]	1.16 ± 0.40 [a]
5S2x	792.33 ± 19.59 [a]	2.16 ± 0.75 [a]
5S2y	1119.83 ± 21.84 [a]	1.33 ± 0.51 [a]
Tiagabine	1276.33 ± 17.50 [a]	1.16 ± 0.40 [a]

* Values are expressed as the Mean ± SD (n = 6); Control: Physiological saline (0.9%) containing 2.5% tween 80; Tiagabine: 10mg/kg, *i.p.;* All the test compounds were administered intraperitoneally at an equimolar dose relative to 10mg/kg tiagabine; [a] $p < 0.05$ compared to control.

5.B.2.2.2. DMCM Induced Seizures in Mice

Only those compounds that exhibited significant antiepileptic activity in the sc-PTZ model were selected for further screening in DMCM induced seizure test. DMCM is a potent convulsant agent having dual effect-augmenting excitatory amino acid and attenuating GABA inhibitory function. DMCM has been identified to possess specific benzodiazepine binding sites [Petersen, 1983]. The outcome of the model was similar to that of sc-PTZ induced seizure model. All the test compounds (**5S2d, 5S2l, 5S2w, 5S2x** and **5S2y**) and standard drug significantly delayed the onset of convulsion **(Table 5.13)**.

Table 5.15. Effect of the test compounds (**Series 2**) on DMCM induced seizures.

Comp.	Latency of seizures (seconds)*
Control	222.33 ± 6.02
5S2d	373.33 ± 8.35 [a]
5S2l	272.33 ± 11.62 [a]
5S2w	417.83 ± 9.17 [a]
5S2x	267.66 ± 13.93 [a]
5S2y	411.50 ± 16.67 [a]
Tiagabine	438.66 ± 10.46 [a]

[*] Values are expressed as the Mean ± SD (n = 6); Control: Physiological saline (0.9%) containing 2.5% tween 80; Tiagabine: 10mg/kg, i.p.; All the test compounds were administered intraperitoneally at an equimolar dose relative to 10mg/kg tiagabine; [a] $p < 0.05$ compared to control.

5.B.2.3. Rota-rod Performance Test in Mice

Drugs acting on CNS do have potential to cause motor incoordination. In order to assess the putative motor incoordination effect of drugs rota rod test is widely used. In this test rodents are placed on a rotating rod and fall off time is measured before and after the drug treatment. A significant decrease in the fall off time indicates the motor incoordination effect of the drug. In this test, all the test compounds were found to be devoid of any adverse effect on muscle coordination **(Table 5.14)**. Standard drug diazepam showed a significant reduction in fall off time.

Table 5.16. Effect of the test compounds (**Series 2**) on rota-rod performance test in mice.

Comp.	Fall off time before treatment (seconds)*	Fall off time after treatment (seconds)*
Control	318.33 ± 11.37	326.66 ± 12.95
5S2d	323.50 ± 6.41	328.16 ± 5.56
5S2w	331.83 ± 15.06	333.33 ± 8.23
5S2y	325.16 ± 16.64	331.83 ± 15.35
Tiagabine	320.83 ± 13.07	322.66 ± 5.98
Diazepam	334.83 ± 15.86	152.66 ± 12.12 [a]

[*] Values are expressed as the Mean ± SD (n = 6); Control: Physiological saline (0.9%) containing 2.5% tween 80; Tiagabine: 10mg/kg, *i.p.;* Diazepam: 4mg/kg, *i.p.;* All the test

compounds were administered intraperitoneally at an equimolar dose relative to 10mg/kg tiagabine; [a] $p < 0.05$ compared to control.

5.B.2.2. Cell viability and neurotoxicity (MTT Assay)

Some antiepileptic drugs and their metabolites have been reported to possess neurotoxicity [Ambrósio *et al*., 2000; Araújo *et al*., 2004; Gao and Chuang 1992; Gao *et al*., 1995; Liu *et al*., 2015; Nonaka *et al*., 1998]. Ideally, antiepileptic drugs should prevent the seizures without producing neuronal toxicity. Therefore, the therapeutic suitability of the most active compounds (**5S2d, 5S2w,** & **5S2y**) and their effects on cell viability was determined in neuroblastoma cell line (SH-SY5Y). The ability of intracellular dehydrogenases to reduce MTT to the formazan is interpreted as the measure of cell viability. The formazan upon solubilization can be measured spectrophotometrically, which is directly proportional to the viable cell number [Lim *et al*., 2015]. The results of the experiment revealed that the MTT reduction was not effected significantly by test compounds thus corresponds to the insignificant cell death in the concentrations ranging from 1-80 µM **(Table 5.17).**

Table 5.17. Cell Viability of the test compounds (**Series 2**) at different concentrations in neuroblastoma cell line (SH-SY5Y).

Comp.	Percentage cell viability[*]				
	1 µm	**10 µm**	**20 µm**	**40 µm**	**80 µm**
5S2d	99.99 ± 0.05	99.95 ± 0.10	99.91 ± 0.12	98.63 ± 0.06	89.25 ± 0.12
5S2w	99.74 ± 0.13	99.46 ± 0.22	96.50 ± 0.38	92.22 ± 0.47	83.52 ± 0.30
5S2y	99.60 ± 0.12	99.40 ± 0.22	95.81 ± 0.38	91.48 ± 0.47	84.92 ± 0.38

[*] Percentage cell viability of SH-SY5Y cells incubated with increasing concentration of test compounds. Values are expressed as the percentage cell viability ± SD of at least five independent experiments.

5.B.2.3. Repeated dose toxicity studies

Compound **5S2w** was evaluated for haematological, hepatic and renal toxic liabilities through their effects on various serum biomarkers. The outcome of the estimation of different haematological and biochemical parameters is summarized in **Table 5.18**

and **5.19** respectively. The results confirmed the safety of the compound **5S2w** at an equimolar dose relative to 10 mg/kg Tiagabine.

Table 5.18. Effect of test compound **(5S2w)** on hematological parameters of mice in 28 days repeated dose toxicity studies

Treatment	Hemoglobin (gm/dl)	Total WBC (X 10^3/µl)	Differential Leukocytes Count (%)				
			Neutrophil	Lymphocyte	Eiosinophil	Monocyte	Basophil
Control	11.71 ± 0.59	5.48 ± 0.49	64.06 ± 2.66	33.38 ± 2.57	1.66 ± 0.30	0.88 ± 0.30	00
5S2w	10.82 ± 0.66	5.07 ± 0.49	65.36 ± 2.20	31.65 ± 1.70	2.10 ± 0.72	0.87 ± 0.13	00
Tiagabine	12.35 ± 0.64	5.58 ± 037	63.75 ± 2.94	33.74 ± 2.75	1.72 ± 0.26	0.78 ± 0.02	00

Values are mean ± SD, WBC= White blood corpuscles.

Table 5.19. Effect of test compound **(5S2w)** on biochemical parameters of mice in 28 days repeated dose toxicity studies

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	AST (U/ml)	ALT (U/ml)	ALP (Unit)	Blood Urea (mg/dl)	Creatinine (mg/dl)	Total Protein (gm/dl)
Control	89.50 ± 3.61	105.55 ± 7.55	42.83 ± 3.43	39.33 ± 2.94	240.83 ± 2.94	12.24 ± 1.27	0.86 ± 0.07	5.15 ± 0.20
5S2w	84.50 ± 4.32	101.66 ± 7.86	40.83 ± 5.70	41.16 ± 3.86	244.33 ± 9.24	11.79 ± 1.76	0.79 ± 0.09	4.80 ± 0.54
Tiagabine	90.16 ± 6.06	100.66 ± 7.00	40.33 ± 4.03	37.50 ± 1.76	243.50 ± 5.31	12.28 ± 1.88	0.85 ± 0.07	5.06 ± 0.31

Values are mean ± SD, AST= Aspartate transaminase, ALT= Alanine transaminase, ALP= Alkaline phosphatase.

5.B.3. COMPUTATIONAL STUDIES

5.B.3.1. Homology modeling of GAT-1

The same homology model of GAT-1 was used to investigate the binding mode interactions of the title compounds of series **2** which was previously constructed for series **1**. The outcomes of the homology modelling were elaborated under section **5.A.3.1.**

5.B.3.2. Molecular Docking Studies

In silico docking studies were performed using the Schrödinger Maestro program to gain insight into the possible mode of protein-ligand interactions using a generated and validated model of GAT1 GABA transporter (PDB Code: 4XP4). The validation of the prepared grid and docking protocols was performed by generating a minimum energy conformer of tiagabine, and it's docking on a prepared grid. The results demonstrate that tiagabine occupied the same active site within the binding pocket leading to its complementary interaction with the amino acid residues within the active site [Jurik et al. 2013, Petrera et al. 2016, Skovstrup et al. 2010]. (**Fig. 5.12.)**

The binding affinity of the active compounds **5S2d, 5S2w** and **5S2y** was determined by using GAT1 GABA transporter modeled protein. The molecular docking studies yielded the best possible conformation for all the ligands **5S2d** (Glide Score: -3.9); **5S2w** (GLIDE Score: -6.2) and **5S2y** (GLIDE Score: -7.3) occupying the similar binding pocket as that of tiagabine (GLIDE Score: -4.6). In the present study, the active binding pocket was selected from literature with the presence of active site amino acid residues Tyr60, Ala61, Gly63, Gly65, Trp68, Arg69, Tyr139, Tyr140, Ile143, Gln291, Phe294, Ser295 and Na atom [Petrera *et al*., 2016].

The docking conformations of the compounds **5S2d, 5S2w,** and **5S2y** reveal salt bridge formation between oxygen (O) atom of carboxyl group and sodium

(Na611). Another oxygen (O) atom present in carboxyl group of compound **5S2y** shown to have additional metal coordination interaction with sodium (Na611). The O atom of the carboxyl group in all the docked ligands interacted through a network of hydrogen bonding with backbone atom of Gly65. Compounds **5S2d** and **5S2w** involved in the hydrogen bonding with side chain hydroxyl groups of Tyr140. Additionally, the NH group of all the ligands was also involved in hydrogen bonding interactions with Phe294 similar to tiagabine. The NH group of compound **5S2y** was additionally involved in π-cationic interaction with Tyr60. The charged interactions with Arg69 and Asp451 were also responsible for stabilizing the aromatic rings of the ligands**.** The detailed interaction results of tiagabine, **5S2d, 5S2w,** and **5S2y** with active site amino acid residues are summarized in **Table 5.20**. Overall, these interactions of all the docked ligands with the modeled protein of GAT1 GABA transporter showed complementary binding with active site amino acids residues as shown in **Fig. 5.13, 5.14 and 5.15**.

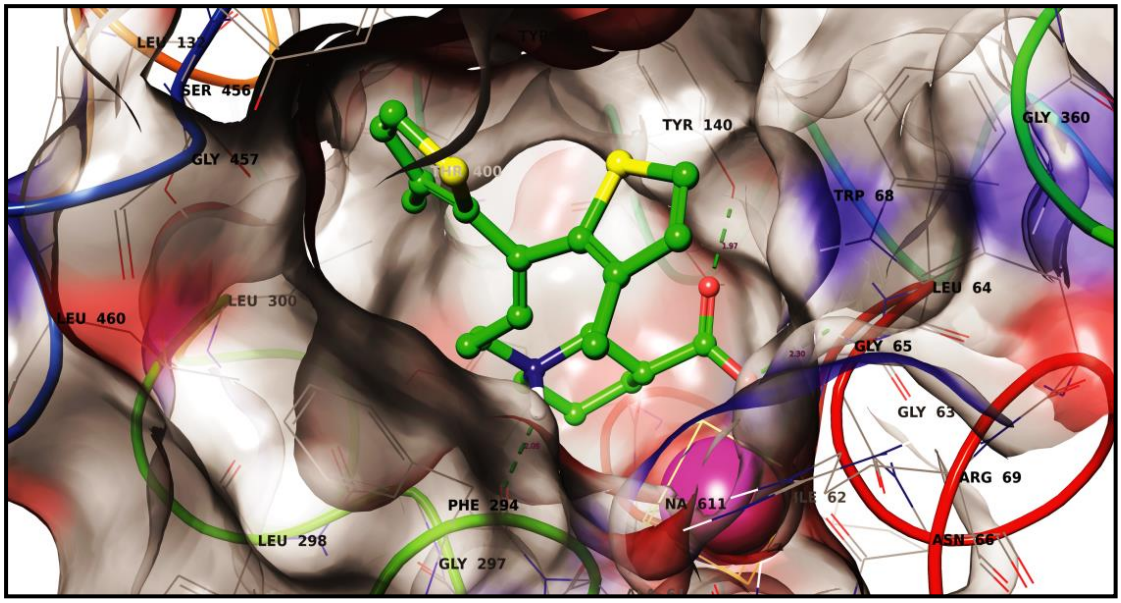


Fig. 5.12. 3D Ribbon structure representation of docking conformation of the tiagabine in the hydrophobic pocket of homology modelled protein structure of GAT1 GABA transporter. Structure of tiagabine is shown as ball and stick model; light brown color surface is showing hydrophobic pocket; blue and red surface is charged surface of the protein; green dotted line is showing H-bonding interactions between active site amino acid residues and ligand.

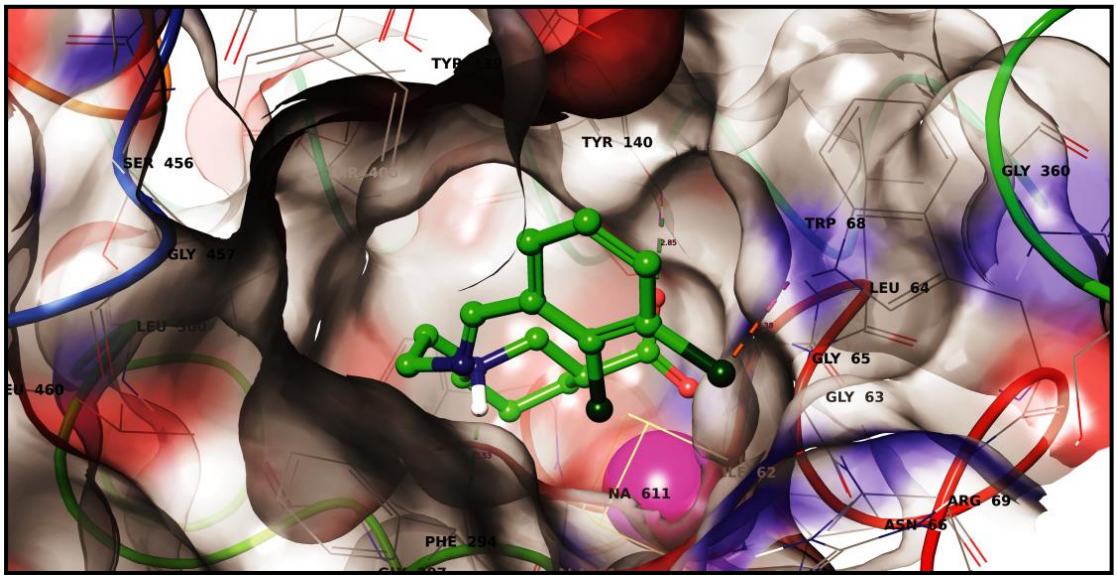


Fig. 5.13. 3D Ribbon structure representation of docking conformation of the compound **5S2d** in the hydrophobic pocket of homology modelled protein structure of GAT1 GABA transporter. Structure of compound **5S2d** is shown as ball and stick model; light brown color surface showing hydrophobic pocket; the blue and red surface is charged surface of the protein; green dotted lines are H-bonding, and the red dotted line is charged interaction between active site amino acid residues and ligand.

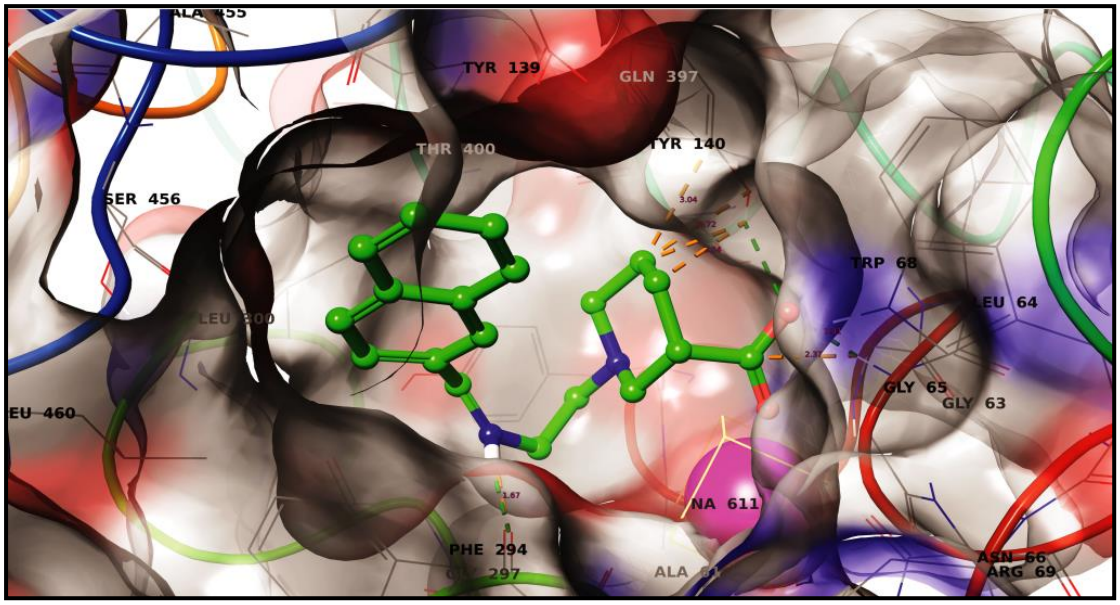


Fig. 5.14. 3D Ribbon structure representation of docking conformation of the compound **5S2w** in the hydrophobic pocket of homology modelled protein structure of GAT1 GABA transporter. Structure of compound **5S2w** is shown as ball and stick model; light brown color surface showing hydrophobic pocket; the blue and red surface is charged surface of the protein; green dotted lines are H-bonding and orange dotted lines are Van der Waals interactions between active site amino acid residues and ligand.

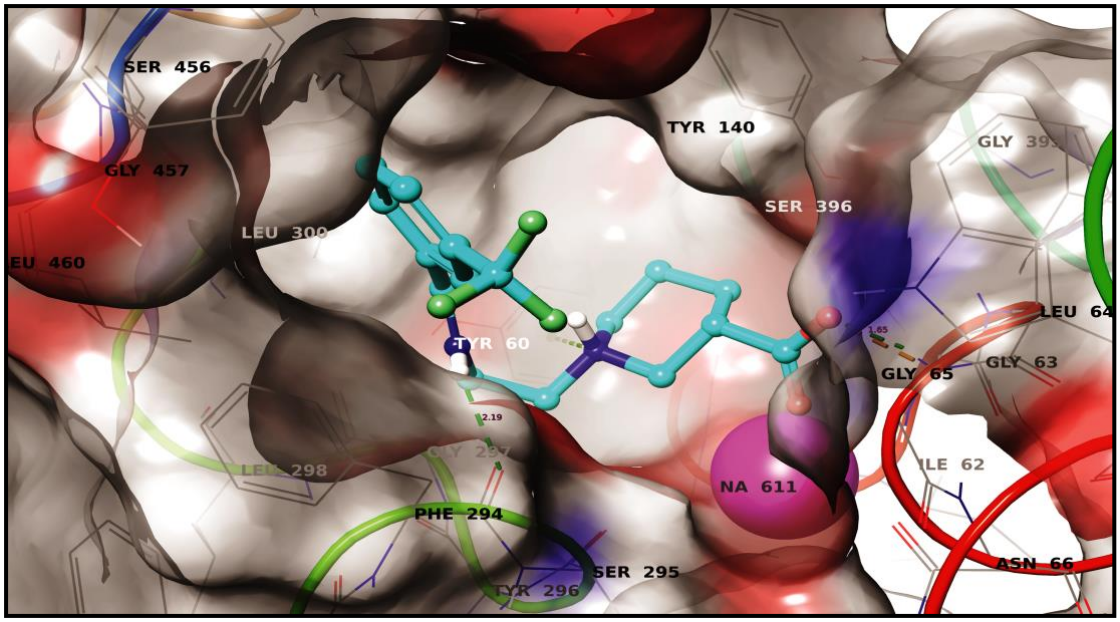


Fig. 5.15. 3D Ribbon structure representation of docking conformation of the compound **5S2y** in the hydrophobic pocket of homology modelled protein structure of GAT1 GABA transporter. Structure of compound **5S2y** is shown as ball and stick model; light brown color surface showing hydrophobic pocket; the blue and red surface is charged surface of the protein; green dotted lines are H-bonding and orange dotted lines are Van der Waals interactions between active site amino acid residues and ligand.

Table 5.20. Details of protein-ligand interactions of **tiagabine, 5S2d, 5S2w**, and **5S2y.**

Comp.	Parameters							
	Glide Score	Interacting residues*						
		H-bonding	Salt bridge	Metal Coordination	π-π cation	Hydrophobic	Polar	Charged
Tiagabine	-4.6	Gly65, Tyr140, Phe294	Na611	None	None	Tyr60, Ala61, Gly63, Trp68, Tyr139, Ile143, Gly297, Phe447	Asn66, Gln291, Ser295, Ser396	Arg69, Asp451
5S2d	-3.9	Gly65, Tyr140, Phe294	Na611	None	None	Tyr60, Ala61, Ile62, Gly63, Leu64, Trp68, Leu136, Tyr139, Gly297, Leu300, Leu460	Asn66, Ser295, Ser396	Arg69, Asp451
5S2w	-6.2	Gly65, Tyr140, Phe294	Na611	None	None	Tyr60, Ala61, Gly63, Leu64, Leu136, Tyr139, Tyr296, Gly297, Leu300, Ala455, Leu460	Asn66, Ser295, Ser396, Ser456	Asp451
5S2y	-7.3	Gly65, Phe294	Na611	Na611	Tyr60	Ala61, ile62, Gly63, Leu64, Leu136, Tyr139, Tyr140, Tyr296, Gly297, Leu300, Ala455, Leu460	Asn66, Ser396, Ser456	Asp451

* All the interactions were observed within 4Å distance.

5.B.3.3. Molecular Dynamics

The dynamics simulation runs of the generated minimized complex of **5S2w** with GABA GAT1 transporter protein of 4XP4 was performed for 50 ns to predict the stability of binding mode interactions. The overall stability of the system was evaluated by RMSD (Root Mean Square Deviation) and RMSF (Root Mean Square Fluctuation) calculations. The results of the RMSD values confirmed that all frames of the complex were in trajectory throughout the simulation with average fluctuation in the range of 1-3 Å (**Fig. 5.16 & 5.17**).

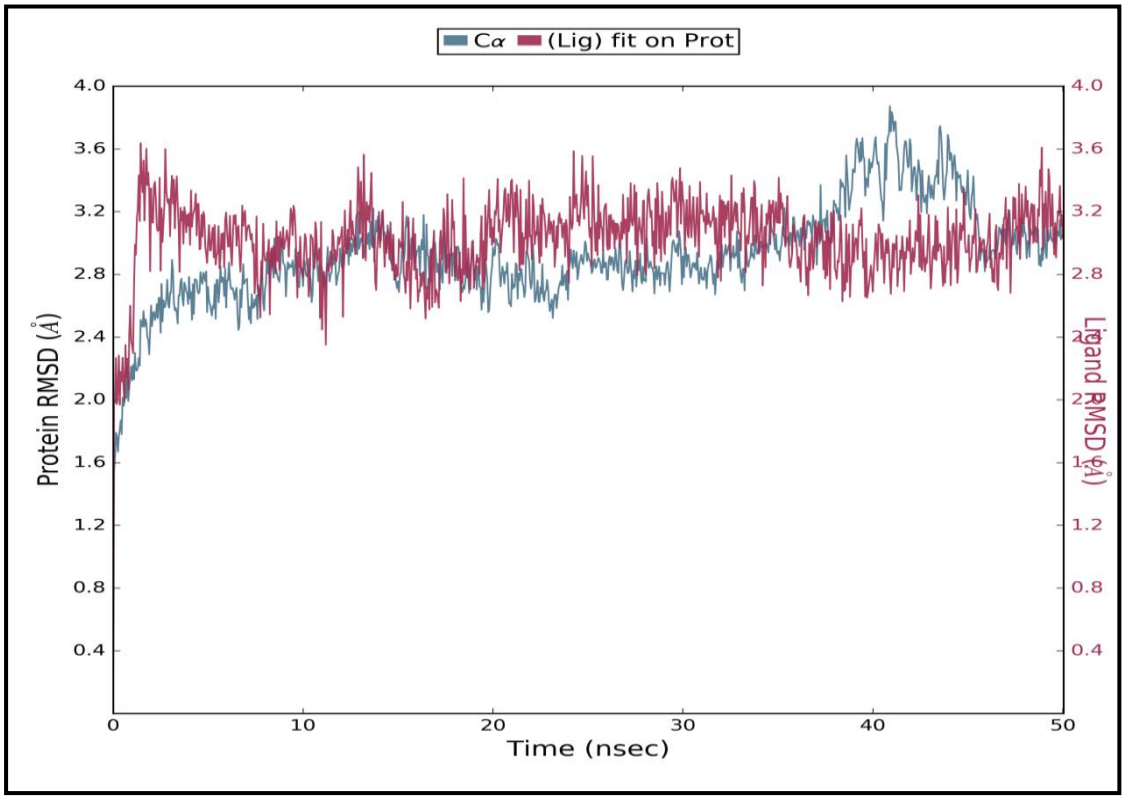


Fig. 5.16. Protein RMSD (Left Y-Axis) and ligand RMSD (Right Y-Axis) values in Å with time in ns (X-Axis) for the molecular dynamics simulation run for the complex of 5w with GAT1 GABA transporter.

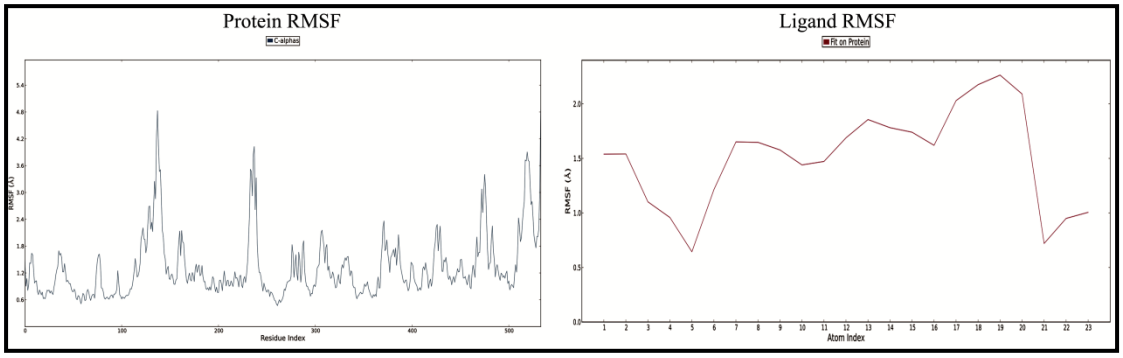


Fig. 5.17. Protein RMSF (Left side) and ligand RMSF (Right side) values in Å with time in nsec.

The graphical representation of binding interactions of compound **5S2w** showed the active site interactions throughout the simulation run **(Fig. 5.18).** The results demonstrated that compound **5S2w** efficiently interacted with active site residues Ala61, Gly65, Asn66, Arg69, Tyr140 and Phe294 through H-bonds. Besides, it also interacted with Phe294 through hydrophobic π-stacking. The carboxylate O-atom also involved in salt bridge formation with Na611 atom. The interaction fraction with individual amino acid residues was also calculated and represented in a stacked bar chart **(Fig. 5.19)**. The interaction fraction is the percentage of total contact for the simulation run. For example, 0.8 suggests that interaction was maintained 80% of the total simulation run.

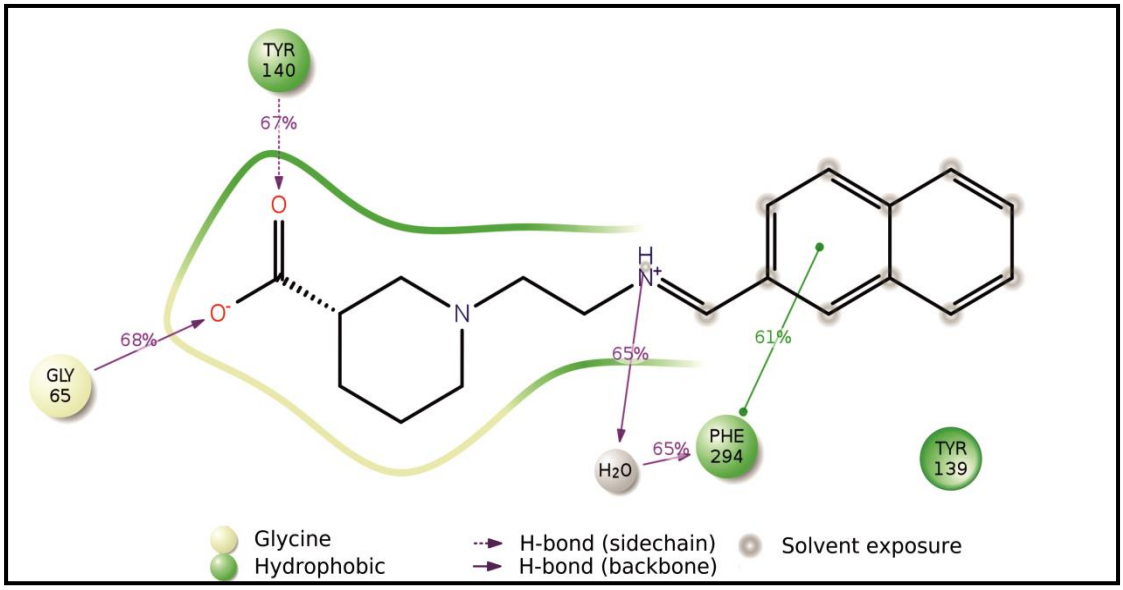


Fig. 5.18. The detailed atomic interactions of ligand **5S2w** with the key active amino acid residues with GABA GAT1 transporter protein.

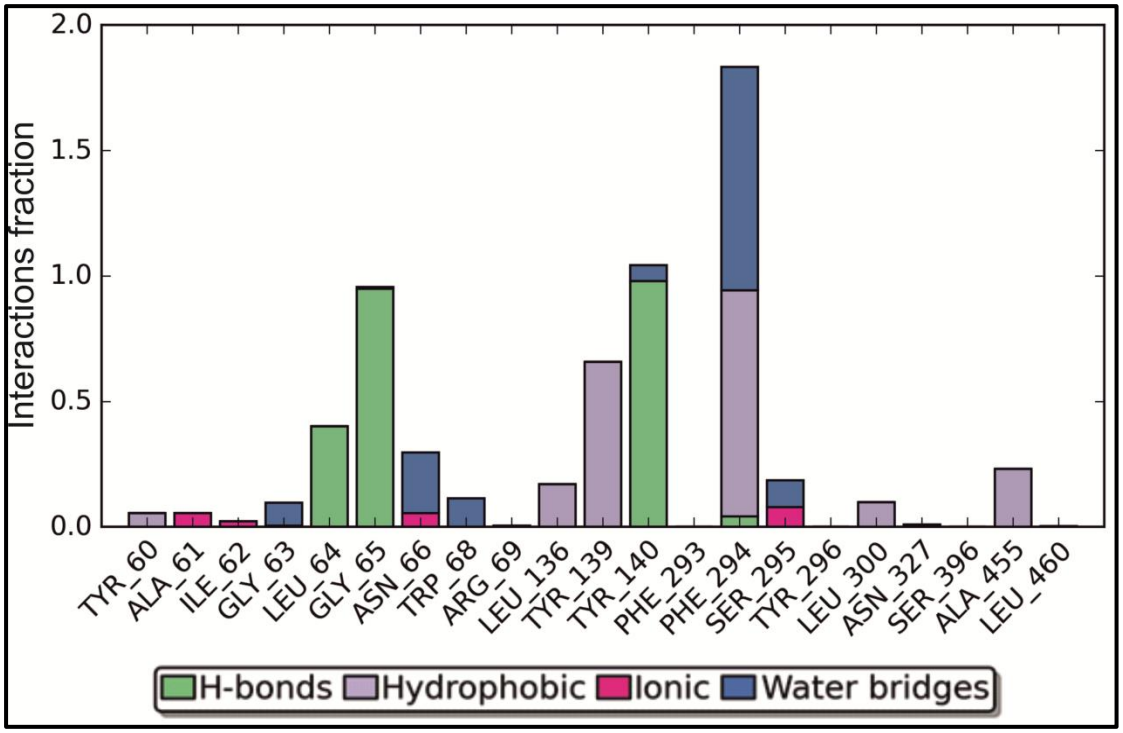


Fig. 5.19. Stacked bar charts of protein interactions with ligand **5S2w** as monitored throughout the MD simulation.

5.B.3.4. Estimation of "Drug-Like" Properties

The results of some principle descriptors for the prediction of in silico "drug likeliness" of the most active compounds (**5S2d, 5S2w,** and **5S2y**) are mentioned in **Table 5.21**. The predicted values for QPlogBB and CNS activity predicted by QikProp method indicated that the selected compounds were found to be active for CNS and might be permeable across blood–brain barrier [Das *et al*., 2014]. These results are comparable with the outcome of PAMPA-BBB assay and experimental log P values. However, the experimental log P values differ with that of the predicted values. The predicted PSA (polar surface area) values were found to be in the range of 58.378- 59.856, which revealed that the selected compounds showed lower polar surface area. Lower PSA is a key requisite for the compounds designed for CNS disorders [Meena *et al*., 2015].

The absence of reactive functional groups that causes decomposition, reactivity, or toxicity problems in vivo was predicted by the outcome of "rtvFG"

value, which was found to be 0 for all the tested compounds. The test compounds demonstrated drug likeliness as per the Lipinski's rule of five (mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤10). QPlogKHSA values for the tested compounds fall within the limit, indicating considerable binding of the compounds with plasma proteins. Overall the predicted parameters revealed that the compounds 4a, 4b, and 4i fulfill drug-like characteristics [Banerjee *et al*., 2016].

Table 5.21. *In silico* drug like properties of the active compounds of **Series 2**.

Comp.	Mol_MW (130-725)	QPlogBB (-3-1.2)	CNS (-2-+2)	QPlogPo/w (-2-6.8)	PSA (7-200)	QP log KHSA (-1.5-1.5)	Lipinski's rule of five (Max. 4)	donor HB (0-6)	accpt HB (2-20)	#rtvFG (0-2)
5S2d	310.39	-0.267	0	1.579	59.856	0.053	0	1	5.5	0
5S2w	329.22	0.059	1	1.47	58.378	-0.106	0	1	5.5	0
5S2y	328.33	-0.005	0	1.625	59.195	-0.041	0	1	5.5	0

Mol_MW: molecular weight;
QPlogBB: Predicted brain/blood partition coefficient;
CNS: Predicted central nervous system activity;
QPlogPo/w: Predicted octanol/water partition coefficient;
PSA: polar surface area;
QPlogKHSA: Prediction of binding to human serum albumin;
Rule of five: No. of violations of Lipinski's rule of five;
donorHB: No. of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution;
accptHB: No. of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution;
#rtvFG: Number of reactive functional group.