Chapter 5

Discovery of 2-substituted Benzo[d]oxazol-5-amine Analogs

5.1 Experimental Work

5.1.1 Rationale of drug design & *in-silico* optimization

The design strategy of multi-target directed benzoxazole derivatives is depicted in Figure 5.1. The cholinergic and $A\beta_{1-42}$ hypotheses are the basic approaches to treat AD and therefore were employed for the scaffold hopping guided MTDL design. The scaffold selection was based on the structural modifications of known anti-AD and neuroprotective agents. The benzoxazole moiety has been established as a privileged scaffold to confer anti-AD property [Cui et al. 2012, Srivastava et al. 2019, Watanabe et al. 2015]. Cholinesterase inhibition studies of Gaussian-based quantitative structure-activity relationship and virtual screening revealed phenyl benzoxazole analogs as potent agents. 1-((4-(benzo[d]oxazol-2-yl)phenyl)amino)-3-((4-chlorophenyl)amino) propan-2-ol (**1A**) derivative exhibited competitive AChE inhibition with IC_{50} of 0.363 μM and Ki = 0.19 μM. ^{18}F labeled phenylbenzoxazole derivatives, 4-(5-(2-(2-(2-[^{18}F]fluoroethoxy)ethoxy) ethoxy)benzo- [*d*]oxazol-2-yl)-N-methylaniline (**2A**), produced high affinity for $A\beta_{1-42}$ aggregates with K_i of 9.3 nM. 5-(5-[^{125}I]iodobenzo[d]oxazol-2-yl)-N,N-dimethylpyridin-2-amine (**3A**) (Ki = 6.9 nM) displayed affinity for $A\beta_{1-42}$ aggregates in *in-vitro* and good penetration to normal mouse brain.

Neuronal protection is considered to be an important factor for anti-AD agents. Piperazine containing compounds (VK-28 and SP-04) are known to exhibit neuronal protection [Popugaeva et al. 2019, Srivastava et al. 2019]. Fenazinel, a neuroprotective piperazine containing analog is a NMDAR antagonist developed for the treatment of ischemic stroke [Li et al. 2009a].

Structural analogs of donepezil (DNZ) with potent cholinesterase inhibition have been reported since the past decade. Further, compounds **4A** and **5A** exhibited potent AChE inhibition with IC_{50} of 29 and 0.36 nM, respectively. All these analogs indicate the therapeutic significance of various functional scaffolds/groups as anti-AD agents.

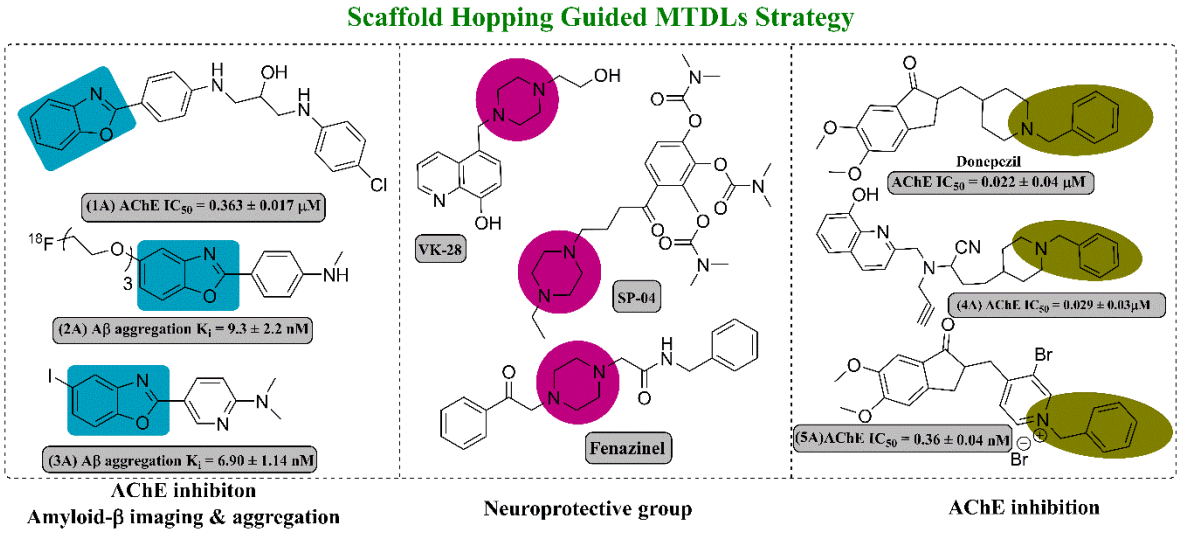


Figure 5.1. Molecular framework of multi-target directed ligand (MTDL) strategy for AD.

5.1.2 *In-silico* studies

5.1.2.1 Molecular docking

All the molecular docking protocols were followed as described in chapter 4, section 4.1.2.2.

5.1.2.2 Prediction of physicochemical properties

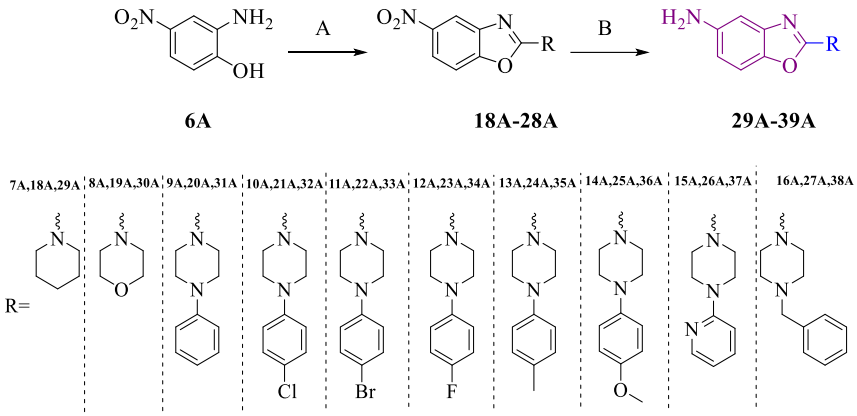
The QikProp in Maestro 10.1 was used to predict physicochemical properties of designed molecules. As QikProp was unsuitable for neutralizing the compounds and generating the descriptors in the normal mode; hence, neutralization of all compounds was essential before performing QikProp. Physicochemical parameters like brain/blood partition coefficient (QPlogBB), octanol/water partition coefficient (QPlogPo/w), Caco-

2 cell permeability (QPPCaco), human oral absorption (%HOA), and IC_{50} value for blockage of hERG K^+ channels (QPlogHERG) were predicted and analyzed.

5.1.3 Synthesis and characterization

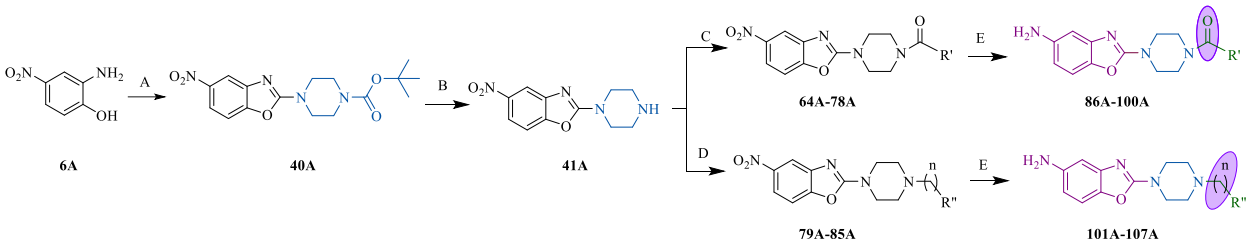
All the reagents used for chemical synthesis were of commercial products and were used without further purification. The experiments were carried out in oven-dried glassware under dry N_2 atmosphere, and standard vacuum techniques were used. All reactions were monitored on silica gel F_{254} TLC aluminium sheets (Merck), and ultraviolet light (254 nm) or iodine vapours were used for visualization of spots. The melting points were determined on a Stuart Melting Point apparatus (SMP10) using open end capillary tubes and reported as uncorrected. The NMR spectra were obtained on Bruker-500 (^1H 500 MHz, ^{13}C 125 MHz) instrument in $CDCl_3$ and DMSO-d_6 solutions at room temperature with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported as ppm (δ values) relative to the internal TMS. Coupling constants (*J*) are reported in hertz (Hz). Multiplicity is defined by s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (doublet of doublet), and m (multiplet). Mass spectra were acquired with a Waters Q-TOF premier-HAB213 instrument equipped with APCI and ESI multimode ionization source. High-performance liquid chromatography (HPLC) was performed with Agilent 1260 infinity II Quaternary LC (a quaternary pump with a DAD HS G7115A detector, λ = 315 nm) using a column of Agilent ZORBAX Eclipse plus C8 (5µm, 4.6 × 250 mm) and acetonitrile:water (9:1) as the mobile phase. The purity of the synthesized compounds was determined using analytical HPLC and was found to be more than 97%.

5.1.3.1 Scheme 1. Synthesis of compounds 29A-39A[a]



[a]Reagents and conditions: (A) Carbon disulfide 1.5 eq, substituted amines (**7A-17A**) 1.5 eq, 110 °C, 3 h; (B) Nitro derivatives (**18A-28A**) 1.0 eq, Iron powder 3.5 eq, Ammonium chloride 2.5 eq, Ethanol : THF: Water (1:1:1) 70 °C, 1.5 h.

5.1.3.2 Scheme 2. Synthesis of compounds 86A-107A[b]



42A,64A,86A; R'= Phenyl
43A,65A,87A; R'= 2-Cl Phenyl
44A,66A,88A; R'= 3-Cl Phenyl
45A,67A,89A; R'= 4-Cl Phenyl
46A,68A,90A; R'= 2-Br Phenyl
47A,69A,91A; R'= 3-Br Phenyl
48A,70A,92A; R'= 4-Br Phenyl
49A,71A,93A; R'= 2-F Phenyl
50A,72A,94A; R'= 3-F Phenyl
51A,73A,95A; R'= 4-F Phenyl
52A,74A,96A; R'= 2-OMe Phenyl
53A,75A,97A; R'= 3-OMe phenyl
54A,76A,98A; R'= 4-OMe Phenyl
55A,77A,99A; R'= 5-benzo[*d*][1,3]dioxyl
56A,78A,100A; R'= 5-Cl,2-OMe Phenyl

57A,79A,101A; n = 1, R" = 4-Cl Phenyl
58A,80A,102A; n = 1, R" = 4-Br Phenyl
59A,81A,103A; n = 1, R" = 4-F Phenyl
60A,82A,104A; n = 1, R" = 4-Me Phenyl
61A,83A,105A; n = 1, R" = 4-OMePhenyl
62A,84A,106A; n = 1, R" = 4-Ispr Phenyl
63A,85A,107A; n = 2, R" = Phenyl

[b]Reagents and conditions: (A) Carbon disulfide 1.5 eq, 1-Boc-piperazine 1.5 eq, 110 °C, 3 h; (B) TFA 5.0 eq, DCM, rt, 15 min; (C) Carboxylic acid (**42A-56A**) 1.2 eq, 1-Hydroxybenzotriazole 2.5 eq, Diisopropylethylamine 2.5 eq, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide.HCl 1.5 eq, DCM, rt, 8 h. (D) Aryl Halide (**57A-63A**) 1.2eq, K_2CO_3 2.0 eq, DMF, rt, 4 h. (E) Nitro derivatives (**64A-78A; 79A-**

85A) 1.0 eq, Iron powder 3.5 eq, Ammonium chloride 2.5 eq, Ethanol : THF: Water (1:1:1) 70 °C, 1.5 h.

5.1.3.3 General procedure for the synthesis of compounds 18A-28A;40A

A mixture of secondary amine (**7A-17A**) /1-Boc-piperazine (1.5 eq) and carbon disulfide (1.5 eq) was taken in a 50-mL round bottomed (R.B) flask with a magnetic stir bar, and stirred thoroughly for 30 min at 0-5 °C, which resulted in the formation of the intermediate dithiocarbamate (DTC). To it, was added 2-amino-4-nitrophenol (**6A**, 1 eq) and the mixture contained in the R.B flask equipped with a condenser, was heated at a temperature of 110 °C in an oil bath for 3 h. After completion of the reaction (monitored by TLC), mixture was subsequently partitioned between ethyl acetate (15 mL) and water (10 mL). The aqueous phase was extracted with ethyl acetate (2 × 5 mL). The combined organic extracts were washed with brine solution, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (60–120 mesh) to afford the desired compounds (**18A**-**28A; 40A**).

5-nitro-2-(piperidin-1-yl)benzo[d]oxazole (**18A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **7A,** obtained as a bright yellow solid, yield- 94%, M.P.- 121 - 122 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.14 (d, *J* = 2Hz, 1H, benzoxazole C_4), 7.98 - 7.96 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 7.29 (d, *J* = 9Hz, 1H, benzoxazole C_7), 3.71 (s, 4H, piperidin-1-yl C_2, C_6), 1.72 (s, 6H, piperidin-1-yl C_3, C_4, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.77 (benzoxazole C_2), 152.71 (benzoxazole C_{7a}), 145.13 (benzoxazole C_{3a}), 144.46 (benzoxazole C_5), 116.82 (benzoxazole C_6), 111.34 (benzoxazole C_4), 108.10 (benzoxazole C_7), 46.67 (piperidin-1-yl C_2, C_6), 25.22 (piperidin-1-yl C_3, C_5), 23.88

(piperidin-1-yl C_4); MS (EI+): m/z calculated for $C_{12}H_{13}N_3O_3$: 247.25, found – 248.65 (M+1).

2-morpholino-5-nitrobenzo[d]oxazole (**19A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **8A,** obtained as a bright yellow solid, yield- 95%, M.P.- 141 - 142 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 2Hz, 1H, benzoxazole C_4), 7.84 - 7.86 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 7.31 (d, *J* = 9Hz, 1H, benzoxazole C_7), 3.84 - 3.82 (m, 4H, morpholine C_2, C_6), 3.79 – 3.77 (m, 4H, morpholine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.52 (benzoxazole C_2), 153.45 (benzoxazole C_{7a}), 145.52 (benzoxazole C_{3a}), 144.27 (benzoxazole C_5), 116.24 (benzoxazole C_6), 111.79 (benzoxazole C_4), 107.72 (benzoxazole C_7), 66.31 (morpholine C_2, C_6), 45.62 (morpholine C_3, C_5); MS (EI+): m/z calculated for $C_{11}H_{11}N_3O_4$: 249.23, found – 250.31 (M+1).

5-nitro-2-(4-phenylpiperazin-1-yl)benzo[d]oxazole (**20A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **9A,** obtained as a yellow solid, yield- 91%, M.P.- 137 – 138 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 2Hz, 1H,benzoxazole C_4), 8.05 - 8.03 (dd, *J* = 9Hz, 2Hz, 1H, benzoxazole C_6), 7.36 - 7.32 (m, 3H, benzoxazole C_7, phenyl C_3, C_5), 7.02 (d, *J* = 8Hz, 2H, phenyl C_2, C_6), 6.99 (t, *J* = 7.5Hz, 1H, phenyl C_4), 3.94 (t, *J* = 5Hz, 4H, piperazine C_3, C_5), 3.36 (t, *J* = 5Hz, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.49 (benzoxazole C_2), 152.72 (benzoxazole C_{7a}), 150.88 (phenyl C_1), 145.30 (benzoxazole C_5), 144.10 (benzoxazole C_{3a}), 129.36 (phenyl C_3, C_5), 121.05 (phenyl C_4), 117.33 (benzoxazole C_6), 117.08 (phenyl C_2, C_6), 111.92 (benzoxazole C_4), 108.44 (benzoxazole C_7), 49.22 (piperazine C_3, C_5), 45.62 (piperazine C_2, C_6); MS (EI+): m/z calculated for $C_{17}H_{16}N_4O_3$: 324.34, found – 325.15 (M+1).

2-(4-(4-chlorophenyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**21A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **10A,** obtained as a yellow solid, yield- 92%, M.P.- 135 – 136 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* =2.5Hz, 1H, benzoxazole C_4), 8.05 - 8.03 (dd, *J* = 2.5 Hz, 1H, benzoxazole C_6), 7.36 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.28 - 7.26 (m, 2H, chlorophenyl C_3, C_5), 6.93 - 6.91 (m, 2H, chlorophenyl C_2, C_6), 3.93 - 3.91 (m, 4H, piperidin-1-yl C_2, C_6), 3.32 - 3.30 (m, 4H, piperidin-1-yl C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.40 (benzoxazole C_2), 152.70 (benzoxazole C_{7a}), 149.48 (4-chlorophenyl C_1), 145.30 (benzoxazole C_{3a}), 144.02 (benzoxazole C_5), 129.23 (4-chlorophenyl C_3, C_5), 126.00 (4-chlorophenyl C_4), 118.29 (4-chlorophenyl C_2, C_6), 117.39 (benzoxazole C_6), 111.97 (benzoxazole C_4), 108.47 (benzoxazole C_7), 49.19 (piperazine C_3, C_5), 45.49 (piperazine C_2, C_6); MS (EI+): m/z calculated for $C_{17}H_{15}ClN_4O_3$: 358.78, found – 359.09 (M+), 361.08 (M+2).

2-(4-(4-bromophenyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**22A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **11A**, obtained as a yellow solid, yield- 93%, M.P.- 163-164 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 8.05 - 8.03 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 7.42 (d, *J* = 8.5Hz, 2H, bromophenyl C_3, C_5), 7.36 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.88 (d, *J* = 9Hz, 2H, bromophenyl C_2, C_6), 3.93 - 3.91 (t, *J* = 5.5Hz, 4H, piperazine C_3, C_5), 3.32 - 3.30 (t, *J* = 5.5Hz, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.40 (benzoxazole C_2), 152.70 (benzoxazole C_{7a}), 149.90 (bromophenyl C_1), 145.31(benzoxazole C_5), 144.02 (benzoxazole C_{3a}), 132.16 (bromophenyl C_3, C_5), 118.64 (bromophenyl C_2, C_6), 117.40 (benzoxazole C_6), 113.31 (bromophenyl C_4), 111.99 (benzoxazole C_4), 108.48

(benzoxazole C_7), 49.01 (piperazine C_3, C_5), 45.46 (piperazine C_2, C_6); MS (EI+): m/z calculated for $C_{17}H_{15}BrN_4O_3$: 403.24, found – 403.36 (M+), 405.28 (M+2).

2-(4-(4-fluorophenyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**23A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **12A,** obtained as a yellow solid, yield- 87%, M.P.- 137-138 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (s, 1H, benzoxazole C_4), 8.05 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6) 7.36 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.05 - 7.01 (m, 2H, fluorophenyl C_3, C_5), 6.97 (br s, 2H, fluorophenyl C_2, C_6) 3.93 (s, 4H, piperazine C_3, C_5), 3.26 (s, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.45 (benzoxazole C_2), 158.86 (fluorophenyl C_4), 156.95 (fluorophenyl C_1), 152.71 (benzoxazole C_{7a}), 147.54 (benzoxazole C_{3a}), 145.30 (benzoxazole C_5), 119.10 (fluorophenyl C_3), 119.03 (fluorophenyl C_5), 117.36 (fluorophenyl C_2), 115.93 (fluorophenyl C_6), 115.75 (benzoxazole C_6), 111.94 (benzoxazole C_4), 108.45 (benzoxazole C_7), 50.20 (piperazine C_2, C_6), 45.68 (piperazine C_3, C_5); MS (EI+): m/z calculated for $C_{17}H_{15}FN_4O_3$: 342.33, found – 343.52 (M+1).

5-nitro-2-(4-(p-tolyl)piperazin-1-yl)benzo[d]oxazole (**24A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **13A,** obtained as a yellow solid, yield- 93%, M.P.- 145-146 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.05 - 8.03 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_6), 7.36 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.15 (d, *J* = 8Hz, 2H, p-tolyl C_3, C_5), 6.93 (d, *J* = 8.5Hz, p-tolyl C_2, C_6), 3.94 - 3.92 (t, *J* = 5.5Hz, 4H, piperazine C_3, C_5), 3.30 - 3.28 (t, *J* = 5.5Hz, 4H, piperazine C_2, C_6), 2.32 (s, 3H, -CH3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.50 (benzoxazole C_2), 152.73 (benzoxazole C_{7a}), 148.77 (p-tolyl C_1), 145.29 (benzoxazole C_5), 144.13 (benzoxazole C_{3a}), 130.71 (p-tolyl C_4), 129.87 (p-tolyl C_3, C_5), 117.40 (p-tolyl C_2, C_4), 117.30 (benzoxazole C_6),

111.89 (benzoxazole C_4), 108.41 (benzoxazole C_7), 49.75 (piperazine C_3, C_5), 45.68 (piperazine C_2, C_6), 20.49 (-CH3); MS (EI+): m/z calculated for $C_{18}H_{18}N_4O_3$: 338.37, found – 339.47 (M+1).

2-(4-(4-methoxyphenyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**25A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **14A**, obtained as a brown solid, yield- 85%, M.P.- 128-129 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.05 - 8.02 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_6), 7.35 (dd, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.99 - 6.96 (m, 2H, methoxyphenyl C_2, C_6), 6.91 - 6.88 (m, 2H, methoxyphenyl C_3, C_5), 3.93 - 3.91 (t, 4H, piperazine C_3, C_5), 3.81 (s, 3H, -OCH_3), 3.23 - 3.21 (t, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.51 (benzoxazole C_2), 154.74 (benzoxazole C_{7a}), 152.73 (methoxyphenyl C_4), 145.28 (benzoxazole C_5), 145.16 (methoxyphenyl C_1), 144.14 (benzoxazole C_{3a}), 119.33 (methoxyphenyl C_2, C_6), 117.28 (benzoxazole C_6), 114.61 (methoxyphenyl C_3, C_5), 111.87 (benzoxazole C_4), 108.41 (benzoxazole C_7), 55.57 (-OCH_3), 50.64 (piperazine C_2, C_6), 45.80 (piperazine C_3, C_5); MS (EI+): m/z calculated for $C_{18}H_{18}N_4O_4$: 354.37, found – 355.42 (M+1).

5-nitro-2-(4-(pyridin-2-yl)piperazin-1-yl)benzo[d]oxazole (**26A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6 A** and **15A,** obtained as a brown solid, yield- 85%, M.P.- 162-163 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.26 - 8.24 (dd, *J* = 5Hz, 1Hz, 1H, pyridin-2-yl C_3), 8.21 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 8.05 - 8.02 (dd, *J* = 2.5Hz, 2Hz, 1H, benzoxazole C_6), 7.58 - 7.55 (m, 1H, pyridin-2-yl C_5), 7.36 (d, *J* = 9Hz, 1H, benzoxazole C_7), 6.75 - 6.72 (m, 2H, pyridin-2-yl C_4, C_6), 3.90 - 3.88 (m, 4H, piperazine C_2, C_6), 3.77 - 3.74 (m, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.54 (benzoxazole C_2), 158.90 (pyridin-2-yl C_1), 152.71 (benzoxazole C_{7a}), 148.09 (pyridin-2-yl C_3), 145.29

(benzoxazole C_5), 144.07 (benzoxazole C_{3a}), 137.84 (pyridin-2-yl C_5), 117.33 (benzoxazole C_6), 114.31 (pyridin-2-yl C_4), 111.90 (benzoxazole C_4), 108.45 (benzoxazole C_7), 107.44 (pyridin-2-yl C_6), 45.30 (piperazine C_2, C_6), 44.74 (piperazine C_3, C_5); MS (EI+):m/z calculated for $C_{16}H_{15}N_5O_3$: 325.33, found – 326.53 (M+1).

2-(4-benzylpiperazin-1-yl)-5-nitrobenzo[d]oxazole (**27A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **16A**, obtained as a yellow solid, yield- 92%, M.P.- 141-142 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.02 - 8.00 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 7.39 - 7.36 (m, 4H, benzyl C_2, C_3, C_5, C_6), 7.33 - 7.30 (m, 2H, benzoxazole C_7, benzyl C_4), 3.78 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.60 (s, 2H, benzyl CH_2), 2.62 (t, 4H, *J* = 5.5 Hz, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_H 163.56 (benzoxazole C_2), 152.71 (benzoxazole C_{7a}), 145.23 (benzoxazole C_{3a}), 144.24 (benzoxazole C_5), 137.44 (benzyl C_1), 129.12 (benzyl C_2, C_6), 128.43 (benzyl C_3, C_5), 127.42 (benzyl C_4), 117.11 (benzoxazole C_6), 111.70 (benzoxazole C_4), 108.29 (benzoxazole C_7), 62.96 (benzyl CH_2), 52.16 (piperazine C_3, C_5), 45.67 (piperazine C_2, C_6); MS (EI+): m/z calculated for $C_{18}H_{18}N_4O_3$: 338.37, found – 339.39 (M+1).

N-(1-benzylpiperidin-4-yl)-5-nitrobenzo[d]oxazol-2-amine (**28A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compounds **6A** and **17A,** obtained as a brown solid, yield- 82%, M.P.- 167-168 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.04 - 8.02 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 7.51 (m, 1H, benzoxazole C_7), 7.36 - 7.29 (m, 5H, phenyl), 3.87 - 3.84 (m, 1H, piperidin C_1), 3.63 (s, 2H, CH_2), 2.99 - 2.97 (m, 2H, piperidin C_3), 2.35 - 2.29 (m, 2H, piperidin C_5), 2.18 – 2.15 (m, 2H, piperidin C_2), 1.80 - 1.74 (m, 2H, piperidin C_6), 1.44 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 162.85

(benzoxazole C_2), 152.36 (benzoxazole C_{7a}), 145.16 (benzoxazole C_5), 143.83 (benzoxazole C_{3a}), 129.42 (phenyl C_1), 128.80 (phenyl C_6), 128.59 (Phenyl C_2), 128.43 (phenyl C_3, C_5), 127.56 (Phenyl C_4), 117.51 (benzoxazole C_6), 111.87 (benzoxazole C_4), 108.39 (benzoxazole C_7), 62.70 (CH_2), 51.76 (piperidin C_1), 31.86 (piperidin C_3), 29.70 (piperidin C_5), 22.35 (piperidin C_6), 14.07 (piperidin C_2); MS (EI+): m/z calculated for $C_{19}H_{20}N_4O_3$: 352.39, found – 353.47 (M+1).

tert-butyl 4-(5-nitrobenzo[d]oxazol-2-yl)piperazine-1-carboxylate (**40A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.3 using compound **6A** and 1-boc-piperazine**,** obtained as a light brown solid, yield- 82%, M.P.- 172-173 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, J = 2.5 Hz, 1H, benzoxazole C_4), 8.04 - 8.02 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 7.34 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 3.75 (t, *J* = 5.5 Hz, 4H, piperazine C_3, C_5), 3.62 (t, *J* = 5.5 Hz, 4H, piperazine C_2, C_6), 1.51 (s, 9H, tert-butyl). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.42 (benzoxazole C_2), 154.46 (-C=O), 152.65 (benzoxazole C_{7a}), 145.30 (benzoxazole C_{3a}), 143.96 (benzoxazole C_5), 117.38 (benzoxazole C_6), 111.98 (benzoxazole C_4), 108.47 (benzoxazole C_7), 80.67 (-CH, tert-butyl), 45.44 (piperazine C_2, C_3, C_5, C_6), 28.37 (-CH_3, tert-butyl); MS (EI+):m/z calculated for $C_{16}H_{20}N_4O_5$: 348.36, found – 349.14 (M+1).

5.1.3.4 Synthesis of 5-nitro-2-(piperazin-1-yl)benzo[d]oxazole (41A)

A solution of *tert*-butyl 4-(5-nitrobenzo[d]oxazol-2-yl)piperazine-1-carboxylate (**40A**) in dichloromethane was cooled to 0 °C under nitrogen environment. Trifluoroaceticacid (5.0 eq) was added drop wise and resulting solution was stirred at room temperature for 6 h. After completion of the reaction (monitored by TLC), volatile solvents were evaporated under reduced pressure and obtained residue was washed with diethyl ether to get lemon-yellow solid compound (**41A**). The obtained lemon-yellow solid was

collected and used further steps without purification. Yield - 94.6%, M.P.- 123-124 °C, ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ_H 8.04 (d, *J* = 2Hz, 1H, benzoxazole C_4) 7.98 - 7.95 (dd, *J* = 8.5, 2.5 Hz, 1H, benzoxazole C_6), 7.63 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 3.59 (t, *J* = 5.5 Hz, 4H, piperazine C_2, C_6), 2.82 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ_C 164.00 (benzoxazole C_2), 152.94 (benzoxazole C_{7a}), 145.07 (benzoxazole C_{3a}), 144.58 (benzoxazole C_5), 117.32 (benzoxazole C_6), 110.77 (benzoxazole C_4), 109.52 (benzoxazole C_7), 46.81 (piperazine C_2, C_6), 45.27 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{11}H_{12}N_4O_3$: 248.24, found – 249.35 (M+1).

5.1.3.5 General procedure for the synthesis of 64A-78A

To a 25 mL round-bottom flask equipped with magnetic stirrer, 5-nitro-2-(piperazin-1-yl)benzo[d]oxazole (**41A,** 1.0 eq), dichloromethane (3ml), diisopropylethylamine (2.5 eq), carboxylic acid (**42A-56A**; 1.2 eq), and 1-Hydroxybenzotriazole (2.5 eq) were added. The contents were then cooled to 10 °C, and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide.HCl (1.5 eq) was added. The reaction mixture was stirred at room temperature for 4-6 h. The progress of reaction was monitored by TLC followed by the addition of water (10-20 ml). The title compounds (**64A-78A**) were isolated via extractive workup with ethylacetate or dichloromethane as solvent.

(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)(phenyl)methanone (**64A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **42A,** obtained as a yellow solid, yield- 94%, M.P.- 155-156 °C, ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ_H 8.08 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.00 - 7.98 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 7.66 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.49 - 7.44 (m, 5H, phenyl), 3.74 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ_C 169.36 (-C=O), 163.29 (benzoxazole C_2), 152.50 (benzoxazole C_{7a}), 144.67 (benzoxazole C_{3a}), 143.86 (benzoxazole C_5), 135.51 (phenyl C_1), 129.74 (phenyl C_4),

128.50 (phenyl C_3, C_5), 127.01 (phenyl C_2, C_6), 117.13 (benzoxazole C_6), 110.62 (benzoxazole C_4), 109.23 (benzoxazole C_7), 45.13 (piperazine C_2, C_3, C_5, C_6); MS (EI+): *m/z* calculated for $C_{18}H_{16}N_4O_4$: 352.35, found – 353.11 (M+1).

(2-chlorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**65A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **43A,** obtained as a yellow solid, yield- 96%, M.P.- 162-163 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 8.07 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 7.99 - 7.97 (dd, *J* = 8.5, 2.5 Hz, 1H, benzoxazole C_6), 7.65 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.57 - 7.55 (dd, *J* = 7, 1.5 Hz, 1H, chlorophenyl C_3), 7.50 - 7.43 (m, 3H, chlorophenyl C_4, C_5, C_6), 3.89 - 3.65 (m, 8H, piperazine). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 165.84 (-C=O), 163.24 (benzoxazole C_2), 152.49 (benzoxazole C_{7a}), 144.65 (benzoxazole C_{3a}), 143.80 (benzoxazole C_5), 135.33 (chlorophenyl C_1), 130.71 (chlorophenyl C_2), 129.47 (chlorophenyl C_4), 129.14 (chlorophenyl C_3), 128.03 (chlorophenyl C_6), 127.68 (chlorophenyl C_5), 117.15 (benzoxazole C_6), 110.64 (benzoxazole C_4), 109.24 (benzoxazole C_7), 45.35 (piperazine C_2), 45.25 (piperazine C_6), 44.93 (piperazine C_3), 40.31 (piperazine C_5); MS (EI+): *m/z* calculated for $C_{18}H_{15}ClN_4O_4$: 386.79, found – 387.21 (M+), 389.02 (M+2).

(3-chlorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**66A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **44A,** obtained as a brown solid, yield- 94%, M.P.- 158-159 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.05 - 8.03 (dd, *J* = 8, 2 Hz, 1H, benzoxazole C_6), 7.48 - 7.40 (m, 3H, benzoxazole C_7 , chlorophenyl C_2, C_6), 7.36 - 7.33 (m, 2H, chlorophenyl C_4, C_5), 3.80 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.17 (-C=O), 163.16 (benzoxazole C_2), 152.64 (benzoxazole C_{7a}), 145.31 (benzoxazole C_{3a}), 143.72 (benzoxazole C_5), 136.59

(chlorophenyl C_1), 134.93 (chlorophenyl C_3), 130.48 (chlorophenyl C_5), 130.18 (chlorophenyl C_4), 127.37 (chlorophenyl C_2), 125.17 (chlorophenyl C_6), 117.64 (benzoxazole C_6), 112.20 (benzoxazole C_4), 108.67 (benzoxazole C_7), 45.56 (piperazine C_2, C_3, C_5, C_6); MS (EI+): *m/z* calculated for $C_{18}H_{15}ClN_4O_4$: 386.79, found – 387.1171 (M+), 389.1156 (M+2).

(4-chlorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**67A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **45A,** obtained as a yellow solid, yield- 926%, M.P.- 165-166 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.20 (d, *J* = 2Hz, 1H, benzoxazole C_4), 8.06 - 8.03 (dd, *J* = 8, 2 Hz, 1H, benzoxazole C_6), 7.47 - 7.41 (m, 4H, chlorophenyl C_2, C_6, C_3, C_5), 7.36 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 3.80 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.73 (-C=O), 163.16 (benzoxazole C_2), 152.64 (benzoxazole C_{7a}), 145.31 (benzoxazole C_{3a}), 143.73 (benzoxazole C_5), 136.54 (chlorophenyl C_4), 133.17 (chlorophenyl C_1), 129.09 (chlorophenyl C_2, C_6), 128.72 (chlorophenyl C_3, C_5), 117.63 (benzoxazole C_6), 112.18 (benzoxazole C_4), 108.66 (benzoxazole C_7), 45.58 (piperazine C_2, C_3, C_5, C_6); MS (EI+): *m/z* calculated for $C_{18}H_{15}ClN_4O_4$: 386.79, found – 387.0769 (M+), 389.0752 (M+2).

(2-bromophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**68A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **46A,** obtained as a brown solid, yield- 96%, M.P.- 154-155 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (s, 1H, benzoxazole C_4), 8.06 (d, *J* = 9 Hz, 1H, benzoxazole C_6), 7.66 (d, *J* = 8 Hz, 1H, benzoxazole C_7), 7.45 - 7.42 (t, *J* = 7.5 Hz, 1H, bromophenyl C_4), 7.36 - 7.31 (m, 3H, bromophenyl C_3, C_5, C_6), 4.13 (br s, 1H, piperazine C_{3a}), 3.91 - 3.82 (m, 4H, piperazine C_2, C_6), 3.75 (br s, 1H, piperazine C_{3b}), 3.48 (br s, 1H, piperazine C_{5a}), 3.40 (br s, 1H, piperazine C_{5b}). ^{13}C NMR (125 MHz,

$CDCl_3$) δ_C 167.98 (-C=O), 163.19 (benzoxazole C_2), 152.65 (benzoxazole C_{7a}), 145.36 (benzoxazole C_{3a}), 143.77 (benzoxazole C_5), 137.20 (bromophenyl C_1), 133.05 (bromophenyl C_3), 130.83 (bromophenyl C_5), 128.02 (bromophenyl C_4), 127.75 (bromophenyl C_6), 119.10 (bromophenyl C_2), 117.62 (benzoxazole C_6), 112.21 (benzoxazole C_4), 108.63 (benzoxazole C_7), 46.01 (piperazine C_2), 45.74 (piperazine C_6), 45.35 (piperazine C_3), 40.97 (piperazine C_5); MS (EI+): *m/z* calculated for $C_{18}H_{15}BrN_4O_4$: 431.25, found – 431.52 (M+), 433.14 (M+2).

(3-bromophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**69A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **47A,** obtained as a lemon yellow solid, yield- 92%, M.P.- 149-150 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 (s, 1H, bromophenyl C_2), 8.07 (d, *J* = 9Hz, 1H, benzoxazole C_6), 7.64 (m, 2H, benzoxazole C_4, C_7), 7.40 - 7.36 (m, 3H, bromophenyl C_4, C_5, C_6), 3.81 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.02 (-C=O), 163.16 (benzoxazole C_2), 152.65 (benzoxazole C_{7a}), 145.36 (benzoxazole C_{3a}), 143.74 (benzoxazole C_5), 136.82 (bromophenyl C_1), 133.42 (bromophenyl C_4), 130.39 (bromophenyl C_2), 130.23 (bromophenyl C_5), 125.62 (bromophenyl C_6), 122.95 (bromophenyl C_3), 117.66 (benzoxazole C_6), 112.25 (benzoxazole C_4), 108.66 (benzoxazole C_7), 45.58 (piperazine C_2), 31.63 (piperazine C_4, C_5, C_6); MS (EI+): *m/z* calculated for $C_{18}H_{15}BrN_4O_4$: 431.25, found – 431.41 (M+), 433.32 (M+2).

(4-bromophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**70A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **48A,** obtained as a lemon yellow solid, yield- 96%, M.P.- 162-163 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (s, 1H, benzoxazole C_4), 8.04 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6), 7.62 (d, *J* = 7.5Hz, 2H, bromophenyl C_2, C_6), 7.36 (d, *J* =

7.5 Hz, 3H, bromophenyl C_3, C_5 benzoxazole C_7), 3.79 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.75 (-C=O), 163.15 (benzoxazole C_2), 152.64 (benzoxazole C_{7a}), 145.30 (benzoxazole C_{3a}), 143.73 (benzoxazole C_5), 133.65 (bromophenyl C_1), 132.05 (bromophenyl C_3, C_5), 128.89 (bromophenyl C_2, C_6), 124.78 (bromophenyl C_4), 117.63 (benzoxazole C_6), 112.17 (benzoxazole C_4), 108.66 (benzoxazole C_7), 45.57 (piperazine C_2, C_3, C_5, C_6); MS (EI+): *m/z* calculated for $C_{18}H_{15}BrN_4O_4$: 431.25, found – 431.0256 (M+), 433.0235 (M+2).

(2-fluorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**71A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **49A,** obtained as a yellow solid, yield- 95%, M.P.- 148-149 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21(s, 1H, benzoxazole C_4), 8.06 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6), 7.48 (m, 2H, fluorophenyl C_4, C_6), 7.36 (d, *J* = 9 Hz, 1H, benzoxazole C_7), 7.30 (s, 1H, fluorophenyl C_5), 7.18 (t, *J* = 8.5 Hz, 1H, fluorophenyl C_3), 4.00 (s, 2H, piperazine C_3), 3.89 (s, 2H, piperazine C_5), 3.77 (s, 2H, piperazine C_2), 3.54 (s, 2H, piperazine C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.53 (-C=O), 163.21 (benzoxazole C_2), 159.08 (fluorophenyl C_2), 152.66 (benzoxazole C_{7a}), 145.35 (benzoxazole C_{3a}), 143.81 (benzoxazole C_5), 132.01(fluorophenyl C_4), 129.44 (fluorophenyl C_5), 125.07 (fluorophenyl C_1), 123.32 (fluorophenyl C_6), 117.59 (benzoxazole C_6), 116.02 (fluorophenyl C_3) , 112.19 (benzoxazole C_4), 108.62 (benzoxazole C_7), 46.31 (piperazine C_2), 45.86 (piperazine C_6), 45.42 (piperazine C_3), 41.41 (piperazine C_5); MS (EI+):*m/z* calculated for $C_{18}H_{15}FN_4O_4$: 370.34, found – 371.51 (M+1).

(3-fluorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**72A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **50A,** obtained as a yellow solid, yield- 92%, M.P.- 132-133

°C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.21 – 8.19 (m, 1H, fluorophenyl C_2), 8.06 - 8.04 (m 1H, benzoxazole C_6), 7.48 - 7.43 (m, 1H, benzoxazole C_4), 7.37 - 7.35 (m, 1H, benzoxazole C_7), 7.24 - 7.17 (m, 3H, fluorophenyl C_4, C_5, C_6), 3.81 (bs, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.32 (-C=O), 163.64 (fluorophenyl C_3), 163.15 (benzoxazole C_2), 152.61 (benzoxazole C_{7a}), 145.37 (benzoxazole C_{3a}), 143.68 (benzoxazole C_5), 136.89 (fluorophenyl C_1), 130.69 (fluorophenyl C_5), 122.74 (fluorophenyl C_6), 117.66 (benzoxazole C_6), 117.51 (fluorophenyl C_4), 114.58 (fluorophenyl C_2), 112.21 (benzoxazole C_4), 108.66 (benzoxazole C_7), 45.61(piperazine); MS (EI+): *m/z* calculated for $C_{18}H_{15}FN_4O_4$: 370.34, found – 371.32 (M+1).

(4-fluorophenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**73A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **51A,** obtained as a yellow solid, yield- 96 %, M.P.- 155-156 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, *J* = 2.5Hz, 1H, benzoxazole C_6), 8.06 - 8.03 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 7.49 - 7.46 (m, 2H, fluorophenyl C_2, C_6), 7.36 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.18 - 7.13 (m, 2H, fluorophenyl C_3, C_5), 3.80 (bs, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.10 (-C=O), 164.89 (fluorophenyl C_4), 163.29 (benzoxazole C_2), 152.72 (benzoxazole C_{7a}), 145.48 (benzoxazole C_{3a}), 143.78 (benzoxazole C_5), 130.89 (fluorophenyl C_1), 129.69 (fluorophenyl C_2), 126.95 (fluorophenyl C_6), 117.92 (fluorophenyl C_5), 117.77 (benzoxazole C_6), 116.15 (fluorophenyl C_3), 112.29 (benzoxazole C_4), 108.78 (benzoxazole C_7), 45.74, (piperazine); MS (EI+): *m/z* calculated for $C_{18}H_{15}FN_4O_4$: 370.34, found – 371.1378 (M+1).

(2-methoxyphenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**74A**): This compound was synthesized as per general procedure described earlier in section

5.1.3.5 using compounds **41A** and **52A,** obtained as a brown solid, yield- 93%, M.P.- 172-173 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.20 (s, 1H, benzoxazole C_4), 8.05 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6), 7.44 (t, *J* = 8Hz, 1H, methoxyphenyl C_4), 7.36 - 7.29 (m, 2H, benzoxazole C_7, methoxyphenyl C_3), 7.07 (t, *J* = 7.5Hz, 1H, methoxyphenyl C_5), 6.98 (d, J = 8.5Hz, methoxyphenyl C_6), 3.88 (br s, 8H, piperazine), 3.72 (s, 3H, -OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 168.18 (-C=O), 163.29 (benzoxazole C_2), 155.26 (methoxyphenyl C_2), 152.62 (benzoxazole C_{7a}), 145.32 (benzoxazole C_{3a}), 143.78 (benzoxazole C_5), 131.06 (methoxyphenyl C_4), 128.27 (methoxyphenyl C_6), 124.80 (methoxyphenyl C_1), 121.26 (benzoxazole C_6), 117.55 (methoxyphenyl C_5), 112.07 (benzoxazole C_4), 111.03 (benzoxazole C_7), 108.59 (methoxyphenyl C_3), 55.62 (-OCH_3), 46.12 (piperazine C_2), 45.94 (piperazine C_3), 45.48 (piperazine C_5), 41.05 (piperazine C_6); MS (EI+): *m/z* calculated for $C_{19}H_{18}N_4O_5$: 382.38, found – 383.52 (M+1).

(3-methoxyphenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**75A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **53A,** obtained as a brown solid, yield- 95%, M.P.- 165-167 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, *J* = 2Hz, 1H, benzoxazole C_6), 8.05 - 8.03 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 7.36 (br s, 1H, methoxyphenyl C_2), 7.34 - 7.34 (m, 1H, benzoxazole C_7), 7.02 - 6.97 (m, 3H, methoxyphenyl C_4, C_5, C_6), 3.85 (br s, 11H, piperazine, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.67 (-C=O), 163.19 (benzoxazole C_2), 159.84 (methoxyphenyl C_3), 152.59 (benzoxazole C_{7a}), 145.34 (benzoxazole C_{3a}), 143.67 (benzoxazole C_5), 136.01 (methoxyphenyl C_1), 129.91 (methoxyphenyl C_5), 118.99 (methoxyphenyl C_6), 117.62 (benzoxazole C_6), 116.03 (methoxyphenyl C_4), 112.61 (benzoxazole C_4), 112.13 (methoxyphenyl C_2),

108.64 (benzoxazole C_7), 55.42 (OCH_3), 45.68 (piperazine); MS (EI+): *m/z* calculated for $C_{19}H_{18}N_4O_5$: 382.38, found – 383.14 (M+1).

(4-methoxyphenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**76A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **54A,** obtained as a brown solid, yield- 87%, M.P.- 177-178 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.20 (s, 1H, benzoxazole C_4), 8.05 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6), 7.44 (d, *J* = 7.5Hz, 2H, methoxyphenyl C_2, C_6), 7.43 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 6.96 (d, *J* = 7.5Hz, 2H, methoxyphenyl C_3, C_5), 3.87 (s, 3H, -OCH_3), 3.80 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.94 (-C=O), 163.23 (benzoxazole C_2), 161.30 (methoxyphenyl C_4), 152.61(benzoxazole C_{7a}), 145.34 (benzoxazole C_{3a}), 143.73 (benzoxazole C_5), 129.29 (methoxyphenyl C_2, C_6), 126.67 (methoxyphenyl C_1), 117.59 (benzoxazole C_6), 114.00 (methoxyphenyl C_3, C_5), 112.11 (benzoxazole C_4), 108.63 (benzoxazole C_7), 55.43 (-OCH_3), 45.67 (piperazine C_2, C_3, C_5, C_6); MS (EI+): *m/z* calculated for $C_{19}H_{18}N_4O_5$: 382.38, found- 383.22 (M+1).

benzo[d][1,3]dioxol-5-yl(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**77A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **55A,** obtained as a yellow solid, yield- 92%, M.P.- 149-150 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2.5Hz, 1H, benzoxazole C_6), 8.03 - 8.01 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 7.34 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.98 - 6.96 (dd, *J* = 8Hz, 2Hz, 1H, benzo[d][1,3]dioxol-5-yl C_6), 6.94 (d, *J* = 1.5Hz, 1H, benzo[d][1,3]dioxol-5-yl C_4), 6.86 - 6.85 (d, *J* = 8Hz, 1H, benzo[d][1,3]dioxol-5-yl C_7), 6.03 (s, 2H, benzo[d][1,3]dioxol-5-yl C_2), 3.77 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.47 (-C=O), 163.36 (benzoxazole C_2), 152.77 (benzoxazole C_{7a}), 149.53 (benzo[d][1,3]dioxol-5-yl C_{7a}), 148.03

(benzo[d][1,3]dioxol-5-yl C_{3a}), 145.48 (benzoxazole C_{3a}), 143.92 (benzoxazole C_5), 128.51 (benzo[d][1,3]dioxol-5-yl C_5), 121.97 (benzo[d][1,3]dioxol-5-yl C_6), 117.71 (benzoxazole C_6), 112.29 (benzoxazole C_4), 108.74 (benzo[d][1,3]dioxol-5-yl C_4), 108.54 (benzoxazole C_7), 108.22 (benzo[d][1,3]dioxol-5-yl C_7), 101.77 (benzo[d][1,3]dioxol-5-yl C_2), 45.78 (piperazine); MS (EI+): *m/z* calculated for $C_{19}H_{16}N_4O_6$: 396.36, found – 397.45 (M+1).

(5-chloro-2-methoxyphenyl)(4-(5-nitrobenzo[d]oxazol-2-yl)piperazin-1-yl)methanone (**78A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.5 using compounds **41A** and **56A,** obtained as a brown solid, yield- 96%, M.P.- 152-153 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2.5Hz, 1H, benzoxazole C_6), 8.03 - 8.01 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 7.36 - 7.32 (m, 2H, 5-chloro-2-methoxyphenyl C_4, benzoxazole C_7), 7.26 (d, *J* = 1Hz, 1H, 5-chloro-2-methoxy phenyl C_6), 6.89 (d, *J* = 8.5 Hz, 1H, 5-chloro-2-methoxyphenyl C_3), 4.05 (br s, 1H, piperazine), 3.84 (br s, 6H, piperazine, OCH_3), 3.72 - 3.70 (t, 2H, piperazine), 3.46 - 3.39 (m, 2H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.65 (-C=O), 163.37 (benzoxazole C_2), 154.02 (5-chloro-2-methoxyphenyl C_2), 152.78 (benzoxazole C_{7a}), 145.48 (benzoxazole C_{3a}), 143.93 (benzoxazole C_5), 130.86 (5-chloro-2-methoxyphenyl C_4), 128.34 (5-chloro-2-methoxyphenyl C_6), 126.51 (5-chloro-2-methoxyphenyl C_5), 126.43 (5-chloro-2-methoxyphenyl C_1), 117.70 (benzoxazole C_6), 112.55 (benzoxazole C_4), 112.27 (5-chloro-2-methoxyphenyl C_3), 108.73 (benzoxazole C_7), 56.17 (OCH_3), 46.22 - 41.24 (piperazine); MS (EI+): *m/z* calculated for $C_{19}H_{17}ClN_4O_5$: 416.82, found - 417.31 (M+), 419.24 (M+2).

5.1.3.6 General procedure for the synthesis of 79A-85A

To a stirred solution of 5-nitro-2-(piperazin-1-yl)benzo[d]oxazole (**41A,** 1.0 eq) and diisopropylethylamine (2.5 eq) in DMF (2ml), aryl halide (**57A-63A;** 1.2 eq) were

added at 10 °C. The reaction mixture was stirred at 100 °C for 2-3 h. After completion of the reaction (monitored by TLC), it was cooled to room temperature, and was diluted with ice cold water (15ml) and ethylacetate (15ml). The separated organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (eluent: ethylacetate and n-hexane) on silica gel (60–120 mesh) to afford the desired compounds **(79A-85A)**.

2-(4-(4-chlorobenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**79A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **57A,** obtained as a brown solid, yield- 92%, M.P.- 156-157 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2.5 Hz, 1H, benzoxazole C_4), 8.02 - 8.00 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 7.49 - 7.47 (m, 2H, chlorobenzyl C_2, C_6), 7.34 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.23 (d, *J* = 8 Hz, 2H, chlorobenzyl C_3, C_5), 3.76 - 3.74 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.56 (s, 2H, benzyl CH_2), 2.61 - 2.59 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.52 (benzoxazole C_2), 152.73 (benzoxazole C_{7a}), 145.20 (benzoxazole C_{3a}), 144.15 (benzoxazole C_5), 136.57 (chlorobenzyl C_1), 132.34 (chlorobenzyl C_2, C_6), 131.57 (chlorobenzyl C_3, C_5), 125.34 (chlorobenzyl C_4), 117.17 (benzoxazole C_6), 111.69 (benzoxazole C_4), 108.32 (benzoxazole C_7), 62.34 (CH_2), 52.14 (piperazine C_2, C_6), 45.63 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{18}H_{17}ClN_4O_3$: 372.81, found - 373.22 (M+), 375.17 (M+2).

2-(4-(4-bromobenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**80A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **58A,** obtained as a pale brown solid, yield- 87%, M.P.- 138-139 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.17 (d, *J* = 2.5 Hz, 1H, benzoxazole C_4), 8.02 - 8.00 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 7.50 - 7.47 (m, 2H, bromobenzyl C_2,

C_6), 7.32 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.25 (d, *J* = 8 Hz, 2H, bromobenzyl C_3, C_5), 3.77 - 3.75 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.54 (s, 2H, benzyl CH_2), 2.60 - 2.58 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.51 (benzoxazole C_2), 152.70 (benzoxazole C_{7a}), 145.20 (benzoxazole C_{3a}), 144.16 (benzoxazole C_5), 136.55 (bromobenzyl C_1), 131.57 (bromobenzyl C_2, C_6), 130.72 (bromobenzyl C_3, C_5), 121.26 (bromobenzyl C_4), 117.19 (benzoxazole C_6), 111.73 (benzoxazole C_4), 108.35 (benzoxazole C_7), 62.20 (CH_2), 52.11 (piperazine C_2, C_6), 45.61 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{18}H_{17}BrN_4O_3$: 417.26, found - 417.35 (M+), 419.11 (M+2).

2-(4-(4-fluorobenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**81A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **59A,** obtained as a yellow solid, yield- 85%, M.P.- 148-149 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.19 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 8.03 - 8.01 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 7.52 - 7.50 (m, 2H, fluorobenzyl C_2, C_6), 7.34 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.26 (d, *J* = 8 Hz, 2H, fluorobenzyl C_3, C_5), 3.71 - 3.70 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.52 (s, 2H, benzyl CH_2), 2.75 - 2.73 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 164.24 (fluorobenzyl C_4), 163.53 (benzoxazole C_2), 152.72 (benzoxazole C_{7a}), 145.25 (benzoxazole C_{3a}), 144.21 (benzoxazole C_5), 137.32 (fluorobenzyl C_1), 130.45 (fluorobenzyl C_2), 129.57 (fluorobenzyl C_6), 119.34 (fluorobenzyl C_5), 117.17 (benzoxazole C_6), 115.32 (fluorobenzyl C_3), 111.28 (benzoxazole C_4), 108.38 (benzoxazole C_7), 62.15 (CH_2), 52.23 (piperazine C_2, C_6), 45.68 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{18}H_{17}FN_4O_3$: 417.26, found - 418.44 (M+1).

2-(4-(4-methylbenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**82A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using

compounds **41A** and **60A,** obtained as a brown solid, yield- 89%, M.P.- 172-173 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 8.02 - 8.00 (dd, *J* = 9Hz, 2,5Hz, 1H, benzoxazole C_6), 7.32 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.25 (d, *J* = 7.5Hz, 2H, methylbenzyl C_2, C_6), 7.19 (d, *J* = 7.5Hz, 2H, methylbenzyl C_3, C_5), 3.77 - 3.75 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.56 (s, 2H, benzyl CH_2), 2.61 - 2.59 (t, *J* = 5Hz, 4H, piperazine C_3, C_5), 2.37 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.54 (benzoxazole C_2), 152.70 (benzoxazole C_{7a}), 145.20 (benzoxazole C_{3a}), 144.21 (benzoxazole C_5), 137.13 (methylbenzyl C_1), 134.24 (methylbenzyl C_4), 129.15 (methylbenzyl C_2, C_6), 129.11 (methylbenzyl C_3, C_5), 117.15 (benzoxazole C_6), 111.68 (benzoxazole C_4), 108.31 (benzoxazole C_7), 62.69 (CH_2), 52.09 (piperazine C_2, C_6), 45.63 (piperazine C_3, C_5), 21.15 (CH_3). MS (EI+): *m/z* calculated for $C_{19}H_{20}N_4O_3$: 352.39, found – 353.28 (M+1).

2-(4-(4-methoxybenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**83A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **61A,** obtained as a pale brown solid, yield- 87%, M.P.- 152-153 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.20 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 8.05 -8.02 (dd, *J* = 8.5 Hz, 2.5Hz, 1H, benzoxazole C_6), 7.37 (d, *J* = 7.5Hz, 2H, methoxybenzyl C_2, C_6), 7.32 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 6.96 (d, *J* = 7.5Hz, 2H, methoxybenzyl C_3, C_5), 3.78 (s, 3H, -OCH_3), 3.7 - 3.71 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.54 (s, 2H, CH_2), 2.68 – 2.67 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.27 (benzoxazole C_2), 161.35 (methoxybenzyl C_4), 152.54 (benzoxazole C_{7a}), 145.27 (benzoxazole C_{3a}), 143.85 (benzoxazole C_5), 128.52 (methoxybenzyl C_2, C_6), 125.82 (methoxybenzyl C_1), 117.47 (benzoxazole C_6), 115.27 (methoxybenzyl C_3, C_5), 111.17 (benzoxazole C_4), 108.57 (benzoxazole C_7), 62.23 (CH_2), 57.36 (-OCH_3), 52.71

(piperazine C_2, C_6), 45.85 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{19}H_{20}N_4O_4$: 368.39, found – 369.20 (M+1).

2-(4-(4-isopropylbenzyl)piperazin-1-yl)-5-nitrobenzo[d]oxazole (**84A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **62A,** obtained as a pale yellow solid, yield- 92%, M.P.- 177-178 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 8.02 - 8.00 (dd, *J* = 9Hz, 2,5Hz, 1H, benzoxazole C_6), 7.32 (d, *J* = 10Hz, 1H, benzoxazole C_7), 7.27 (d, *J* = 5Hz, 2H, isopropylbenzyl C_2, C_6), 7.23 (d, *J* = 5Hz, 2H, isopropylbenzyl C_3, C_5), 3.78 - 3.76 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.57 (s, 2H, benzyl CH_2), 2.98 – 2.89 (m, 1H, CH), 2.62 – 2.59 (t, *J* = 5Hz, 4H, piperazine C_3, C_5), 1.29 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.57 (benzoxazole C_2), 152.71 (benzoxazole C_{7a}), 148.12 (isopropylbenzyl C_4), 145.23 (benzoxazole C_{3a}), 144.25 (benzoxazole C_5), 134.68 (isopropylbenzyl C_1), 129.11 (isopropylbenzyl C_3, C_5), 126.46 (isopropylbenzyl C_2, C_6), 117.11 (benzoxazole C_6), 111.69 (benzoxazole C_4), 108.28 (benzoxazole C_7), 62.72 (CH_2), 52.15 (piperazine C_2, C_6), 45.67 (piperazine C_3, C_5), 33.80 (CH), 24.02 (2*CH_3); MS (EI+): *m/z* calculated for $C_{21}H_{24}N_4O_3$: 380.45, found – 381.37 (M+1).

5-nitro-2-(4-phenethylpiperazin-1-yl)benzo[d]oxazole (**85A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.6 using compounds **41A** and **63A,** obtained as a pale brown solid, yield- 89%, M.P.- 152-153 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.16 (d, *J* = 2Hz, 1H, benzoxazole C_6), 8.06 - 8.04 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 7.73 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.37 - 7.26 (m, 5H, 4-phenethylpiperazin-1-yl), 4.32 (br s, 2H, -CH_2), 3.69 (bs, 8H, piperazine), 3.07 (bs, 2H, -CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.85 (benzoxazole C_2), 152.72 (benzoxazole C_{7a}), 145.97 (benzoxazole C_{3a}), 143.38 (benzoxazole C_5), 138.64 (4-phenethylpiperazin-1-yl C_1), 129.19 (4-phenethylpiperazin-1-yl C_3, C_5),

129.06 (4-phenethylpiperazin-1-yl C_2, C_6), 126.36 (4-phenethylpiperazin-1-yl C_4), 117.65 (benzoxazole C_6), 112.38 (benzoxazole C_4), 108.56 (benzoxazole C_7), 56.08 (-CH_2), 47.94 (piperazine), 33.53 (-CH_2); MS (EI+): *m/z* calculated for $C_{19}H_{20}N_4O_3$: 352.39, found – 353.40 (M+1).

5.1.3.7 General procedure for the synthesis of 29A-39A; 86A-107A

To a stirred solution of nitro compounds (**18A-28A; 64A-85A**, 1.0 eq) in 2 ml of ethanol:tetrahydrofuran (1:1), iron powder was added. Ammoniumchloride (2.5 eq) in 1 ml distilled water was added drop wise and reaction mixture was heated to 70 °C for 1.5 to 3 h. The progress of reaction was monitored by TLC. After completion of the reaction, mixture was passed through celite bed filter and washed twice with ethylacetate (2*10ml). The combined organic phases were passed through sodium sulphate and evaporated under reduced pressure. The obtained crude residue was further purified by column chromatography (eluent: ethylacetate and n-hexane) on silica gel (60–120 mesh) to afford the title compounds **(29A-39A; 86A-107A).**

2-(piperidin-1-yl)benzo[d]oxazol-5-amine (**29A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **18A** and obtained as a pale Brown solid, yield- 92%, M.P.- 165-166 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.05 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 6.64 (d, *J* = 2.5 Hz, benzoxazole C_6), 6.42 – 6.39 (dd, *J* = 8.5Hz, 2Hz, 1H, benzoxazole C_4), 3.69 (s, 4H, piperidin-1-yl C_2, C_6), 1.75 (s, 6H, piperidin-1-yl C_3, C_4, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 162.57 (benzoxazole C_2), 153.24 (benzoxazole C_{7a}), 146.57 (benzoxazole C_{3a}), 143.44 (benzoxazole C_5), 115.54 (benzoxazole C_7), 111.27 (benzoxazole C_4), 108.34 (benzoxazole C_6), 46.28 (piperidin-1-yl C_2, C_6), 24.65 (piperidin-1-yl C_3, C_5), 23.62 (piperidin-1-yl C_4); MS (EI+): *m/z* calculated for $C_{12}H_{15}N_3O$: 217.27, found - 218.34 (M+1).

2-morpholinobenzo[d]oxazol-5-amine (**30A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **19A** and obtained as a yellow Solid, yield- 92%, M.P.- 142 – 143 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.06 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 6.78 (d, *J* = 2.5 Hz, benzoxazole C_6), 6.37 – 6.35 (dd, *J* = 8.5Hz, 2Hz, 1H, benzoxazole C_4), 3.86 - 3.84 (m, 4H, morpholine C_2, C_6), 3.56 – 3.54 (m, 4H, morpholine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.41 (benzoxazole C_2), 153.32 (benzoxazole C_{7a}), 145.21 (benzoxazole C_{3a}), 144.35 (benzoxazole C_5), 116.57 (benzoxazole C_7), 111.36 (benzoxazole C_4), 107.45 (benzoxazole C_6), 64.24 (morpholine C_2, C_6), 43.47 (morpholine C_3, C_5); MS (EI+): *m/z* calculated for $C_{12}H_{15}N_3O$: 219.24, found - 220.11 (M+1).

2-(4-phenylpiperazin-1-yl)benzo[d]oxazol-5-amine (**31A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **20A** and obtained as a brown solid, yield- 85%, M.P.- 172-173 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.48 (d, *J* = 8Hz, 2H, phenyl C_2, C_6), 7.35 (d, *J* = 8Hz, 2H, phenyl C_3, C_5), 7.02 (d, *J* = 2Hz, 1H, benzoxazole C_7), 6.87 - 6.64 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 6.36 - 6.32 (m, 2H, benzoxazole C_4, phenyl C_4), 3.57 (t, *J* = 5.5Hz, 4H, piperazine C_3, C_5), 3.27 (t, *J* = 5.5Hz, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.27 (benzoxazole C_2), 152.14 (benzoxazole C_{7a}), 151.37 (phenyl C_1), 145.56 (benzoxazole C_5), 144.45 (benzoxazole C_{3a}), 129.74 (phenyl C_3, C_5), 121.53 (phenyl C_4), 117.27 (benzoxazole C_7), 117.10 (phenyl C_2, C_6), 111.74 (benzoxazole C_4), 107.94 (benzoxazole C_6), 49.76 (piperazine C_3, C_5), 45.57 (piperazine C_2, C_6); MS (EI+):*m/z* calculated for $C_{17}H_{18}N_4O$: 294.36, found – 295.42 (M+1).

2-(4-(4-chlorophenyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**32A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **21A** and obtained as a brown solid, yield- 86%, M.P.- 178-179 °C, ^{1}H NMR

(500 MHz, $CDCl_3$) δ_H 7.59 - 7.57 (m, 2H, chlorophenyl C_3, C_5), 7.42 – 7.40 (m, 2H, chlorophenyl C_2, C_6), 7.07 (d, *J* = 2Hz, 1H, benzoxazole C_7), 6.84 - 6.61 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 6.37 - 6.33 (m, 2H, benzoxazole C_4, phenyl C_4), 3.91 - 3.89 (m, 4H, piperidin-1-yl C_2, C_6), 3.46 - 3.44 (m, 4H, piperidin-1-yl C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.32 (benzoxazole C_2), 152.67 (benzoxazole C_{7a}), 148.52 (4-chlorophenyl C_1), 145.24 (benzoxazole C_{3a}), 144.51 (benzoxazole C_5), 127.84 (4-chlorophenyl C_3, C_5), 123.86 (4-chlorophenyl C_4), 119.54 (4-chlorophenyl C_2, C_6), 117.71 (benzoxazole C_7), 111.47 (benzoxazole C_4), 108.51 (benzoxazole C_6), 47.52 (piperazine C_3, C_5), 44.72 (piperazine C_2, C_6); MS (EI+): *m/z* calculated for $C_{17}H_{17}ClN_4O$: 328.80, found – 329.1453 (M+), 331.1408 (M+2).

2-(4-(4-bromophenyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**33A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **22A** and obtained as a pale brown solid, yield- 88%, M.P.- 154-155 °C, ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.67 (d, *J* = 8.5Hz, 2H, bromophenyl C_3, C_5), 7.54 (d, *J* = 9Hz, 2H bromophenyl C_2, C_6), 7.14 (d, *J* = 2.5Hz, 1H, benzoxazole C_7), 6.75 - 6.73 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 6.37 (d, *J* = 8.5Hz, 1H, benzoxazole C_4), 3.92 - 3.90 (t, *J* = 5.5Hz, 4H, piperazine C_3, C_5), 3.43 - 3.41 (t, *J* = 5.5Hz, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.47 (benzoxazole C_2), 152.72 (benzoxazole C_{7a}), 149.82 (bromophenyl C_1), 145.24 (benzoxazole C_5), 144.07 (benzoxazole C_{3a}), 133.74 (bromophenyl C_3, C_5), 119.27 (bromophenyl C_2, C_6), 117.54 (benzoxazole C_7), 114.27 (bromophenyl C_4), 111.52 (benzoxazole C_4), 108.34 (benzoxazole C_6), 49.56 (piperazine C_3, C_5), 44.48 (piperazine C_2, C_6); MS (EI+):*m/z* calculated for $C_{17}H_{17}BrN_4O$: 373.25, found – 373.41 (M+), 375.34 (M+2).

2-(4-(4-fluorophenyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**34A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using

compound **23A** and obtained as a brown solid, yield- 81%, M.P.- 167-168 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.48 - 7.46 (m, 2H, fluorophenyl C_3, C_5), 7.32 (br s, 2H, fluorophenyl C_2, C_6), 7.17 (s, 1H, benzoxazole C_7), 6.82 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6) 6.42 (d, *J* = 8.5 Hz, 1H, benzoxazole C_4), 3.94 (s, 4H, piperazine C_3, C_5), 3.37 (s, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.38 (benzoxazole C_2), 158.72 (fluorophenyl C_4), 156.84 (fluorophenyl C_1), 152.73 (benzoxazole C_{7a}), 147.62 (benzoxazole C_{3a}), 145.31 (benzoxazole C_5), 118.24 (fluorophenyl C_3), 119.71 (fluorophenyl C_5), 117.28 (fluorophenyl C_2), 115.34 (fluorophenyl C_6), 115.27 (benzoxazole C_7), 111.38 (benzoxazole C_4), 108.37 (benzoxazole C_6), 50.38 (piperazine C_2, C_6), 47.42 (piperazine C_3, C_5); MS (EI+):*m/z* calculated for $C_{17}H_{17}FN_4O$: 312.35, found – 313.57 (M+1).

2-(4-(p-tolyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**35A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **24A** and obtained as a brown solid, yield- 84%, M.P.- 177-178 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 (d, *J* = 8Hz, 2H, p-tolyl C_3, C_5), 7.37 (d, *J* = 8.5Hz, 2H, p-tolyl C_2, C_6), 7.05 (d, *J* = 2Hz, 1H, benzoxazole C_7), 6.73 – 6.71 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_6), 6.42 (d, *J* = 9Hz, 1H, benzoxazole C_4), 3.82 - 3.80 (t, *J* = 5.5Hz, 4H, piperazine C_3, C_5), 3.37 - 3.35 (t, *J* = 5.5Hz, 4H, piperazine C_2, C_6), 2.74 (s, 3H, -CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.62 (benzoxazole C_2), 152.37 (benzoxazole C_{7a}), 149.64 (p-tolyl C_1), 145.32 (benzoxazole C_5), 144.50 (benzoxazole C_{3a}), 131.37 (p-tolyl C_4), 128.67 (p-tolyl C_3, C_5), 117.34 (p-tolyl C_2, C_4), 117.27 (benzoxazole C_7), 111.45 (benzoxazole C_4), 107.87 (benzoxazole C_6), 49.34 (piperazine C_3, C_5), 45.18 (piperazine C_2, C_6), 20.65 (-CH_3); MS (EI+):*m/z* calculated for $C_{18}H_{20}N_4O$: 308.39, found – 309.37 (M+1).

2-(4-(4-methoxyphenyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**36A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **25A** and obtained as a brown solid, yield- 92%, M.P.- 164-165 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.82 – 7.79 (m, 2H, methoxyphenyl C_2, C_6), 7.64 – 7.61 (m, 3H, methoxyphenyl C_3, C_5, benzoxazole C_7), 7.05 -7.03 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_6), 6.82 – 6.80 (dd, *J* = 8.5Hz, 1H, benzoxazole C_4), 3.98 - 3.97 (t, 4H, piperazine C_3, C_5), 3.85 (s, 3H, -OCH_3), 3.42 - 3.40 (t, 4H, piperazine C_2, C_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.34 (benzoxazole C_2), 154.42 (benzoxazole C_{7a}), 153.53 (methoxyphenyl C_4), 145.31 (benzoxazole C_5), 145.72 (methoxyphenyl C_1), 144.17 (benzoxazole C_{3a}), 119.54 (methoxyphenyl C_2, C_6), 117.23 (benzoxazole C_7), 114.57 (methoxyphenyl C_3, C_5), 111.82 (benzoxazole C_4), 108.47 (benzoxazole C_6), 55.25 (-OCH_3), 50.15 (piperazine C_2, C_6), 44.94 (piperazine C_3, C_5); MS (EI+): *m/z* calculated for $C_{18}H_{20}N_4O_2$: 324.38, found – 325.42 (M+1).

2-(4-(pyridin-2-yl)piperazin-1-yl)benzo[d]oxazol-5-amine (**37A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **26A** and obtained as a brown solid, yield- 85%, M.P.- 183-184 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.17 - 8.15 (dd, *J* = 5Hz, 1Hz, 1H, pyridin-2-yl C_3), 7.98 - 7.95 (m, 1H, pyridin-2-yl C_5), 7.62 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.21 – 7.19 (m, 3H, benzoxazole C_4, pyridin-2-yl C_4, C_6), 7.05 - 7.02 (dd, *J* = 2.5Hz, 2Hz, 1H, benzoxazole C_6), 3.92 - 3.90 (m, 4H, piperazine C_2, C_6), 3.73 - 3.71 (m, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.27 (benzoxazole C_2), 158.74 (pyridin-2-yl C_1), 152.74 (benzoxazole C_{7a}), 149.17 (pyridin-2-yl C_3), 145.24 (benzoxazole C_5), 144.04 (benzoxazole C_{3a}), 136.27 (pyridin-2-yl C_5), 117.21 (benzoxazole C_7), 115.47 (pyridin-2-yl C_4), 111.25 (benzoxazole C_4), 108.41 (benzoxazole C_6), 106.95 (pyridin-2-yl C_6),

47.67 (piperazine C_2, C_6), 44.31 (piperazine C_3, C_5); MS (EI+):*m/z* calculated for $C_{16}H_{17}N_5O$: 295.35, found – 296.27 (M+1).

2-(4-benzylpiperazin-1-yl)benzo[d]oxazol-5-amine (**38A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **27A** and obtained as a brown solid, yield-86 %, M.P.- 163-164 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.02 (d, *J* = 2Hz, 1H, benzoxazole C_7), 7.85 - 7.82 (m, 4H, benzyl C_2, C_3, C_5, C_6), 7.63 (d, *J* = 2Hz, 1H, benzoxazole C_4), 7.33 - 7.30 (m, 2H, benzoxazole C_6, benzyl C_4), 3.82 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.54 (s, 2H, benzyl CH_2), 2.86 (t, 4H, *J* = 5.5 Hz, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.75 (benzoxazole C_2), 152.63 (benzoxazole C_{7a}), 145.34 (benzoxazole C_{3a}), 144.15 (benzoxazole C_5), 136.37 (benzyl C_1), 129.86 (benzyl C_2, C_6), 128.75 (benzyl C_3, C_5), 125.34 (benzyl C_4), 117.32 (benzoxazole C_7), 111.61 (benzoxazole C_4), 108.33 (benzoxazole C_6), 63.51 (benzyl CH_2), 53.25 (piperazine C_3, C_5), 43.28 (piperazine C_2, C_6); MS (EI+):*m/z* calculated for $C_{18}H_{20}N_4O$: 308.39, found – 309.20 (M+1).

N2-(1-benzylpiperidin-4-yl)benzo[d]oxazole-2,5-diamine (**39A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **28A** and obtained as a brown solid, yield- 76%, M.P.- 157-158 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.12 (d, *J* = 2Hz, 1H, benzoxazole C_7), 8.02 - 8.00 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 7.62 - 7.55 (m, 5H, phenyl), 7.42 (m, 1H, benzoxazole C_7), 4.92 (br s, 2H, NH_2), 3.72 - 3.69 (m, 1H, piperidin C_1), 3.48 (s, 2H, CH_2), 2.84 - 2.82 (m, 2H, piperidin C_3), 2.37 - 2.35 (m, 2H, piperidin C_5), 2.16 – 2.13 (m, 2H, piperidin C_2), 1.92 - 1.89 (m, 2H, piperidin C_6), 1.47 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.46 (benzoxazole C_2), 152.41 (benzoxazole C_{7a}), 145.34 (benzoxazole C_5), 143.27 (benzoxazole C_{3a}), 129.17 (phenyl C_1), 128.62 (phenyl C_6), 128.57 (Phenyl C_2), 128.42 (phenyl C_3, C_5), 126.27 (Phenyl C_4), 117.28 (benzoxazole

C_7), 111.87 (benzoxazole C_4), 108.45 (benzoxazole C_6), 65.24 (CH_2), 51.32 (piperidin C_1), 31.54 (piperidin C_3), 29.22 (piperidin C_5), 23.61 (piperidin C_6), 13.85 (piperidin C_2); MS (EI+): *m/z* calculated for $C_{19}H_{22}N_4O$: 322.41, found – 323.54 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(phenyl)methanone (**86A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **64A** and obtained as a yellow solid, yield- 86%, M.P.- 146-147 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.57 - 7.52 (m, 5H, phenyl), 7.21 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.00 - 6.98 (dd, *J* = 9, 2.5 Hz, 1H, benzoxazole C_6), 6.52 (d, *J* = 2Hz, 1H, benzoxazole C_4), 3.57 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 169.29 (-C=O), 163.15 (benzoxazole C_2), 152.47 (benzoxazole C_{7a}), 144.32 (benzoxazole C_{3a}), 143.48 (benzoxazole C_5), 136.15 (phenyl C_1), 129.37 (phenyl C_4), 128.10 (phenyl C_3, C_5), 127.32 (phenyl C_2, C_6), 115.25 (benzoxazole C_7), 110.14 (benzoxazole C_4), 109.37 (benzoxazole C_6), 46.57 (piperazine C_2, C_3, C_5, C_6); MS (EI+):*m/z* calculated for $C_{18}H_{18}N_4O_2$: 322.37, found – 323.20 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(2-chlorophenyl)methanone (**87A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **65A** and obtained as a yellow solid, yield- 82%, M.P.- 151-152 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.64 - 7.62 (dd, *J* = 7, 1.5 Hz, 1H, chlorophenyl C_3), 7.59 - 7.51 (m, 3H, chlorophenyl C_4, C_5, C_6), 3.72 - 3.65 (m, 8H, piperazine). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 166.24 (-C=O), 163.35 (benzoxazole C_2), 152.51 (benzoxazole C_{7a}), 144.42 (benzoxazole C_{3a}), 143.74 (benzoxazole C_5), 133.93 (chlorophenyl C_1), 130.25 (chlorophenyl C_2), 126.28 (chlorophenyl C_4), 126.14 (chlorophenyl C_3), 124.68 (chlorophenyl C_6), 127.11 (chlorophenyl C_5), 117.32 (benzoxazole C_7), 110.20 (benzoxazole C_4), 109.44 (benzoxazole C_6), 55.26 (piperazine C_2), 47.94 (piperazine

C_6), 46.34 (piperazine C_3), 44.71 (piperazine C_5); MS (EI+):*m/z* calculated for $C_{18}H_{17}ClN_4O_2$: 356.81, found – 357.26 (M+), 359.30 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(3-chlorophenyl)methanone (**88A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **66A** and obtained as a pale brown solid, yield- 87%, M.P.- 135-136 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.47 -7.45 (m, 2H, chlorophenyl C_2, C_6), 7.42 - 7.39 (m, 1H, chlorophenyl C_4), 7.34 - 7.32 (td, *J* = 7.5Hz, 1.5Hz, 1H, chlorophenyl C_5), 7.07 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.74 (d, *J* = 2Hz, benzoxazole C_6), 6.43 - 6.41 (dd, *J* = 8.5Hz, 2Hz, benzoxazole C_4), 3.52 - 3.48 (q, 4H, piperazine C_2, C_6), 1.24 - 1.21 (t, *J* = 7Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.10 (-C=O), 162.18 (benzoxazole C_2), 143.66 (benzoxazole C_5), 142.69 (benzoxazole C_{3a}), 136.89 (chlorophenyl C_3), 134.88 (benzoxazole C_{7a}), 130.30 (chlorophenyl C_1), 130.08 (chlorophenyl C_4, C_5), 127.35 (chlorophenyl C_2), 125.14 (chlorophenyl C_6), 108.95 (benzoxazole C_7), 108.71 (benzoxazole C_4), 103.40 (benzoxazole C_6), 65.85 (piperazine C_2, C_6), 15.26 (piperazine C3, C_5); MS (EI+): *m/z* calculated for $C_{18}H_{17}ClN_4O_2$: 356.81, found - 357.16 (M+), 359.11 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-chlorophenyl)methanone (**89A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **67A** and obtained as a brown solid, yield- 88%, M.P.- 149-150 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.57 - 7.52 (m, 4H, chlorophenyl C_2, C_6, C_3, C_5), 7.36 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.20 (d, *J* = 2Hz, 1H, benzoxazole C_4), 7.07 - 7.04 (dd, *J* = 8, 2 Hz, 1H, benzoxazole C_6), 3.75 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.52 (-C=O), 163.25 (benzoxazole C_2), 152.11 (benzoxazole C_{7a}), 145.24 (benzoxazole C_{3a}), 143.81 (benzoxazole C_5), 137.32 (chlorophenyl C_4), 133.54 (chlorophenyl C_1), 129.12 (chlorophenyl C_2,C_6), 128.33 (chlorophenyl C_3,C_5), 117.45

(benzoxazole C_7), 112.58 (benzoxazole C_4), 108.24 (benzoxazole C_6), 45.37 (piperazine C_2, C_3, C_5, C_6); MS (EI+):*m/z* calculated for $C_{18}H_{17}ClN_4O_2$: 356.81, found – 357.11 (M+), 359.20 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(2-bromophenyl)methanone (**90A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **68A** and obtained as a brown solid, yield- 85%, M.P.- 163-164 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.53 - 7.50 (t, *J* = 7.5 Hz, 1H, bromophenyl C_4), 7.37 - 7.32 (m, 3H, bromophenyl C_3 C_5, C_6), 7.05 (s, 1H, benzoxazole C_7), 6.75 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 6.52 - 6.50 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 4.17 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.92 (-C=O), 163.27 (benzoxazole C_2), 145.38 (benzoxazole C_{7a}), 143.36 (benzoxazole C_{3a}), 142.54 (benzoxazole C_5), 134.20 (bromophenyl C_1), 133.24 (bromophenyl C_3), 131.11 (bromophenyl C_5), 126.02 (bromophenyl C_4), 125.75 (bromophenyl C_6), 119.10 (bromophenyl C_2), 110.74 (benzoxazole C_7), 108.52 (benzoxazole C_4), 105.62 (benzoxazole C_6), 46.07 (piperazine); MS (EI+):*m/z* calculated for $C_{18}H_{17}BrN_4O_2$: 401.26, found – 401.21 (M+), 403.34 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(3-bromophenyl)methanone (**91A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **69A** and obtained as a brown solid, yield- 82%, M.P.- 166-167 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.51 (s, 1H, bromophenyl C_2), 7.46 - 7.42 (m, 3H, bromophenyl C_4, C_5, C_6), 7.21 (d, *J* = 9Hz, 1H, benzoxazole C_7), 6.97 – 6.95 (m, 2H, benzoxazole C_4, C_6), 3.81 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.32 (-C=O), 163.33 (benzoxazole C_2), 144.42 (benzoxazole C_{7a}), 143.73 (benzoxazole C_{3a}), 143.21 (benzoxazole C_5), 137.52 (bromophenyl C_1), 132.63 (bromophenyl C_4), 130.74 (bromophenyl C_2), 130.20 (bromophenyl C_5), 124.37 (bromophenyl C_6), 122.44

(bromophenyl C_3), 111.51 (benzoxazole C_7), 108.51 (benzoxazole C_4), 105.57 (benzoxazole C_6), 45.58 (piperazine C_2); MS (EI+):*m/z* calculated for $C_{18}H_{17}BrN_4O_2$: 401.26, found – 401.17 (M+), 403.26 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone (**92A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **70A** and obtained as a brown solid, yield- 82%, M.P.- 158-159 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 - 7.60 (d, *J* = 8.5Hz, 2H, bromophenyl C_2, C_6), 7.35 - 7.33 (d, *J* = 8.5Hz, 2H, bromophenyl C_3, C_5), 7.07 - 7.05 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.73 (d, *J* = 2Hz, 1H, benzoxazole C_4), 6.42 - 6.40 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 3.72 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.68 (-C=O), 162.20 (benzoxazole C_2), 143.72 (benzoxazole C_{7a}), 143.55 (benzoxazole C_{3a}), 142.68 (benzoxazole C_5), 133.95 (bromophenyl C_1), 131.97 (bromophenyl C_3, C_5), 128.86 (bromophenyl C_2, C_6), 124.57 (bromophenyl C_4), 108.93 (benzoxazole C_7), 108.65 (benzoxazole C_4), 103.36 (benzoxazole C_6), 45.54 (piperazine); MS (EI+):*m/z* calculated for $C_{18}H_{17}BrN_4O_2$: 401.26, found – 401.0952 (M+), 403.0924 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(2-fluorophenyl)methanone (**93A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **71A** and obtained as a brown solid, yield- 86%, M.P.- 144-145 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.49 - 7.46 (t, *J* = 7.5 Hz, 1H, fluorophenyl C_4), 7.35 - 7.30 (m, 3H, fluorophenyl C_3 C_5, C_6), 7.04 (s, 1H, benzoxazole C_7), 6.75 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 6.48 - 6.46 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 3.97 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_H 169.72 (-C=O), 163.11 (benzoxazole C_2), 159.37 (fluorophenyl C_2), 145.24 (benzoxazole C_{7a}), 143.62 (benzoxazole C_{3a}), 142.25 (benzoxazole C_5), 132.14 (fluorophenyl C_5), 131.24 (fluorophenyl C_6), 129.35

(fluorophenyl C_1), 126.74 (fluorophenyl C_4), 115.21 (fluorophenyl C_3), 109.05 (benzoxazole C_7), 108.57 (benzoxazole C_4), 105.14 (benzoxazole C_6), 46.12 (piperazine); MS (EI+):*m/z* calculated for $C_{18}H_{17}FN_4O_2$: 340.36, found –341.48 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(3-fluorophenyl)methanone (**94A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **72A** and obtained as a pale brown solid, yield – 82%, M.P.- 177-178 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.50 (s, 1H, fluorophenyl C_2), 7.48 - 7.44 (m, 3H, fluorophenyl C_4, C_5, C_6), 7.15 (d, *J* = 9Hz, 1H, benzoxazole C_7), 6.84 – 6.80 (m, 2H, benzoxazole C_4, C_6), 3.67 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.32 (-C=O), 163.78 (fluorophenyl C_3), 163.32 (benzoxazole C_2), 144.51 (benzoxazole C_{7a}), 143.75 (benzoxazole C_{3a}), 143.56 (fluorophenyl C_1), 143.32 (benzoxazole C_5), 132.41 (fluorophenyl C_5), 124.33 (fluorophenyl C_6), 116.74 (fluorophenyl C_4), 115.87 (fluorophenyl C_2), 109.24 (benzoxazole C_7), 108.51 (benzoxazole C_4), 105.41 (benzoxazole C_6), 45.52 (piperazine C_2); MS (EI+):*m/z* calculated for $C_{18}H_{17}FN_4O_2$: 340.36, found –341.30 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-fluorophenyl)methanone (**95A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **73A** and obtained as a brown solid, yield- 76%, M.P.- 168-169 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.49 - 7.45 (m, 2H, fluorophenyl C_2, C_6), 7.17 - 7.13 (m, 2H, fluorophenyl C_3, C_5), 7.06 (d, *J* = 8.5Hz, 1H, benzoxazole C_7) 6.73 (d, *J* = 2Hz, 1H, benzoxazole C_4), 6.42 - 6.40 (dd, *J* = 8.5Hz, 2.5Hz, 1H, benzoxazole C_6), 3.72 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.79 (-C=O), 164.65 (fluorophenyl C_4), 162.23 (benzoxazole C_2), 143.76 (benzoxazole C_{7a}), 143.57 (benzoxazole C_{3a}), 142.67 (benzoxazole C_5), 131.18 (fluorophenyl C_1), 129.53 (fluorophenyl C_6), 129.47 (fluorophenyl C_2), 115.92 (fluorophenyl C_3), 115.74 (fluorophenyl C_5), 108.92

(benzoxazole C_7), 108.60 (benzoxazole C_4), 103.34 (benzoxazole C_6), 45.61 (piperazine); MS (EI+):*m/z* calculated for $C_{18}H_{17}FN_4O_2$: 340.36, found –341.1633 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(2-methoxyphenyl)methanone (**96A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **74A** and obtained as a brown solid, yield- 83%, M.P.- 144-145 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.46 – 7.43 (t, *J* = 8Hz, 1H, methoxyphenyl C_4), 7.36 - 7.30 (m, 2H, benzoxazole C_7, methoxyphenyl C_3), 7.24 (t, *J* = 7.5Hz, 1H, methoxyphenyl C_5), 6.98 (d, *J* = 8.5Hz, 1H, methoxyphenyl C_6), 6.76 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 6.43 (d, *J* = 8.5 Hz, 1H, benzoxazole C_6), 3.85 (br s, 8H, piperazine), 3.74 (s, 3H, -OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.98 (-C=O), 163.15 (benzoxazole C_2), 155.53 (methoxyphenyl C_2), 145.36 (benzoxazole C_{7a}), 143.47 (benzoxazole C_{3a}), 142.81 (benzoxazole C_5), 131.20 (methoxyphenyl C_4), 128.51 (methoxyphenyl C_6), 125.10 (methoxyphenyl C_1), 119.72 (methoxyphenyl C_5), 109.05 (benzoxazole C_7), 108.59 (methoxyphenyl C_3), 108.07 (benzoxazole C_4), 105.14 (benzoxazole C_6), 55.37 (-OCH_3), 45.24 (piperazine); MS (EI+):*m/z* calculated for $C_{19}H_{20}N_4O_3$: 352.39, found –353.45 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(3-methoxyphenyl)methanone (**97A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **75A** and obtained as a brown solid, yield- 86%, M.P.- 167-168 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.41 (s, 1H, methoxyphenyl C_2), 7.32 – 7.26 (m, 3H, methoxyphenyl C_4, C_5, C_6), 7.16 - 7.14 (m, 1H, benzoxazole C_7), 6.92 (d, *J* = 2Hz, 1H, benzoxazole C_6), 6.66 - 6.64 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 3.84 (bs, 8H, piperazine), 3.68 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.53 (-C=O), 163.15 (benzoxazole C_2), 159.57 (methoxyphenyl C_3), 145.35 (benzoxazole C_{7a}),

143.28 (benzoxazole C_{3a}), 142.81 (benzoxazole C_5), 136.23 (methoxyphenyl C_1), 129.82 (methoxyphenyl C_5), 119.37 (methoxyphenyl C_6), 116.87 (methoxyphenyl C_4), 112.64 (methoxyphenyl C_2), 109.21 (benzoxazole C_7), 108.51 (benzoxazole C_4), 105.34 (benzoxazole C_6), 55.47 (OCH_3), 46.25 (piperazine); MS (EI+):*m/z* calculated for $C_{19}H_{20}N_4O_3$: 352.39, found –353.38 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-methoxyphenyl)methanone (**98A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **76A** and obtained as a brown solid, yield- 85%, M.P.- 164-165 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.44 (d, *J* = 8.5Hz, 2H, methoxyphenyl C_2, C_6), 7.06 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.97 (d, *J* = 8.5Hz, 2H, methoxyphenyl C_3, C_5), 6.73 (d, *J* = 2Hz, 1H, benzoxazole C_4), 6.41 - 6.39 (dd, *J* = 8Hz, 2Hz, 1H, benzoxazole C_6), 3.87 (s, 3H, -OCH_3), 3.72 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.71 (-C=O), 162.31 (benzoxazole C_2), 161.13 (methoxyphenyl C_4), 143.76 (benzoxazole C_{7a}), 143.50 (benzoxazole C_{3a}), 142.66 (benzoxazole C_5), 129.26 (methoxyphenyl C_2, C_6), 127.17 (methoxyphenyl C_1), 113.93 (methoxyphenyl C_3, C_5), 108.89 (benzoxazole C_7), 108.55 (benzoxazole C_4), 103.33 (benzoxazole C_6), 55.40 (-OCH_3), 45.66 (piperazine); MS (EI+):*m/z* calculated for $C_{19}H_{20}N_4O_3$: 352.39, found –353.20 (M+1).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(5-chloro-2-methoxyphenyl)methanone (**99A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **77A** and obtained as a brown solid, yield- 79%, M.P.- 172-173 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.59 (d, *J* = 1Hz, 1H, 5-chloro-2-methoxyphenyl C_6), 7.37 (d, *J* = 8.5Hz, 1H, 5-chloro-2-methoxyphenyl C_4), 7.12 (d, *J* = 8.5 Hz, 1H, 5-chloro-2-methoxyphenyl C_3), 7.05 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.78 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 6.52 - 6.49 (dd, *J* = 8Hz, 2Hz, 1H,

benzoxazole C_6), 3.82 (s, 3H, OCH_3), 3.46 - 3.39 (m, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 168.42 (-C=O), 162.57 (benzoxazole C_2), 156.31 (5-chloro-2-methoxyphenyl C_2), 143.72 (benzoxazole C_{7a}), 143.46 (benzoxazole C_{3a}), 142.56 (benzoxazole C_5), 131.11 (5-chloro-2-methoxyphenyl C_4), 128.64 (5-chloro-2-methoxyphenyl C_6), 126.54 (5-chloro-2-methoxyphenyl C_5), 122.68 (5-chloro-2-methoxyphenyl C_1), 115.41 (5-chloro-2-methoxyphenyl C_3), 108.82 (benzoxazole C_7), 108.25 (benzoxazole C_4), 103.41 (benzoxazole C_6), 56.34 (-OCH_3), 46.66 (piperazine); MS (EI+):*m/z* calculated for $C_{19}H_{19}ClN_4O_3$: 386.84, found –387.12 (M+), 389.32 (M+2).

(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(benzo[d][1,3]dioxol-5-yl)methanone (**100A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **78A** and obtained as a brown solid, yield- 88%, M.P.-168-169 °C, ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.58 – 7.55 (dd, *J* = 8Hz, 2Hz, 1H, benzo[d][1,3]dioxol-5-yl C_6), 7.48 (d, *J* = 1.5Hz, 1H, benzo[d][1,3]dioxol-5-yl C_4), 7.23 – 7.20 (d, *J* = 8Hz, 1H, benzo[d][1,3]dioxol-5-yl C_7), 7.07 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 6.75 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 6.48 - 6.44 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_6), 6.21 (s, 2H, benzo[d][1,3]dioxol-5-yl C_2), 3.82 (br s, 8H, piperazine). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.15 (-C=O), 162.72 (benzoxazole C_2), 149.52 (benzo[d][1,3]dioxol-5-yl C_{7a}), 148.21 (benzo[d][1,3]dioxol-5-yl C_{3a}), 143.54 (benzoxazole C_{7a}), 143.48 (benzoxazole C_{3a}), 142.52 (benzoxazole C_5), 129.42 (benzo[d][1,3]dioxol-5-yl C_5), 121.51 (benzo[d][1,3]dioxol-5-yl C_6), 115.50 (benzo[d][1,3]dioxol-5-yl C_4), 108.95 (benzoxazole C_7), 108.35 (benzo[d][1,3]dioxol-5-yl C_7), 108.24 (benzoxazole C_4), 103.27 (benzoxazole C_6), 101.62 (benzo[d][1,3]dioxol-5-yl C_2), 45.27 (piperazine); MS (EI+):*m/z* calculated for $C_{19}H_{18}N_4O_4$: 366.38, found – 367.51 (M+1).

2-(4-(4-chlorobenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**101A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **79A** and obtained as a brown solid, yield- 82%, M.P.- 165-166 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.78 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.35 - 7.33 (m, 2H, chlorobenzyl C_2, C_6), , 7.25 (d, *J* = 8 Hz, 2H, chlorobenzyl C_3, C_5), 6.82 (d, *J* = 2.5 Hz, 1H, benzoxazole C_4), 6.64 – 6.61 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 3.84 - 3.82 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.72 (s, 2H, benzyl CH_2), 2.67 - 2.65 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 162.47 (benzoxazole C_2), 145.34 (benzoxazole C_{7a}), 143.65 (benzoxazole C_{3a}), 143.24 (benzoxazole C_5), 138.14 (chlorobenzyl C_1), 135.46 (chlorobenzyl C_2, C_6), 134.78 (chlorobenzyl C_3, C_5), 125.55 (chlorobenzyl C_4), 110.32 (benzoxazole C_7), 108.37 (benzoxazole C_4), 104.75 (benzoxazole C_6), 62.21 (CH_2), 54.32 (piperazine C_2, C_6), 46.68 (piperazine C_3, C_5); MS (EI+):*m/z* calculated for $C_{18}H_{19}ClN_4O$: 342.83, found –343.17 (M+), 345.50 (M+2).

2-(4-(4-bromobenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**102A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **80A** and obtained as a brown solid, yield- 84%, M.P.- 158-159 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.64 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.49 - 7.46 (m, 2H, bromobenzyl C_2, C_6), 7.35 (d, *J* = 8 Hz, 2H, bromobenzyl C_3, C_5), 6.84 (d, *J* = 2.5 Hz, 1H, benzoxazole C_4), 6.75 – 6.72 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 3.75 - 3.73 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.64 (s, 2H, benzyl CH_2), 2.65 - 2.63 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 162.45 (benzoxazole C_2), 145.61 (benzoxazole C_{7a}), 143.37 (benzoxazole C_{3a}), 142.81 (benzoxazole C_5), 136.55 (bromobenzyl C_1), 132.41 (bromobenzyl C_2, C_6), 131.63 (bromobenzyl C_3, C_5), 123.57 (bromobenzyl C_4), 110.15 (benzoxazole C_7), 108.22 (benzoxazole C_4), 104.74

(benzoxazole C_6), 62.35 (CH_2), 52.67 (piperazine C_2, C_6), 45.30 (piperazine C_3, C_5). MS (EI+):*m/z* calculated for $C_{18}H_{19}BrN_4O$: 387.28, found –387.54 (M+), 389.14 (M+2).

2-(4-(4-fluorobenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**103A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **81A** and obtained as a pale brown solid, yield- 86%, M.P.- 178-179 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.53 - 7.50 (m, 2H, fluorobenzyl C_2, C_6), 7.32 (d, *J* = 8 Hz, 2H, fluorobenzyl C_3, C_5), 6.72 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 6.67 – 6.64 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, benzoxazole C_6), 3.72 - 3.71 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.54 (s, 2H, benzyl CH_2), 2.69 - 2.66 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 164.62 (fluorobenzyl C_4), 163.24 (benzoxazole C_2), 145.34 (benzoxazole C_{7a}), 143.34 (benzoxazole C_{3a}), 143.01 (benzoxazole C_5), 135.74 (fluorobenzyl C_1), 130.33 (fluorobenzyl C_2), 129.64 (fluorobenzyl C_6), 118.61 (fluorobenzyl C_5), 117.20 (fluorobenzyl C_3), 110.17 (benzoxazole C_7), 108.27 (benzoxazole C_4), 104.74 (benzoxazole C_6), 62.54 (CH_2), 52.37 (piperazine C_2, C_6), 45.42 (piperazine C_3, C_5); MS (EI+):*m/z* calculated for $C_{18}H_{19}FN_4O$: 326.38, found –327.51 (M+1).

2-(4-(4-methylbenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**104A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **82A** and obtained as a brown solid, yield- 83%, M.P.- 182-183 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.65 (d, *J* = 9Hz, 1H, benzoxazole C_7), 7.34 (d, *J* = 7.5Hz, 2H, methylbenzyl C_2, C_6), 7.15 (d, *J* = 7.5Hz, 2H, methylbenzyl C_3, C_5), 6.75 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 6.63 – 6.60 (dd, *J* = 9Hz, 2,5Hz, 1H, benzoxazole C_6), 3.75 - 3.73 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.52 (s, 2H, benzyl CH_2), 2.64 - 2.62 (t, *J* = 5Hz, 4H, piperazine C_3, C_5), 2.35 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.38 (benzoxazole C_2), 145.42 (benzoxazole C_{7a}), 143.64 (benzoxazole C_{3a}), 143.15

(benzoxazole C_5), 135.51 (methylbenzyl C_1), 134.32 (methylbenzyl C_4), 129.68 (methylbenzyl C_2, C_6), 129.25 (methylbenzyl C_3, C_5), 110.27 (benzoxazole C_7), 108.35 (benzoxazole C_4), 103.75 (benzoxazole C_6), 62.37 (CH_2), 52.31 (piperazine C_2, C_6), 45.47 (piperazine C_3, C_5), 21.35 (CH_3); MS (EI+):*m/z* calculated for $C_{19}H_{22}N_4O$: 322.41, found –323.34 (M+1).

2-(4-(4-methoxybenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**105A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **83A** and obtained as a brown solid, yield- 81%, M.P.- 192-193 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.64 (d, *J* = 8.5 Hz, 1H, benzoxazole C_7), 7.32 (d, *J* = 7.5Hz, 2H, methoxybenzyl C_2, C_6), 6.92 (d, *J* = 7.5Hz, 2H, methoxybenzyl C_3, C_5), 6.72 (d, *J* = 2 Hz, 1H, benzoxazole C_4), 6.57 – 6.54 (dd, *J* = 8.5 Hz, 2.5Hz, 1H, benzoxazole C_6), 3.82 (s, 3H, -OCH_3), 3.75 - 3.73 (t, *J* = 5 Hz, 4H, piperazine C_2, C_6), 3.52 (s, 2H, CH_2), 2.66 – 2.64 (t, *J* = 5 Hz, 4H, piperazine C_3, C_5). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.42 (benzoxazole C_2), 161.28 (methoxybenzyl C_4), 145.24 (benzoxazole C_{7a}), 143.45 (benzoxazole C_{3a}), 143.15 (benzoxazole C_5), 130.47 (methoxybenzyl C_2, C_6), 128.24 (methoxybenzyl C_1), 117.34 (methoxybenzyl C_3, C_5), 110.34 (benzoxazole C_7), 108.45 (benzoxazole C_4), 103.62 (benzoxazole C_6), 62.37 (CH_2), 57.52 (-OCH_3), 52.42 (piperazine C_2, C_6), 45.20 (piperazine C_3, C_5); MS (EI+):*m/z* calculated for $C_{19}H_{22}N_4O_2$: 338.41, found –339.32 (M+1).

2-(4-(4-isopropylbenzyl)piperazin-1-yl)benzo[d]oxazol-5-amine (**106A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **84A** and obtained as a brown solid, yield- 79%, M.P.- 157-158 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.65 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.36 (d, *J* = 5Hz, 2H, isopropylbenzyl C_2, C_6), 7.26 (d, *J* = 5Hz, 2H, isopropylbenzyl C_3, C_5), 6.76 (d, *J* = 2.5Hz, 1H, benzoxazole C_4), 6.45 – 6.42 (dd, *J* = 9Hz, 2,5Hz, 1H, benzoxazole C_6),

3.75 - 3.73 (t, *J* = 5Hz, 4H, piperazine C_2, C_6), 3.54 (s, 2H, benzyl CH_2), 2.72 – 2.70 (m, 1H, CH), 2.63 – 2.60 (t, *J* = 5Hz, 4H, piperazine C_3, C_5), 1.28 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 163.62 (benzoxazole C_2), 148.20 (isopropylbenzyl C_4), 145.38 (benzoxazole C_{7a}), 143.45 (benzoxazole C_{3a}), 143.17 (benzoxazole C_5), 134.53 (isopropylbenzyl C_1), 129.35 (isopropylbenzyl C_3, C_5), 125.31 (isopropylbenzyl C_2, C_6), 110.32 (benzoxazole C_7), 108.45 (benzoxazole C_4), 103.75 (benzoxazole C_7), 62.35 (CH_2), 52.54 (piperazine C_2, C_6), 45.20 (piperazine C_3, C_5), 33.78 (CH), 24.21 (2*CH_3); MS (EI+):*m/z* calculated for $C_{21}H_{26}N_4O$: 350.47, found – 351.50 (M+1).

2-(4-phenethylpiperazin-1-yl)benzo[d]oxazol-5-amine (**107A**): This compound was synthesized as per general procedure described earlier in section 5.1.3.7 using compound **85A** and obtained as a brown solid, yield- 85%, M.P.- 185-186 °C, ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.69 (d, *J* = 8.5Hz, 1H, benzoxazole C_7), 7.42 - 7.37(m, 5H, 4-phenethylpiperazin-1-yl), 6.68 (d, *J* = 2Hz, 1H, benzoxazole C_4), 6.71 – 6.68 (dd, *J* = 9Hz, 2.5Hz, 1H, benzoxazole C_4), 4.33 (br s, 2H, -CH_2), 3.72 (br s, 8H, piperazine), 3.10 (br s, 2H, -CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δC 163.65 (benzoxazole C_2), 145.42 (benzoxazole C_{7a}), 143.51 (benzoxazole C_{3a}), 143.17 (benzoxazole C_5), 138.35 (4-phenethylpiperazin-1-yl C_1), 129.52 (4-phenethylpiperazin-1-yl C_3, C_5), 128.34 (4-phenethylpiperazin-1-yl C_2, C_6), 126.81 (4-phenethylpiperazin-1-yl C_4), 110.24 (benzoxazole C_7), 108.41 (benzoxazole C_4), 103.30 (benzoxazole C_6), 56.53 (-CH_2), 47.92 (piperazine), 33.14 (-CH_2). MS (EI+):*m/z* calculated for $C_{19}H_{22}N_4O$: 322.41, found – 323.56 (M+1).

5.1.4 Biological evaluation

5.1.4.1 Cholinesterase inhibition assay (AChE and BuChE)

Cholinesterase inhibitory activity of all thirty-three analogs were evaluated spectrometrically using the reported protocol [Gutti et al. 2019]. Enzymes, *ee*AChE and *eq*BuChE were purchased from Sigma Aldrich (CAS No.9000-81-1, 9001-08-5 respectively). Concisely, stock concentrations (1 mg/ml) of inhibitors were prepared in DMSO and from those, six different working concentrations (0.001 µM, 0.01 µM, 0.1 µM, 1 µM, 10 µM, and 50 µM) of test compounds were used to determine IC_{50}. The final assay volume consisted of 100 µL of DTNB (0.0005M), 50 µL of AChE (0.5 U mL^{-1}) or 50 µL of BuChE (0.5 U mL^{-1}) and substrate *i.e.* ATCI (for AChE, 0.00375M, 20 µL) or BTCI (for BuChE, 0.00375M, 20 µL). The test solutions and DTNB were pre-incubated for 10 min followed by the addition of enzyme. After 30min, the substrate was added into it. The formation of yellow colour (5-thio-2-nitrobenzoate anion) as a result of a reaction of DTNB with thiocholines was monitored for 1 min a change in absorbance at 415 nm till 20 min on Synergy HTX multi-mode reader (BioTek, USA). Blank readings were taken 2 µL DMSO, instead of test compound, and DNZ (0.001 – 50 µM) was used as the reference standard. All the assays were carried out in triplicates (three independent runs), and the IC_{50} values of all the tested compounds were calculated graphically using the log concentration-percentage inhibition curves (Graph Pad Prism 5.01).

The enzyme kinetics study was performed to establish the nature of ChEs inhibition and binding pattern of the most potent compound **92A**. Seven different concentrations of ATCI (0.25 - 5 µM) were used to evaluate kinetic parameters (K_m and V_{max}). Compound **92A** was used in three different concentrations for AChE (25, 50, and 75 nM) and BuChE (0.5, 1, and 1.5 µM). Each concentration of inhibitor was evaluated with seven

different concentrations of ATCI. The inhibitory activity was measured for 10 min at an interval of 2 min in presence and absence of test compounds. The velocity of the enzyme kinetic reaction was obtained by plotting product formed during 10 min analysis and V_{max}, K_m were measured by Michaelis-Menten nonlinear regression graph. Lineweaver-Burk reciprocal linear regression plots were plotted to determine the mechanism of inhibition by GraphPad Prism version 5.01. The enzyme kinetic assay was performed in triplicate.

5.1.4.2 PAMPA-BBB assay

The details of assay protocol is mentioned in chapter 4 section 4.1.4.2. The concentration of drug in acceptor, donor and reference wells were determined by UV spectroscopy. Each sample was scanned to at least five different wavelengths and in three independent runs.

5.1.4.3 Propidium iodide displacement assay

The details of assay protocol is mentioned in chapter 4 section 4.1.4.3. Fluorescence intensity was measured after 10 min of the reaction, at excitation and emission wavelengths of 535 nm and 595 nm, respectively using microplate reader (HTX multi-mode reader, BioTek, USA).

5.1.4.4 $A\beta_{1-42}$ inhibition- thioflavin T assay

$A\beta_{1-42}$ peptide was purchased from Sigma (India). A lyophilized aliquot (1 mg) of $A\beta_{1-42}$ was dissolved in 80 µl of 1% NH_4OH and then in 920 µl of sterile phosphate-buffered saline (PBS, pH 7.4) to get stock solution of concentration 1 mg/ml and was stored at -80 °C. The working solution of $A\beta_{1-42}$ (10 µM) was prepared by further dilution with PBS solution.

The test compound **92A** was initially dissolved in DMSO, and final dilutions (5 µM, 10 µM, and 20 µM) were made in PBS solution. Different concentrations of $A\beta_{1-42}$:

inhibitors (10:5, 10:10; and 10:20 μM) were tested by ThT fluorescence method [Jan et al. 2010b, Kumar et al. 2018a]. For self-induced $A\beta_{1-42}$ aggregation inhibition, the mixture of $A\beta_{1-42}$ (10 μM; 10 μl) with 50μM PBS pH 7.4 and incubated at 37 °C for 48 h with or without compound **92A** (5 μM, 10 μM, and 20 μM; 10 μl). After completion of the incubation time, 178 μL of 20 μM ThT was added and the fluorescence intensity was immediately measured at the excitation and emission wavelengths of 450 nm and 485 nm, respectively.

For AChE-induced $A\beta_{1-42}$ aggregation inhibition, the $A\beta_{1-42}$ (10 μM; 2 μl) and AChE (230 μM, 16 μl) from electric eel was incubated with or without tested compounds (5 μM, 10 μM, and 20 μM; 2 μl) at 37 °C for 48 h. After incubation, 178 μL of 20 μM ThT was added and fluorescence intensity was measured (same as in self-induced). Blank measurements were made by using PBS instead of $A\beta_{1-42}$ with or without inhibitor. The results were reported as normalized fluorescence intensity (NFI) with respect to control. All the experiments were performed in triplicates and in three independent runs.

5.1.4.5 Neuroprotection studies of *(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone* (92A) on SH-SY5Y cell lines

The neuroprotection property of compound **92A** was evaluated against SH-SY5Y neuroblastoma cell lines by the MTT assay according to the literature procedure with minor modifications [Chierrito et al. 2017]. Briefly, the cell lines (density 1 x 10^5 cells/wells) were plated in 96 well plates and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Cells were treated with $A\beta_{1-42}$ (10 μM) for another 24 h. During this period, the medium was replaced with fresh culture medium. The test compounds in different concentration range (DNZ – 10, 20 μM; compound **92A** - 10, 20, 40, and 80 μM) were added, and cells were incubated for 72 h. After that, 20 μl of MTT reagent was added, and the cells were incubated for an additional 2 h. The purple

colored formazan crystals obtained were solubilized in dissolving solvent (100 µl DMSO). The absorbance was measured at 570 nm, and the % cell viability was calculated. Each treatment was executed in triplicate and data are presented as percentage of the control.

5.1.4.6 *In-vivo* behavioral studies

5.1.4.6.1 Animals, housing and materials

The male Wistar rats, weighing 200 ± 15 g were purchased from Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed on a 12 h light/dark cycle under environmentally controlled temperature (25 ± 2 °C) and humidity (55 ± 10% RH). They were allowed to acclimatize for one week with free access to food and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee of the university (Banaras Hindu University, Varanasi, India) (Dean/2017/CAEC/265). Scopolamine hydrobromide, donepezil (DNZ), and sodium carboxy methyl cellulose (SCMC) were procured from commercial sources. 0.5% SCMC solution was prepared in HPLC grade water.

5.1.4.6.2 LD_{50} determination

The female Wistar rats were used for determination of LD_{50} of compound **92A.** According to the OECD test guidelines for chemicals, at fixed dosages of 5, 50 and 300 mg/kg of compound **92A** was administered and monitored for 72 h. The body weight changes and mortality parameters were observed. The LD_{50} of compound **92A** was calculated as per the guidelines.

5.1.4.6.3 Scopolamine-induced amnesia model in rats

5.1.4.6.3.1 Y-Maze test

Y-maze test is widely used to assess the spatial working memory in amnesic rodents. The experimental groups included control, scopolamine, compound **92A** (2.5, 5, and 10

mg/kg) and DNZ (5 mg/kg). Compound **92A** and DNZ was administered for 7 days, whereas 0.5% sodium carboxy methyl cellulose was administered to control and scopolamine groups. On the seventh day, Scopolamine hydrobromide (0.5 mg/kg) was dissolved in water and injected intraperitoneally to all the animals except the control group. After 30 min of scopolamine administration, spatial working memory was assessed by using the Y-maze apparatus. The animals were introduced to the center of the Y-maze with closed novel arm, the animal was allowed to freely explore the arms for 5 min. The arms were cleaned and wiped with 70% of alcohol after completion of each test. When the animal subject crossed all four paws into the arm was considered as an arm entry. The improvement in learning and memory is directly proportional to increased spontaneous alternations (i.e., three consecutive arm entries). Percentage spontaneous alternation was calculated by using the formula: [number of alternations/(total arm entries-2)]X100 [Gutti et al. 2019].

5.1.4.6.3.2 *Ex-vivo* biochemical analysis

After the Y-maze test, all animals were sacrificed immediately through cervical dislocation and the whole brain was collected and washed with saline solution for *ex-vivo* AChE activity. The brain was homogenized in 10 mM PBS (pH 7.4) and centrifuged for 15 min at 4350 g force at 4 °C, the supernatants were further used. AChE activity was assessed in the brain of an animal as per the reported protocols [Gutti et al. 2019]. Concisely, 100 µL of the supernatant was incubated with 15 mM of freshly prepared ATCI (100µL) in presence of 2.7 ml of PBS for 5 min. After the addition of 100 µL of 1.5 mM DTNB, absorbance was recorded immediately at 415 nm. The rate of hydrolysis was calculated as µM of substrate hydrolyzed/min/mg of protein. All the experiments were performed in triplicate.

5.1.4.6.4 Morris water maze test

The ICV injection of $A\beta_{1-42}$ was used to induce the learning and memory impairment in rats [Zhang et al. 2015]. Briefly, the rats were randomly divided into four groups (n=6) including sham (control), $A\beta_{1-42}$ control, compound **92A** (10 mg/kg, p.o.), and DNZ (5 mg/kg, p.o.). The $A\beta_{1-42}$ was dissolved in sterile saline solution. Each animal was anaesthetized with a mixture of ketamine and xylazine at a dose of 90, and 9 mg/kg, i.p. respectively and placed on stereotaxic apparatus. The ear bars were set symmetrically, and scalp was incised and retracted. The head position was adjusted to place bregma and lambda. The hole was drilled through the skull, and stereotaxic coordinates were set related to bregma (-0.5 mm anteroposterior, + 1.2 mm mediolateral, -3.2 mm dorsoventral with incision bar set at -3.3 mm). $A\beta_{1-42}$ (5 µL, 4 µM) was infused into all the animals except sham group through a Hamilton microsyringe at 2 µL/min carefully. The Hamilton microsyringe was kept for another 5 min to prevent efflux of infused solution. In the sham group, saline was infused in place of $A\beta_{1-42}$. All the animals were given special care after post-surgery until recovery.

After 7 days post-surgery recovery, compound **92A** and DNZ treatments were continued for next seven days. The Morris water maze test is widely used to assess spatial learning and memory. The test apparatus (121 cm diameter, 62 cm height, and 32 cm depth) was filled with water (25 ± 2 ^{0}C) and titanium dioxide (TiO_2) was used to make the water opaque. The pool was equally divided into four quadrants, and a hidden platform was submerged 1cm in the fourth quadrant. The animal was placed in one of quadrant facing towards the wall and allowed to swim for 120 s to find the hidden platform. The experiment was continued for five consecutive days with four training trials per day (intra trail interval is 10 min). On sixth day, before probe trial, platform was removed, and each animal was allowed to swim 120 sec to assess the spatial

memory. All experiments were recorded and analyzed using the ANY-MAZE^{TM} software.

After completion of the probe trial, animals were sacrificed immediately through cervical dislocation, and the isolated brain samples were washed with saline solution. The whole brains were fixed in 4% paraformaldehyde solution at 4 °C overnight. The brain tissue samples were stored in 15% sucrose (in PBS) and then 30 % sucrose (PBS) until tissue sinks. Cryomold of each sample was prepared separately with tissue freezing medium. The frozen brain tissues were sectioned (sagittal) with a thickness of 20 µm on Leica CM1520 cryostat (cryosectioning). The sections were collected on microscopic slides and stained with hematoxylin and eosin [Fischer et al. 2008]. Images were taken under Olympus CX21iLED microscope with a magnification of 4X.

5.2 Results and Discussion

5.2.1 Molecular docking

Molecular docking studies were performed using Glide XP module of Schrödinger Maestro 2018.1 to understand the molecular interactions between benzoxazole derivatives targeting acetylcholinesterase (PDB ID: 4EY7). All the thirty-three derivatives were docked into the active site of AChE and the results were analyzed based on key interactions and glide docking score. 3-D and 2-D views of ligand **92A** (glide score: -15.0 kcal/mol) were presented in Figure 5.2 (A and B), respectively. In-depth analysis of binding pattern of compound **92A** positioned in the AChE active sites displayed H-bonding of free amine functionality with TYR133 and GLU202 residues at anionic subsite (AS).

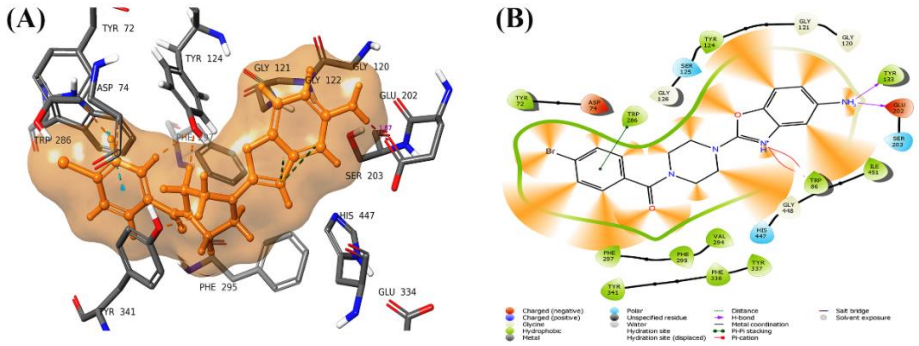


Figure 5.2. Molecular binding pattern of compound **92A** (A) 3D and (B) 2D in the AChE (PDB ID: 4EY7) active site. Key interacting residues are shown in line.

Additionally, the nitrogen atom of benzoxazole ring formed hydrophobic interactions (TRP86, PHE338) and π-cation interactions (TRP86) at AS. The *para* bromo phenyl ring, oriented towards peripheral anionic site, formed hydrophobic interactions (TYR72, TYR124, TRP286, and TYR341) and π-π stacking (TRP286) with key residues. Moreover, piperazine and benzoxazole rings were extended into the catalytic active site (CAS) and formed polar interactions with HIS447 and SER203 and SER125. All the compounds were further subjected to *in-silico* physicochemical property prediction.

5.2.2 Prediction of physicochemical properties

To target central nervous system (CNS), detailed analysis of physicochemical properties is essential from the perspective of developing drug-like analogs. Barrier mechanisms in the human brain are complex and prevent the entry of biologically active molecules into it [Saunders et al. 2016].

Therefore, Qikprop module of Schrödinger was employed and compared with Lipinski's rule of five. Some other physicochemical parameters like brain/blood partition coefficient (QPlogBB), octanol/water partition coefficient (QPlogPo/w), Caco-2 cell permeability (QPPCaco), human oral absorption (%HOA), and IC_{50} value for blockage of hERG K^+ channels (QPlogHERG) were also predicted (Table. 5.1). All

compounds exhibited acceptable drug-likeness properties with appreciable BBB permeation capacity.

5.2.3 Synthetic methodology and characterization

The designed compounds were synthesized as per the schemes 1 and 2. Scheme 1 demonstrates the synthesis of compounds **29A-39A**. The reaction of 2-amino-4-nitrophenol with different amine derivatives (**7A-17A**) provided the cyclized 5-nitro benzoxazole intermediates (**18A-28A**). The reduction of nitro group by using ammonium chloride and iron powder yielded compounds **29A-39A**.

Compounds **86A-107A** were synthesized by following scheme 2. The starting material 2-amino-4-nitrophenol was reacted with 1-Boc-piperazine to afford *tert*-butyl 4-(5-nitrobenzo[d]oxazol-2-yl)piperazine-1-carboxylate (**40A**). The removal of boc protecting group with trifluoroacetic acid (TFA) afforded the key intermediate (**41A**) in good yield. Finally, the key intermediate (**41A**) was reacted with various carboxylic acids (**42A-56A**) and aryl halides (**57A-63A**) to get target compounds **86A-100A** and **101A-107A,** respectively**.** The amide derivatives (**86A-100A**) were prepared by using N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazolehydrate (HOBt) as a catalyst in dichloromethane. The alkyl linker derivatives (**101A-107A**) were prepared by reacting with potassium carbonate in dimethylformamide at room temperature. Structures of all target compounds are represented in Table 5.2 and characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS, and HRMS.

5.2.4 *In-vitro* cholinesterase inhibition and SAR studies

In cholinergic synapse, 80 % of ACh is hydrolyzed by AChE, while BuChE plays a secondary role [Lane et al. 2004]. Thus, concurrent inhibition of both ChEs would be essential for anti-AD agents and accordingly all the designed target compounds were

evaluated for their inhibitory activities with DNZ as a reference molecule. Both the enzymes from the animal sources (AChE from electric eel, *ee*AChE, and BuChE from equine serum, *eq*BuChE) were utilized in the study, considering the higher degree of sequence homology and lower cost compared with human enzymes. The inhibitory potencies of designed compounds are included in Table 5.2. The selectivity index (SI) data demonstrated selective inhibition towards AChE. The compounds bearing aryl substitutions without any linkers (**29A-37A**) exhibited moderate inhibitory activities, whereas compounds containing one carbon linker (**38A, 39A; 101A-106A**) showed better activities. Compound **107A** (two carbon linker) displayed moderate inhibitions for both AChE (IC_{50} = 0.393 ± 0.012 μM) and BuChE (IC_{50} = 0.957 ± 0.072 μM) with 2.4- fold selectivity. Conversely, compounds containing carbonyl group as a linker between piperazine and aryl moieties showed potent ChEs inhibitory profiles with moderate to excellent selectivity index. Compound **92A** (*ee*AChE IC_{50} = 0.052 ± 0.010 μM; *eq*BuChE IC_{50} = 1.085 ± 0.035 μM; SI = 20.8 fold) and compound **95A** (*ee*AChE IC_{50} = 0.073 ± 0.010 μM; *eq*BuChE IC_{50} = 0.817 ± 0.021 μM; SI = 11.1 fold) possessed the most significant inhibitory profile among all the analogs, indicating that bromo and fluoro aryl substitutions are crucial for AChE inhibition. Specifically, the *para* substituted carbonyl containing derivatives showed better inhibition as compared to *ortho* and *meta* substituents. Electron donating groups (Cl, Br, and F) significantly influenced the ChEs inhibitions in comparision to the electron withdrawing groups (-OCH_3, -CH_3, and Isopropyl). Further, disubstituted aryl ring produced decrease in activity (*ee*AChE IC_{50} = 0.616 ± 0.010 μM; *eq*BuChE IC_{50} = 1.517 ± 0.044 μM; SI = 2.4 fold). BuChE inhibitory profile of compounds without linker was moderately higher and there was no substantial difference in between one carbon linker and carbonyl containing analogs. It may be because of the different structural and functional aspects

Table 5.1: Prediction of physicochemical properties.

Comp. No	QPlogBB[a]	QPlogPo/w[b]	QPPCaco[c]	%HOA[d]	QPlogHERG[e]
29A	-0.17	2.05	1784.81	100	-4.18
30A	-0.13	1.20	1785.41	92.18	-3.74
31A	-0.37	3.29	1446.31	100	-5.99
32A	-0.21	3.79	1446.07	100	-5.91
33A	-0.08	3.83	1780.69	100	-5.73
34A	-0.15	3.12	1452.35	100	-5.35
35A	-0.39	3.60	1446.62	100	-5.92
36A	-0.44	3.38	1460.58	100	-5.87
37A	-0.39	2.97	1376.86	100	-5.99
38A	-0.01	2.73	433.59	90.15	-6.81
39A	-0.14	2.80	421.51	90.33	-6.78
86A	-0.71	2.56	774.38	93.65	-6.03
87A	-0.51	3.05	852.39	100	-5.82
88A	-0.54	3.10	793.74	100	-5.92
89A	-0.54	3.10	796.05	100	-5.92
90A	-0.51	3.10	843.34	100	-5.81
91A	-0.56	3.16	766.10	100	-5.99
92A	-0.53	3.18	796.11	100	-5.95
93A	-0.59	2.78	808.40	95.25	-5.85
94A	-0.61	2.82	768.07	95.11	-5.92
95A	-0.59	2.84	795.31	95.50	-5.87
96A	-0.76	2.45	752.26	92.79	-5.70
97A	-0.79	2.46	776.71	93.09	-5.95
98A	-0.78	2.49	794.16	93.40	-5.92
99A	-0.66	2.18	797.19	91.65	-5.47
100A	-0.61	3.17	753.06	96.98	-5.64
101A	0.15	3.22	433.82	92.99	-6.72
102A	0.16	3.29	433.53	93.43	-6.76
103A	0.09	2.97	433.84	91.54	-6.79
104A	-0.03	3.03	431.03	91.86	-6.73
105A	-0.09	2.83	431.45	90.69	-6.72
106A	-0.11	3.65	428.08	95.41	-6.72
107A	-0.11	3.07	408.38	91.67	-6.91
DNZ	0.11	4.42	893.18	100	-6.75

[a]QPlogBB, predicted brain/blood partition coefficient (−3.0 to 1.2). [b]QPlogPo/w, predicted octanol/water partition coefficient (−2.0 to 6.5). [c]QPPCaco, predicted Caco-2 cell permeability (<25 poor, >500 great). [d]%HOA. predicted human oral absorption (>80% is high, <25% is poor). [e]QPlogHERG, predicted IC_{50} value for blockage of hERG K^+ channels (below −5).

of both ChEs. The compounds without carbonyl linker (**29A-39A**; **101A-107A**) showed moderate to low selectivity towards AChE comparable to DNZ having 42.8 folds AChE selectivity. The mechanistic inhibition study of compound **92A** on both the ChEs was carried out through kinetic parameters like maximal velocity (V_{max}), inhibitory concentration (Ki) and Michalis –menten dissociation constant. The reciprocal Lineweaver-Burk plot of compound **92A** on AChE (Figure 5.3 A; 25, 50, and 75 nM) and BuChE (Figure 5.3 B; 0.5, 1, and 1.5 µM) demonstrated the decreased pattern of V_{max} and K_m. The intersection point of trendlines in both graphs evidenced that the compound displayed non-competitive type of ChEs inhibition. Inhibitory concentrations (ki) on AChE (30 nM) and BuChE (0.86 µM) were calculated with the help of Dixon plot (Figure 5.4).

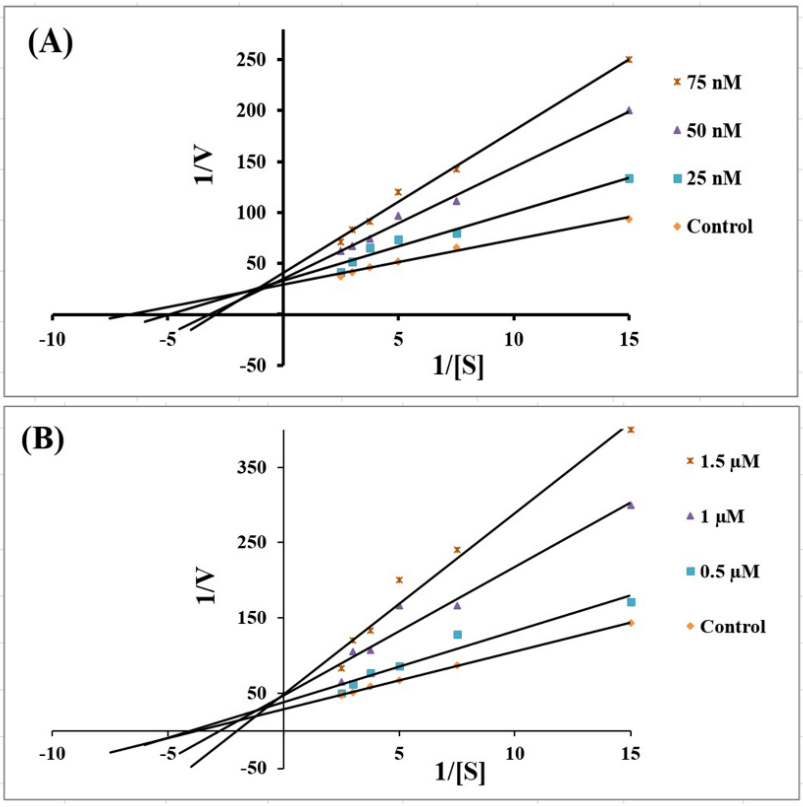
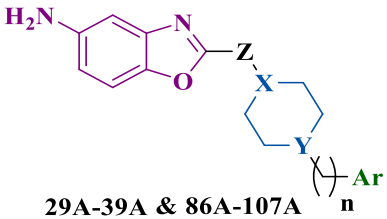


Figure 5.3. Lineweaver-Burk plot for the kinetic study of (A) AChE and (B) BuChE inhibition by compound **92A**.

5.2.5 *In-vitro* blood-brain barrier permeation assay

For AD drugs, permeation through blood-brain barrier is the major prerequisite. All the synthesized derivatives were subjected to the assessment of BBB permeation by using parallel artificial membrane permeation assay (PAMPA) [Gutti et al. 2019]. The permeability of nine commercially available drugs was used as a benchmark and were evaluated for BBB permeability (*Pe*) as reported in our previously published data [Gutti et al. 2019]. The *Pe* values of compounds (**29A-39A & 86A-107A**) are listed in Table 5.2. Among all the derivatives, alkyl linker derivative (compound **107A;** *Pe* = 13.42 ± 0.036 x 10^{-6} cm s^{-1}) showed better BBB permeability and could be attributed to the increased linker chain. The single carbon linker compounds (**102A**; *Pe* = 11.27 ± 0.064 x 10^{-6} cm s^{-1} and **106A**; *Pe* = 11.57 ± 0.011 x 10^{-6} cm s^{-1}) and amide derivative (Compound **92A;** *Pe* = 10.80 ± 0.055 x 10^{-6} cm s^{-1}) are also have appreciable BBB permeability. Moreover, all tested compounds could cross BBB *in-vitro*, with good permeation potentials.

Table 5.2 Structures, cholinesterase (*ee*AChE and *eq*BuChE) inhibitory potential and PAMPA-BBB assay of tested compounds.



29A-39A & 86A-107A

Comp. No	Ar	X	Y	Z	n	IC_{50} (µM) ± SE[a]		SI[b]	Pe (10^{-6} cm s^{-1})[c]	Permeability prediction (CNS+/-) [d]
						*ee*AChE IC_{50} in µM	*eq*BuChE IC_{50} in µM			
29A	-	N	CH_2	-	0	5.680 ± 0.012	24.212 ± 0.012	4.2	9.40 ± 0.05	CNS+
30A	-	N	O	-	0	7.031 ± 0.017	32.296 ± 0.052	4.5	7.44 ± 0.078	CNS+
31A	Phenyl	N	N	-	0	2.093 ± 0.034	16.763 ± 0.017	8.0	10.30 ± 0.049	CNS+
32A	4-Cl Phenyl	N	N	-	0	1.959 ± 0.009	11.102 ± 0.012	5.6	10.64 ± 0.099	CNS+
33A	4-Br Phenyl	N	N	-	0	1.055 ± 0.008	8.762 ± 0.018	8.3	10.54 ± 0.21	CNS+
34A	4-F Phenyl	N	N	-	0	1.445 ± 0.012	6.476 ± 0.021	4.4	10.69 ± 0.15	CNS+
35A	4-Me Phenyl	N	N	-	0	4.176 ± 0.012	9.012 ± 0.071	2.1	10.40 ± 0.047	CNS+
36A	4-OMe Phenyl	N	N	-	0	2.097 ± 0.009	12.971 ± 0.014	6.1	10.51 ± 0.168	CNS+
37A	Pyridin-2-yl	N	N	-	0	3.310 ± 0.015	15.126 ± 0.019	4.5	9.44 ± 0.12	CNS+
38A	Phenyl	N	N	-	1	0.921 ± 0.009	6.757 ± 0.027	7.3	10.16 ± 0.044	CNS+
39A	Phenyl	C	N	N	1	0.559 ± 0.010	2.272 ± 0.018	4.0	9.47 ± 0.093	CNS+
86A	Phenyl	N	N	-	C=O	0.801 ± 0.057	6.052 ± 0.025	7.5	9.52 ± 0.155	CNS+
87A	2-Cl Phenyl	N	N	-	C=O	0.559 ± 0.009	4.856 ± 0.016	8.6	10.33 ± 0.010	CNS+
88A	3-Cl Phenyl	N	N	-	C=O	0.241 ± 0.011	4.562 ± 0.035	18.9	10.36 ± 0.044	CNS+
89A	4-Cl Phenyl	N	N	-	C=O	0.109 ± 0.013	3.317 ± 0.049	30.4	10.39 ± 0.03	CNS+
90A	2-Br Phenyl	N	N	-	C=O	0.445 ± 0.061	6.193 ± 0.016	13.9	10.63 ± 0.11	CNS+
91A	3-Br Phenyl	N	N	-	C=O	0.163 ± 0.011	2.676 ± 0.019	16.4	10.53 ± 0.11	CNS+
92A	4-Br Phenyl	N	N	-	C=O	0.052 ± 0.010	1.085 ± 0.035	20.8	10.80 ± 0.055	CNS+
93A	2-F Phenyl	N	N	-	C=O	0.337 ± 0.007	8.392 ± 0.049	24.9	9.55 ± 0.19	CNS+
94A	3-F Phenyl	N	N	-	C=O	0.231 ± 0.091	5.539 ± 0.026	23.9	9.51 ± 0.165	CNS+

95A	4-F Phenyl	N	N	-	C=O	0.073 ± 0.010	0.817 ± 0.021	11.1	9.67 ± 0.004	CNS+
96A	2-OMe Phenyl	N	N	-	C=O	0.927 ± 0.051	7.379 ± 0.042	7.9	9.21 ± 0.001	CNS+
97A	3-OMe Phenyl	N	N	-	C=O	0.550 ± 0.009	11.736 ± 0.062	21.3	9.54 ± 0.089	CNS+
98A	4-OMe Phenyl	N	N	-	C=O	0.275 ± 0.010	7.327 ± 0.017	26.6	9.71 ± 0.034	CNS+
99A	benzo[d][1,3]dioxyl	N	N	-	C=O	0.321 ± 0.012	1.365 ± 0.021	4.2	8.82 ± 0.145	CNS+
100A	5-Cl,2-OMe Phenyl	N	N	-	C=O	0.616 ± 0.010	1.517 ± 0.044	2.4	9.64 ± 0.001	CNS+
101A	4-Cl Phenyl	N	N	-	1	0.747 ± 0.005	3.852 ± 0.061	5.1	10.71 ± 0.039	CNS+
102A	4-Br Phenyl	N	N	-	1	0.379 ± 0.010	2.972 ± 0.092	7.8	11.27 ± 0.064	CNS+
103A	4-F Phenyl	N	N	-	1	0.525 ± 0.005	3.112 ± 0.036	5.9	10.49 ± 0.14	CNS+
104A	4-Me Phenyl	N	N	-	1	0.807 ± 0.006	5.186 ± 0.017	6.4	10.61 ± 0.059	CNS+
105A	4-OMe Phenyl	N	N	-	1	0.748 ± 0.006	6.327 ± 0.012	8.4	9.49 ± 0.149	CNS+
106A	4-Ispr Phenyl	N	N	-	1	0.943 ± 0.014	11.274 ± 0.037	11.9	11.57 ± 0.011	CNS+
107A	Phenyl	N	N	-	2	0.393 ± 0.012	0.957 ± 0.072	2.4	13.42 ± 0.036	CNS+
DNZ	--	-	-	-	-	0.022 ± 0.042	0.941 ± 0.026	42.8	8.32 ± 0.014	CNS+

[a]Results are reported as the mean IC_{50} ± SEM (n = 3).

[b]Selectivity ratio for AChE = (IC_{50} of *eq*BuChE)/(IC_{50} of *ee*AChE). DNZ= Donepezil.

[c]Data are expressed as the mean ± SEM (n = 3).

[d]Compounds with $Pe > 4.324 \times 10^{-6}$ cm s^{-1} could cross the BBB (CNS+). Compounds with $Pe < 1.846 \times 10^{-6}$ cm s^{-1} could not cross the BBB (CNS−), and compounds with 1.846×10^{-6} cm $s^{-1} < Pe < 4.324 \times 10^{-6}$ cm s^{-1}show uncertain BBB permeation (CNS±).

Table 5.3. Propidium iodide displacement assay.

Comp. No	**Displacement of Propidium iodide from AChE PAS (% inhibition)[a]**	
	At 10 μM	At 50 μM
89A	18.27 ± 2.99	33.73 ± 2.86
92A	20.40 ± 2.63	40.74 ± 2.02
95A	17.27 ± 2.16	27.85 ± 2.16
107A	17.34 ± 3.11	35.23 ± 2.41
DNZ	20.96 ± 1.43	37.73 ± 2.46

[a]Data are expressed as the standard deviation (SD) of three independent experiments.

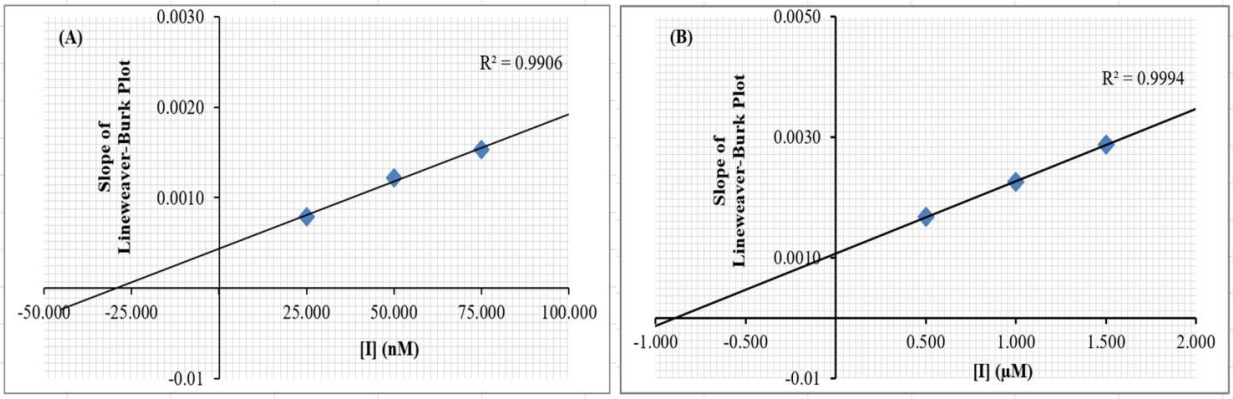


Figure 5.4. Dixon plot of compound **92A** showing the Ki value as negative intercept on X-axis of the Dixon plot for (A) AChE and (B) BUChE.

5.2.6 Propidium iodide displacement assay

Molecular docking studies of compound **92A** demonstarted key interactions with PAS active site of AChE. Based on the *in-vitro* cholinesterase activities and BBB permeability assay, representative compounds (**89A**, **92A**, **95A** and **107A**) were selected for AChE PAS binding affinity assessment by using propidium iodide displacement assay (Table 5.3). Propidium iodide (PI) is a PAS specific ligand of AChE [Taylor and Lappi 1975]. The test compounds/DNZ (10 and 50 µM concentration) were treated with AChE in the presence of PI. The compound **92A** showed considerably uniform displacement of PI at 10 µM (20.40 ± 2.63 %) but higher in case of 50 µM (40.74 ± 2.02 %) as compared to DNZ (10 µM = 20.96 ± 1.43%; 50 µM = 37.73 ± 2.46 %). Compound **89A** (10 µM = 18.27 ± 2.99%; 50 µM = 33.73 ± 2.86 %), **95A** (10 µM = 17.27 ± 2.16%; 50 µM = 27.85 ± 2.16 %), **107A** (10 µM = 17.34 ± 3.11%; 50 µM = 35.23 ± 2.41 %) exhibited low displacement of PI when compared to DNZ. The PI displacement assay results of compound **92A** at PAS active site of AChE are in agreement with molecular docking studies.

5.2.7 Self-induced and AChE induced $A\beta_{1-42}$ aggregation

The cholinesterase inhibitors which bind to PAS region are capable of preventing $A\beta_{1-42}$ production and deposition [Inestrosa et al. 2005]. We further studied the regulation of self- and AChE-induced $A\beta_{1-42}$ aggregation compound **92A** by thioflavin T (ThT) fluorescence method. The experiment was carried out at three different concentration ratios of $A\beta_{1-42}$: compound **92A**/DNZ (10:5, 10:10, and 10:20 μM). The results were described as normalized fluorescence intensity (NFI) (Figure 5.5.A and 5.5.B), and DNZ used as a reference. Compound **92A**, at a concentration ratio of 10:5 and 10:10 μM, exhibited moderate anti-aggregatory property. However, at a concentration ratio of 10:20 μM, it showed the higher anti-aggregatory property in AChE induced experiment, when compared to self-induced. These results indicated that compound **92A** effectively inhibited the $A\beta_{1-42}$ aggregation caused by AChE and had the PAS-AChE binding ability.

5.2.8 Neuroprotection studies of *(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone* (92A) on SH-SY5Y cell lines

The *in-vitro* cell line studies have been routinely used as these can be helpful in primary optimization and mechanistic investigations before animal-based toxicology studies [Allen et al. 2005a]. The evaluation of neuroprotection effect of compound **92A** (10, 20, 40, and 80 μM) on human neuroblastoma SH-SY5Y cell lines was assessed through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) assay (Figure 5.6). $A\beta_{1-42}$ peptide (10 μM), which is the most amyloidogenic and neurotoxic isoform of Aβ was used to promote the toxicity on cell lines. The main hypothesis behind this experiment is to examine whether compound **92A** is involved in the reduction of $A\beta_{1-42}$ aggregation, and had a less toxic effect of $A\beta_{1-42}$ and cell line protection with no cytotoxic effects. The results suggested that the treatment of SH-SY5Y cells with $A\beta_{1-42}$

peptide significantly decreased the cell viability. DNZ did not show cytotoxicity profiles at concentrations of 10, 20 µM. On the other hand, compound **92A** (10 and 20 µM) demonstrated no significant difference with $A\beta_{1-42}$ peptide treatment. However, at higher concentrations of 40 and 80 µM, the compound **92A** exerted better cell recoveries and significant difference with $A\beta_{1-42}$ peptide treatment. Thus, it was evident that the treatment of SH-SY5Y cell lines ($A\beta_{1-42}$ peptide treated) with compound **92A** exhibited neuroprotective property.

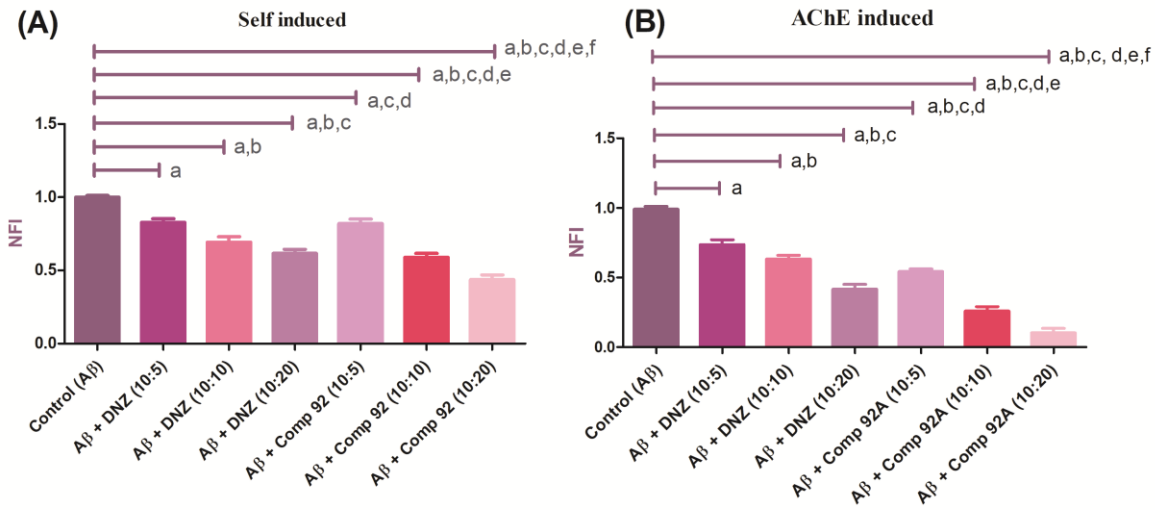


Figure 5.5. Effect of compound **92A** on $A\beta_{1-42}$ aggregation. (A) Self-induced and (B) AChE-induced $A\beta_{1-42}$ aggregation. Bars are showing the normalized fluorescence intensity (NFI) as the mean ± SEM of three separate experiments. $^{a}p< 0.05$ compared to control, $^{b}p< 0.05$ compared to Aβ + DNZ (10:5), $^{c}p< 0.05$ compared to Aβ + DNZ (10:10), $^{d}p< 0.05$ compared to Aβ + DNZ (10:20), $^{e}p< 0.05$ compared to Aβ + comp **92A** (10:5), $^{f}p< 0.05$ compared to Aβ + comp **92A** (10:10) (One-way ANOVA followed by Newman - Keuls test).

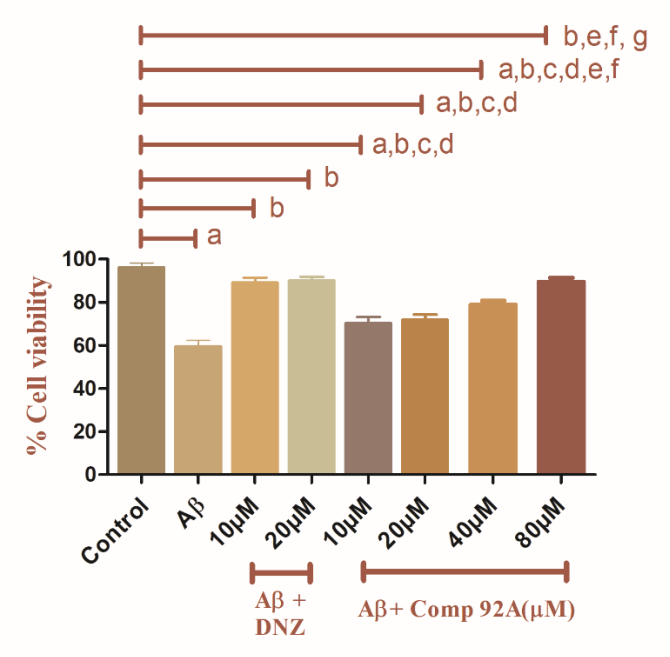


Figure 5.6. Neuroprotection assay on SH-SY5Y cell lines with compound **92A**. Bars display the mean ± SEM for three different experiments. [a]p< 0.05 compared to control, [b]p< 0.05 compared to Aβ, [c]p< 0.05 compared to Aβ + DNZ (10 μM), [d]p< 0.05 compared to Aβ + DNZ (20 μM), [e]p< 0.05 compared to Aβ + comp **92A** (10 μM), [f]p< 0.05 compared to Aβ + comp **92A** (20 μM), [g]p< 0.05 compared to Aβ + comp **92A** (40 μM) (One-way ANOVA followed by Newman - Keuls test).

5.2.9 *In-vivo* behavioral studies of *(4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone* (92A)

5.2.9.1 Scopolamine-induced amnesia models for testing cognition enhancement in rats

Behavioral studies, to evaluate cognition and memory improvement potency, are the utmost requirement for anti-AD agents. The aptness of compound **92A** to improve scopolamine induced cognition impairment in rats was carried out in Y-maze apparatus [Klinkenberg and Blokland 2010]. The dose of the compounds was fixed at half of the lethal dose (LD_{50}) data (Table 5.4 to 5.6). The effects of compound **92A** (2.5, 5, and 10 mg/kg) and DNZ (5 mg/kg) on spontaneous alternation behavior was assessed (Figure 5.7.A). On the 7th day trial, Y-maze test was performed to evaluate the effect of test compounds and spontaneous alternations were calculated. The percentage of

spontaneous alternation was significantly decreased in scopolamine group in comparison to control group. A dose-dependent increase in % spontaneous alternations was observed in compound **92A** treated groups. The compound at a dose of 10 mg/kg showed statistically no significant difference with DNZ (5 mg/kg) treated group. These results revealed that administration of compound **92A** (10 mg/kg) led to a substantial improvement of the cognitive and memory impairment due to the scopolamine-induced cholinergic deficit.

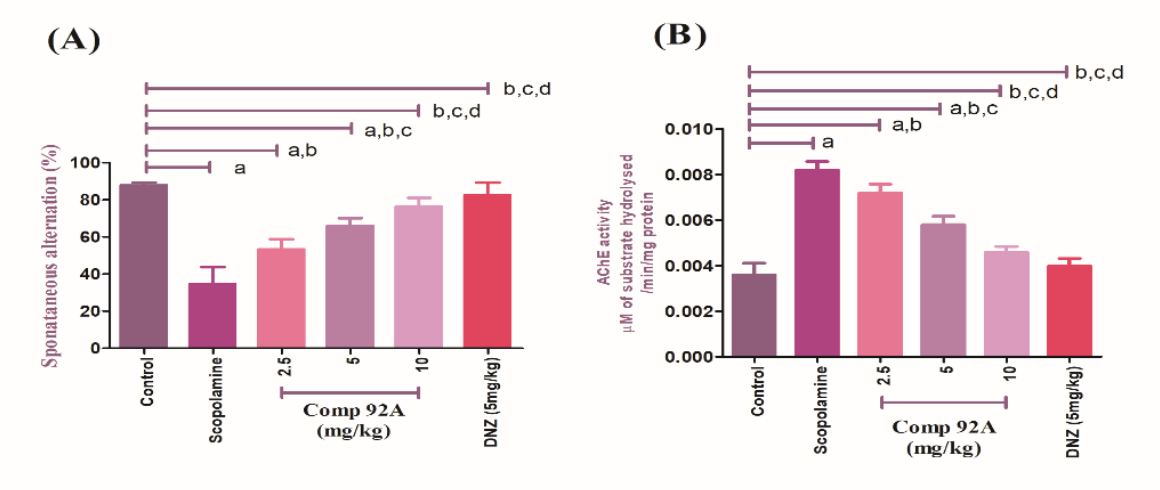


Figure 5.7. Effect of compound **92A** and donepezil on scopolamine-induced cognition and memory impairment. (A) Spontaneous alternations (%); (B) Analysis of AChE activity- rate of substrate hydrolyzed. [a]p< 0.05 compared to control; [b]p< 0.05 compared to scopolamine; [c]p< 0.05 compared to compound **92A** at dose of 2.5 mg/kg; [d]p< 0.05 compared to compound **92A** at dose of 5 mg/kg; [e]p < 0.05 compared to compound **92A** at dose of 10 mg/kg. (One-way ANOVA followed by Newman - Keuls test).

5.2.9.2 *Ex- vivo* biochemical analysis

To further verify the effect of compound **92A** on neurochemical levels, we determined the AChE activity by the *ex-vivo* study as per Ellman's protocol [Ellman et al. 1961]. The AChE activities of different groups are depicted in Figure 5.7.B. Higher levels of AChE were observed in scopolamine treated group. However, in compound **92A** (2.5, 5, and 10 mg/kg) treated groups, a dose-dependent decrease was observed. There was no statistically significant difference between compound **92A** (10 mg/kg) and DNZ (5

mg/kg) groups was observed. The results of *ex-vivo* biochemical analysis suggested that compound **92A** possessed significant brain AChE inhibitory potential.

Table 5.4. Protocol for LD_{50} determination of compound **92A**.

Test substance 1. Physical nature 2. Code	 Brown Solid BOZ-AM-4Br (compound **92A**)
Vehicle	0.5% Sodium carboxy methyl cellulose
Test animals 1. Sex 2. Number	Rat Female 3
Test conditions 1. Starting Dose 2. Dosing volumes 3. Time & date of dosing	 300 milligram/kilogram 0.5 milliliter 09:00 AM 01/05/2019

Table 5.5. Effect of compound **92A** on the body weight of the rat at the dose of 300 mg/kg.

Group	Body weight (gram) on 01/05/2019 at 09:00 AM	Body weight (gram) on 02/05/2019 at 09:00 AM	Body weight (gram) on 03/05/2019 at 09:00 AM
1	215	217	214
2	200	205	207
3	218	215	215

Table 5.6. The onset of toxicity with compound **92A** in the period of 72h.

Group	Body weight changes (gram)			Onset of toxicity	Reversibility	Date & time of death
	01/05/2019	02/05/2019	03/05/2019			
1	00	02	03	01/05/2019, 05:00 PM	No	03/05/2019, 11:00 AM
2	00	05	02	01/05/2019, 05:00 PM	No	03/05/2019, 05:00 PM
3	00	03	00	01/05/2019, 05:00 PM	No	03/05/2019, 05:00 PM

Rationale for the selection of the starting dose: No animal death was observed at 5mg/kg dose however 1 death was observed at 50mg/kg.

Discussion and interpretation of results: Animals were dosed as per the OECD guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method) at 5mg/kg, 50 mg/kg and 300 mg/kg doses. **All animals died at 300mg/kg dose within 72 hrs.**

Conclusions: As per OECD guideline (Annex 2b) **LD_{50} = 200mg/kg**.

5.2.9.3 $A\beta_{1-42}$ induced ICV rat model: Morris water maze test

After the preliminary evaluation of cognition-improving effect of compound **92A** by spontaneous alternation behavior in Y-maze, we further performed learning and memory assessments using Morris water maze test involving daily oral administration of compound **92A** (10 mg/kg) to ICV-$A\beta_{1-42}$ male Wistar rats, suffering from learning and memory dysfunction, with DNZ as a positive control. Cerebral aggregation of $A\beta_{1-42}$ peptide is the primary pathological condition in AD. Aβ plaques initiate the synaptotoxicity and neurodegeneration by activating several biochemical cascades (Oxidative stress, inflammation, and apoptosis etc) resulting in loss of memory and cognitive function [Walsh and Selkoe 2004]. Intracerebroventricular (ICV) infusion of $A\beta_{1-42}$ peptide into the rat brain mimics the AD like behavior which is similar to human AD [Harkany et al. 1998]. The aggregation of $A\beta_{1-42}$ deposits induce neuronal-inflammation and microglial activation, which leads to the learning and memory deficits in rats. This robust animal model allows preclinical evaluation of drugs targeting $A\beta_{1-42}$ cascade and provides understanding about $A\beta_{1-42}$ toxicity [Van Dam and De Deyn 2011]. During the training trails, the mean escape latency for the Wistar rats in each group declined gradually (Figure 5.8.A). However, the $A\beta_{1-42}$ group spent more time to find a hidden platform, which was located at the center of the 4^{th} quadrant (Figure 5.8.D). After the last training trial, the retention of memory was predicted by a special probe trail, with the removal of the platform from the pool. The time spent in the platform quadrant (Figure 5.8.B) and the total number of entries to platform zone (fourth quadrant; Figure 5.8.C) were assessed. Notably, time spent in the platform zone (Figure 5.8.B) was greater for DNZ (5 mg/kg) treated group and compound **92A** (10 mg/kg) treated group in comparison to $A\beta_{1-42}$ group. Search accuracy of the compound **92A** treated animals was assessed by the total number of platform crossings (Figure

5.8.C), and it showed no significant difference with DNZ treated group. Removal of the platform led the animal to increase in the 'number of entries to platform zone' and 'time spent in the platform quadrant' in DNZ and compound **92A** treated groups (Figure 5.8.E). However, $A\beta_{1-42}$ group exhibited less 'number of entries to platform zone' and 'time spent in the platform quadrant'. These results suggested that compound **92A** (10 mg/kg) and DNZ (5 mg/kg) led to an improvement of spatial memory and cognitive abilities when compared with $A\beta_{1-42}$ group.

Hippocampus plays pivotal role in cognition and memory. Administration of $A\beta_{1-42}$ causes significant loss of neurons and disordered arrangement, results in cognition and memory deficit. The brain tissue pattern in control, $A\beta_{1-42,}$ and test compound (DNZ / compound **92A**) treated groups were further observed by histopathological examination (Figure 5.9).

Hematoxylin and eosin staining was performed to investigate the morphology of neuronal cells. In the control group, the neurons in the hippocampus region remained intact and well organized. In the $A\beta_{1-42}$ group, the neuron arrangement was disordered, and vacuolar fibers were observed.

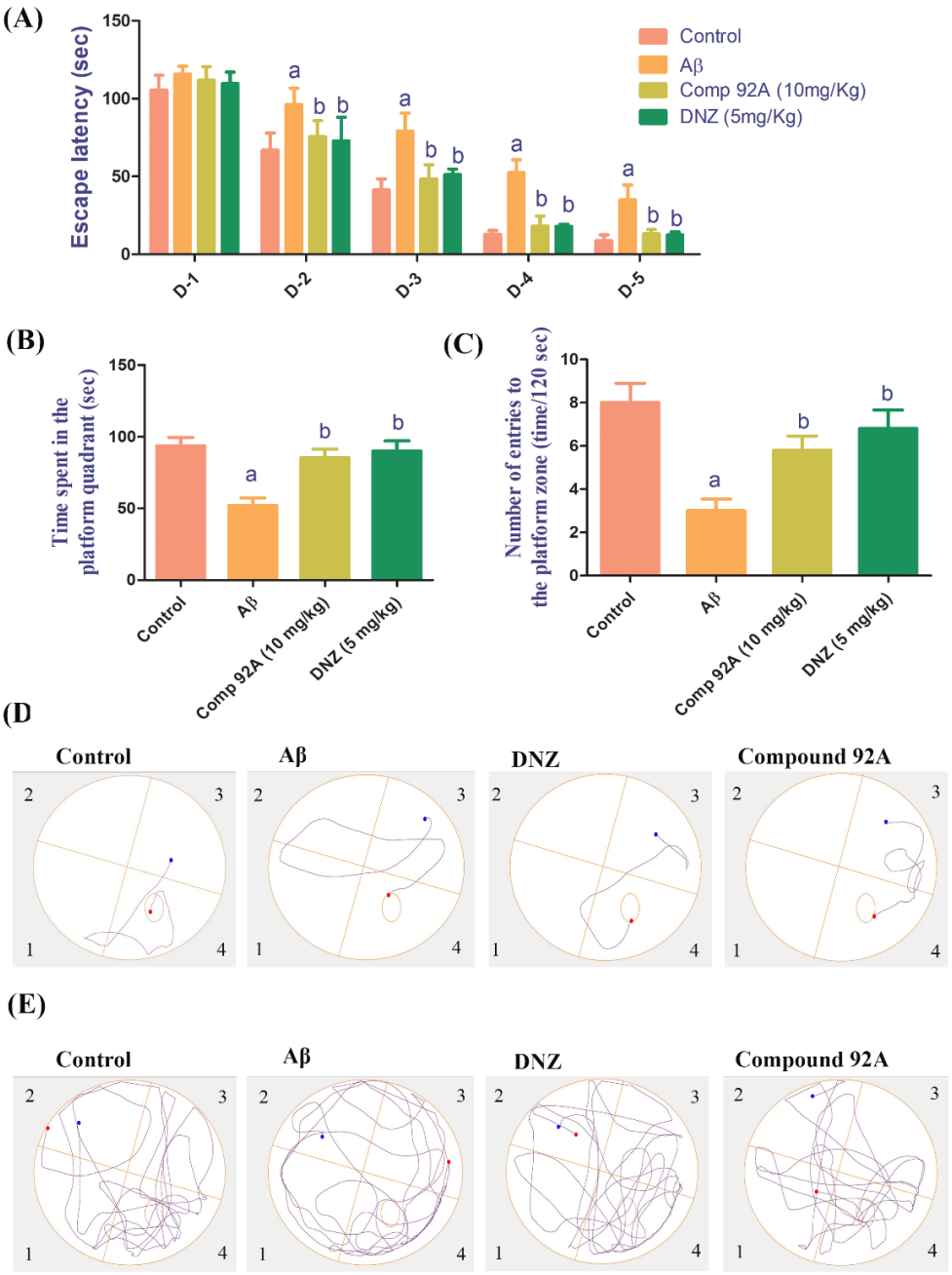


Figure 5.8. Protective effect of compound **92A** on $A\beta_{1-42}$ induced memory deficits the Morris water maze test. (A) Escape latency during the training trials in the MWM tests; (B) Time spent in the platform quadrant in the probe trail; (C) The number of entries to platform zone during the probe trial; Representative swimming tracks showing the (D) Training trials; and (E) Probe trails for the control (sham), $A\beta_{1-42}$, DNZ and compound **92A** (10 mg/kg) respectively. The data are presented as the mean ± SEM with a one-way ANOVA and two-way ANOVA, n = 6 per group, $^{a}p < 0.001$ vs. control, and $^{b}p < 0.05$ vs. on $A\beta_{1-42}$.

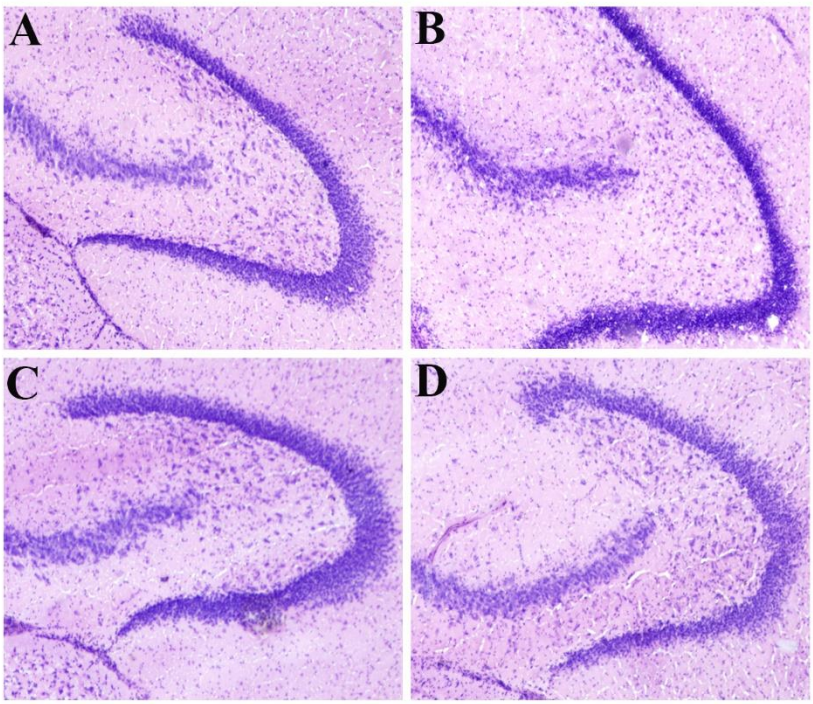


Figure 5.9. Representative images of histomorphological appearance at hippocampal region of the control (A), $A\beta_{1-42}$ group (B), administration of DNZ (C) and compound **92A** (D) treated groups. Hematoxylin and eosin staining, original magnification: 4X.

However, the degeneration and disordered arrangement in the DNZ (5 mg/kg) and compound **92A** (10 mg/kg) groups were lower and it was evidently found that test compound is potential to restore the neuronal structure and function.

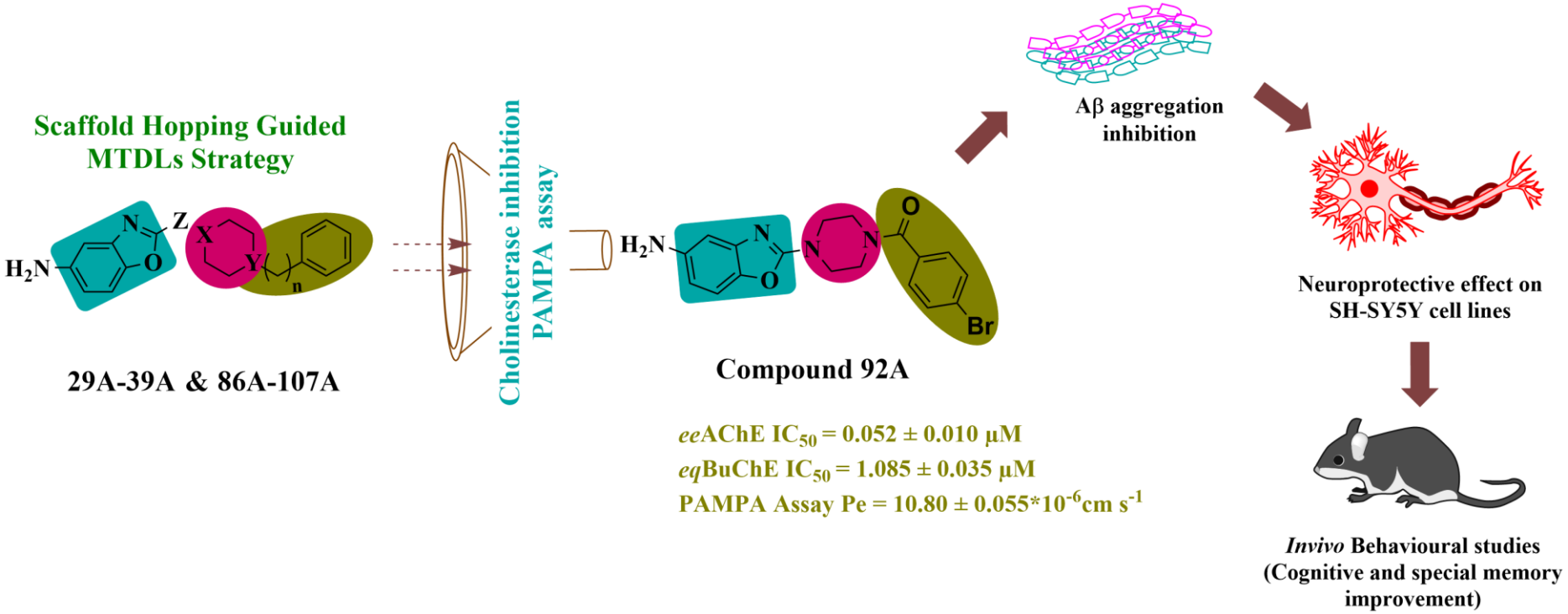


Figure 5.10. Overview of discovery of 2-substituted benzo[d]oxazol-5-amine analogs.