8 Summary and Conclusions

Novel 3,6-diphenyl-1,4-*bis*(phenylsulfonyl)piperazine-2,5-dione derivatives were designed and synthesized by amide coupling followed by cyclization to piperazine ring. The scaffold had BBB penetrating ability along with AChE and MMP-2 inhibition potential. Compounds **38**, **39**, **46**, **53**, **54**, **55**, **47**, **56** and **50** showed promising inhibition potential against AChE while compounds **52**, **38**, **45**, **47**, **56** and **50** showed high potency against MMP-2 inhibition. Further, compounds **52** and **46** showed highest inhibition potential among all for AChE with IC_{50} = 32.45±0.044 nM, 28.65±0.029 nM, BuChE, IC_{50} = 157.95±0.264, 160.58±0.082 and MMP-2 IC_{50} = 36.83±0.015 nM, 19.57±0.005 nM, respectively. These derivatives also demonstrated critical interaction with targets in molecular modeling studies. In enzyme kinetics with AChE, lead compounds **52** and **46** showed noncompetitive inhibition and preferentially bound to the free enzyme ($\alpha > 1$), while MMP-2 was found to be inhibited competitively. Both the compounds were more potent than standard donepezil in AChE-induced Aβ aggregation study at dose of 20 μM. Neuroprotection assay of compound **46**, in MC65 cell lines indicated that it had approximately equal potency as TC for inhibition of ROS induced neurodegeneration. Metal chelation property of compound **46** was helpful in MMP-2 inhibition and AChE-induced Aβ aggregation.

The working memory assessment of compounds **52** and **46** could enhance the spontaneous alteration. Compound **52** showed a significant increase in spontaneous alteration score at 10 mg/kg, while **46** had same response at half dose (5 mg/kg) indicating that there was improvement in immediate working memory. Compound **52**

reduced neophobia and anxiety at doses of 10 and 5 mg/kg as compared to scopolamine treated rats. It also displayed significant anti-anxiolytic activity which was monitored by observing the time spent by the animals in the novel arm. Compounds **52** and **46** showed significant dose-related response in passive avoidance test. Compound **52** exhibited significant increase in TLT at 10 mg/kg while similar response was observed at half (5 mg/kg) dose of compound **46**. Further, no significant difference in the mitochondrial membrane potential of brain in treated animals was observed compared with control. The histopathological studies of brain of scopolamine treated animals revealed the complete absence of neurodegenerative lessons in treated and control groups.

It is evident from the study that compounds **52**, **53** and **55** have anxiolytic activity at different doses. Compound **52** was most active at 1mg/kg dose. It stimulated amygdalar serotonergic and noradrenergic systems. The activity may be mediated through alterations in amygdalar 5HT2A facilitated serotonergic response. Further, it exhibited GABAA mediated anxiolytic response in different animal models and lacked sedative adverse effect. Thus, compound **52** may serve as a potential drug candidate for the treatment of anxiety.

Adamantyl (**16**-**27**) and their 1,4-disubstituted 1,2,3-triazole (**54**-**73**) derivatives were designed, synthesized and screened for its biological activity. The derivatives were substituted with biphenyl groups and various linkers. Compound **22** showed the maximum activity in the adamantyl (**16**-**27**) series. The results indicated that compounds containing unsubstituted amantadine (**21**, **22**) were better for the MMP-2 enzyme binding. Presence of five carbon linker along with substitution at para position of the biphenyl group increased the activity. Three, five di-substituted methyl containing compounds (**23**-**26**) were also found to be active but it was lower than the

unsubstituted amantadines. Electron withdrawing groups at para position of biphenyl along with five carbon linker increased the activity in this series. Hydrogen bond donors at third position of amantadine decreased the activity. The detailed docking pose analysis of hydroxy group containing compounds (**17**-**20**) reveled that hydrophobic amino acid residues were present around the group. These residues showed favorable interactions with compounds (**23**-**26**) containing methyl at third and fifth positions. Triazoles containing compounds having electron withdrawing groups at para position of biphenyl group along with four carbon linker showed better activity (compound **55**). Displacement of chloro group from biphenyl para position to triazoleyl phenyl decreased the activity slightly (compound **54**). Further, increasing the carbon length of linker decreased the activity due to increase in free rotation and unfavorable receptor penetration (compound **58**-**61**). Three hydroxyl and three, five dimethyl containing compounds showed reduced enzyme activity (compound **62**-**73**). Although, the decrease in the activity of compounds **62**-**73** were observed, but still the compounds were produced descent IC_{50} values (18-76 μM due to presence of triazole).

The $A\beta_{1-42}$ aggregation at ratio 10:20 μM for compounds **16**-**27** was in range of 72-92%. Most of the compounds (**16**-**20**) showed $A\beta_{1-42}$ aggregation in the range of 72-80 % range. Amantadine containing compounds (**16**-**27**) having hydroxyl group at third position showed better anti-aggregation activity against peptide due to the presence of lone pair on the hydroxyl group. Moderate antioxidant and neuroprotecting activity was in compounds **16**-**27**. Triazole group is reported for its antioxidant and neuroprotective activity. It was assumed that the activity by triazole will lead to disaggregation of $A\beta_{1-42}$. Compounds (**54**-**73**) containing triazole showed much better activity against $A\beta_{1-42}$ aggregation. These compounds also possessed superior antioxidant and cell viability potentials.

Compounds **19-23** produced mild activity against AChE, BuChE and were feebly active against BACE1. Compound **32**, of six amino quinoline series containing hydroxyl group at fourth position and two carbon linker, was found to be most active among quinolinyl alkyl piperazine (scheme I) series of compounds (**19-31**) followed by compound **27**. This series was further optimized to improve the potency against AChE, BuChE and BACE1. The activity of compounds were increased by 200 times due to shifting the position of piperazine. Compounds **72**, **73**, **75** and **76** were found to be more potent than their previous analogues. Compound **76**, of series of compounds **68-88** was found to be more active but its BBB permeability and pharmacokinetic properties were not much impressive. These results led to the synthesis of compounds **93-102**, which showed very good activity, BBB permeability and ADME properties. Compound **95** of the series can be considered as lead compound of the series with balanced potency and drugability.