

## INDEX

Contents	Page No.
<b>List of Figures</b>	xii
<b>List of Tables</b>	xxii
<b>Abbreviations and Symbols</b>	xxiii
<b>Preface</b>	xxvii
<b>CHAPTER 1. INTRODUCTION</b>	1
1.1 Alzheimer's disease	1
1.2 Pathophysiology involved in AD	2
1.2.1 Cholinergic hypothesis	3
1.2.2 Amyloid beta (A $\beta$ ) hypothesis	5
1.2.3 Tau hypothesis	6
1.2.4 Excitotoxic hypothesis	8
1.2.5 Oxidative stress hypothesis	8
1.2.6 Mitochondrial hypothesis	9
1.2.7 Neuroinflammation	10
1.2.8 Dyrk1A hypothesis	11
1.2.9 Apolipoprotein E (APO $\epsilon$ 4) hypothesis	12
1.3 Available treatment for AD	12
1.4 Newer therapeutic approaches to develop compounds for the treatment of AD	12
1.4.1 Multitarget approach	14
1.4.2 Computer-aided drug design approach	15
1.4.3 Molecular hybridization	16
1.5 Design hypothesis in the present study	16
<b>CHAPTER 2. REVIEW OF LITERATURE</b>	19
2.1. 1,3,4-Oxadiazoles as multitarget directed ligands in AD	19
2.2. Piperazines and Benzylpiperidine as MTDL	27
<b>CHAPTER 3. RATIONALE, OBJECTIVES AND PLAN OF WORK</b>	45
3.1. Rationale and objectives	45
3.1.1 Designing of part I (Series I) Molecular hybrids	46
3.1.2 Designing of part II (Series II & III) Molecular hybrids	47
3.2. Plan of work	50
3.3 Significance of the study	51
<b>CHAPTER 4. EXPERIMENTAL</b>	52
4.1 Computational studies	52
4.1.1 Pharmacophore modeling	52

Contents	Page No.
4.1.2 Virtual screening and docking-post processing (DPP)	52
4.1.3 Mechanics-Generalized Born Surface Area (MM-GBSA)	53
4.1.4 Molecular docking study	53
4.1.5 Molecular dynamics and simulations study	54
4.1.6 In silico drug likeness determination	54
4.1.7 DFT and Fukui function calculations	55
4.2 Synthesis	55
4.2.1 Chemicals and reagents	55
4.2.1.1 Series-I : 3-NH linked benzylpiperazine derivatives of 5-phenyl-1,3,4-oxadiazole 2-thione ( <b>SD 1-17</b> )	55
4.2.1.2 General procedure A for the synthesis of compound ( <b>B1-17, 2 and 2a-q</b> ).	56
4.2.1.3 General procedure B for the synthesis of compound ( <b>C1-17, 3 and 3a-q</b> ).	56
4.2.1.4 General procedure for the synthesis of compound ( <b>SD 1-17</b> )	57
4.2.2 Series-II : 3-NH linked substituted piperazine derivatives of 5-phenyl-1,3,4-oxadiazole 2-thione ( <b>4 a-j</b> )	57
4.2.2.1 General procedure for synthesis of compounds ( <b>4a-j</b> )	58
4.2.3 Series-III : 2-thiol linked N-(1-benzylpiperidin-4-yl)-2-chloroacetamide derivatives of 5-phenyl-1,3,4-oxadiazole 2-thione 1,3,4-oxadiazoles tethered with an —NH linker ( <b>5a-q</b> )	58
4.2.3.1 General procedure for synthesis of compound <b>NPBC</b>	58
4.2.3.2 General procedure for synthesis of compounds ( <b>5a-q</b> )	59
4.3 Characterization of the synthesized compounds	59
4.3.1 Melting point	59
4.3.2 TLC ( $R_f$ value)	59
4.3.3 FT-IR	60
4.3.4 $^1\text{H}$ NMR and $^{13}\text{C}$ NMR	60
4.3.5 Mass spectra	60
4.3.6 Single crystal X-Ray Crystallography	60
4.3.7 Determination of percentage purity by HPLC	61
4.4 Biological Evaluation	61
4.4.1 Pharmacology ( <i>In vitro</i> studies)	61
4.4.1.1 Human Cholinesterase (hAChE and hBChE) inhibition assay to determine $\text{IC}_{50}$ values	61
4.4.1.2 Enzyme kinetics study	62
4.4.1.3 BACE-1 inhibition study	62
4.4.1.4 Propidium iodide displacement assay	63

Contents	Page No.
4.4.1.5 Parallel artificial membrane permeation (PAMPA) assay	63
4.4.1.6 Neuroprotective studies on SH-SY5Y cell lines	64
4.4.1.6.1 Differentiation of SH-SY5Y cell lines	64
4.4.1.6.2 MTT assay	64
4.4.1.6.3 Effect of drug and standard on the morphology of differentiated SH-SY5Y cells	65
4.4.1.7 Anti-A $\beta$ aggregation activity	65
4.4.1.7.1 Thioflavin T assay	65
4.4.1.7.2 Confocal microscopy	66
4.4.1.7.3 AFM study	66
4.4.1.7.4 SEM analysis	67
4.4.2 <i>In vivo</i> behavioral and <i>ex vivo</i> studies	67
4.4.2.1 Animals	67
4.4.2.2 Acute oral toxicity study	67
4.4.2.3 Scopolamine-induced amnesia models for testing cognition enhancement in rat/mice	68
4.4.2.4 <i>Ex Vivo and biochemical analysis in scopolamine-induced model</i>	69
4.4.2.5 qRT-PCR analysis of proinflammatory cytokines (TNF- $\alpha$ and IL- $\beta$ ) in scopolamine-induced model	71
4.4.2.6 A $\beta$ -induced Morris water maze test	72
4.4.2.7 Western-blot analysis	73
4.4.2.8 Immunohistochemistry	73
4.4.2.9 Brain tissue histopathology	74
<b>CHAPTER 5. RESULTS AND DISCUSSION</b>	<b>75</b>
5.1 PART-I: SERIES I	75
5.1.1 Chemistry	75
5.1.1.1 Synthesis of Series I ( <b>SD 1-17</b> ): Substituted 1,3,4-oxadiazole-2-thione linked benzylpiperazine derivatives	75
5.1.1.2 Characterization of the synthesized compounds (Series I, <b>SD 1-17</b> )	76
5.1.2 Biological evaluation	83
5.1.2.1 Pharmacology ( <i>In vitro</i> studies)	83
5.1.2.2 <i>In vivo</i> and <i>ex vivo</i> studies	94
5.1.3 In silico studies	101
5.2 PART-II: SERIES II–V	111
5.2.1 Computational studies	111
5.2.1.1 Pharmacophore modelling	111
5.2.1.2 Virtual screening	112
5.2.1.3 Molecular docking	112
5.2.1.4 MMGB-SA	116

<b>Contents</b>	<b>Page No.</b>
5.2.1.5 Molecular dynamics simulation	117
5.2.1.6 Density Function calculation	123
5.2.1.7 In-silico prediction of Drug likeness	125
5.2.2 Chemistry	126
5.2.2.1 Synthesis of Series II (4a-j) and III (5a-q) compounds: 1.3.4-oxadiazole-2-thiol linked with substituted piperazines and N-(1-benzylpiperidin-4-yl)-2- chloroacetamide (NBPC)	126
5.2.2.2 Characterization of the synthesized compounds (Series II and III)	128
5.2.3 Biological evaluation	143
5.2.3.1 <i>In vitro</i> studies	143
5.2.3.2 <i>In vivo</i> and <i>ex vivo</i> studies	157
<b>CHAPTER 6. SUMMARY AND CONCLUSION</b>	<b>171</b>
6.1. Summary and Conclusion	171
6.2 Scope and future directions	174
<b>CHAPTER 7. REFERENCES</b>	<b>176</b>
<b>CHAPTER 8. APPENDIX</b>	<b>199</b>
8.1. <sup>1</sup> H and <sup>13</sup> C spectra of representative synthesized compounds	199
8.2. Mass spectra of representative synthesized compounds	223
8.3 HPLC chromatograms of representative synthesized compounds	230
<b>LIST OF PUBLICATIONS</b>	<b>233</b>