

Table of Contents

<i>List of figures</i>	i-v
<i>List of tables</i>	vi-vii
<i>List of schemes</i>	viii
<i>List of abbreviations</i>	ix
<i>List of symbols</i>	x
<i>Preface</i>	xi

Chapters	Page No.
1. Introduction	1-14
1.1. Alzheimer's disease (AD)	1-2
1.2. Statistics of AD	2-3
1.3. Pathophysiology of AD	3-12
1.3.1. Role of acetyl and butyrylcholines in AD	3-5
1.3.2. Role of amyloid beta (A β) and tau proteins in AD	5-7
1.3.3. Role of metals in AD	7-9
1.3.4. Role of oxidative stress in AD	9-11
1.3.5. Role of β -secretase in AD	11-12
1.4. Current drug targets for AD	12-14
1.4.1. Cholinesterases (ChEs)	12-13
1.4.1.N-methyl-D-aspartate (NMDA) receptor	14
2. Literature Review	15-50
2.1. Current therapies for AD	15-17
2.1.1. Cholinesterase inhibitors (ChEIs)	15-16
2.1.2. Noncompetitive NMDA antagonists	16-17
2.2. Antioxidant therapy for AD	17-18
2.3. Ferulic acid (FA) and AD	18-21
2.4. FA as a potential therapeutic agent for AD	21-24
2.4.1. Water-soluble ferulic acid (FA) derivatives	21
2.4.2. Dimeric derivative of FA	22
2.4.3. Development of FA ester as a memory enhancer	23-24
2.5. FA based hybrid molecules for AD	25-51
2.5.1. FA-Donepezil based hybrid molecules for AD	24-29
2.5.1.1. FA-alkylamine-benzylpiperidine hybrids (FAHs)	24-25
2.5.1.2. N-benzylpiperidine (NBP)-FA hybrid derivatives	25-26
2.5.1.3. FA-O-alkylamine derivatives	26
2.5.1.4. FA-N-alkyl benzyl piperidinediamide derivative	27
2.5.2. FA-tacrine based hybrid molecules for AD	30-34
2.5.2.1. FA-tacrine alkylenediamine hybrid molecules (TFAHs)	30
2.5.2.2. FA-tacrine-diamide side-chain derivatives	30
2.5.2.3. FA-ethylenediamine tacrine derivatives	31
2.5.2.4. FA-tacrine-melatonin hybrids (FATMHs)	32
2.5.2.5. FA-tacrine piperazine amine side chain hybrids	33
2.5.2.6. FA tacrine-nitric oxide donor trihybrid derivatives	33-34
2.5.3. FA-rivastigmine derivatives	37
2.5.4. FA-N-benzyl-piperidine and N, N-dibenzyl (N-methyl) amine derivatives	37-38
2.5.5. FA and memantine derivatives	38-39
2.5.6. FA-isoquinoline hybrids	39-40

2.5.7. FA- <i>O</i> -carbamoyl ferulamide derivative.....	40-41
2.5.8. FA-carbazole hybrid derivatives.....	41-42
2.5.9. FA-tertiary amine side chain derivatives.....	42-43
2.5.10. FA-mamoquin hybrids.....	43
2.5.11. Isosorbide-2-benzylcarbamate-5-ferulate or lipoic acid ester derivatives...	47
2.5.12. FA and 1,3,4-oxadiazole derived hybrids.....	48
2.5.13. 7- <i>O</i> -Esters of taxifolin and FA derived hybrids.....	48-49
2.5.14. FA-quinolone hybrids.....	49
2.5.15. FA-berberine hybrid analogs.....	49-50
3. Objectives, Rational and Plan of Work.....	51-57
3.1. Objectives and Rationale.....	53-54
3.2. Plan of Work.....	56
3.2. Schemes for the synthesis of FA template based novel compounds	57-58
4. Results and Discussion.....	58-138
4.1. Chemistry involved in synthesizing the first series of compounds.....	58-59
4.2. Biological evaluation of the first series of compounds.....	59
4.2.1. <i>In-vitro</i> cholinesterases inhibition (AChE and BChE) studies.....	59-68
4.2.2. Evaluation of antioxidant property.....	69-70
4.2.3. <i>In-vitro</i> cholinesterases (AChE and BChE) inhibition kinetic studies with the lead molecules.....	71
4.2.4. Measurement of propidium iodide displacement from the peripheral anionic site (PAS) of AChE.....	72
4.2.5. Evaluation of iron chelation property of the lead molecules.....	72-74
4.2.6. Evaluation of A β ₁₋₄₂ aggregation modulation activity of lead molecule	75-76
4.2.7. Molecular docking study with AChE and BChE.....	77-78
4.2.8. Molecular dynamics (MD) simulation studies with AChE.....	78-79
4.2.9. Molecular dynamics (MD) simulation studies with BChE.....	80-81
4.2.10. Calculation of physicochemical parameters.....	81-83
4.2.11. Evaluation of cytotoxicity of lead molecule.....	83-84
4.2.12. Neuroprotection studies with 7a (F24) on H ₂ O ₂ induced cell death and morphological alterations.....	84-87
4.2.13. Intracellular ROS modulation studies with 7a (F24)	87-88
4.2.14. Protective effects of 7a (F24) against H ₂ O ₂ -induced DNA fragmentation and apoptosis.....	88-89
4.2.15. Neuroprotection Studies against A β ₁₋₄₂ induced aggregation modulation property of 7a (F24)	90
4.2.16. <i>In-vitro</i> blood-brain barrier (BBB) permeation study.....	91
4.2.17. Evaluation of <i>in-vivo</i> efficacy of 7a in the AD model of Drosophila..	92-93
4.2.18. Molecular docking and dynamics simulations studies of 7a (F24) with A β ₁₋₄₂ fibrils	94-96
4.2.19. Evaluation of A β ₁₋₄₂ fibrils disintegration property of 7a (F24) using molecular dynamics simulations.....	96-99
4.2.20. Principal component analysis (PCA) of protofibril and protofibril- 7a (F24) complex.....	99-100
4.2.21. Acute toxicity studies on mice.....	101
4.2.22. Evaluation of <i>in-vivo</i> efficacy of 7a in scopolamine-induced AD mice model	
4.2.23. Ex-vivo neurochemicals estimation and antioxidant property evaluation..	103
4.2.24. Evaluation of <i>in-vivo</i> efficacy of 7a (F24) in scopolamine-induced AD mice model (Watermaze).....	105-107
4.3.1. Chemistry involved in synthesizing the second series of compounds.....	108
4.3.2. Biology involved with the second series of compounds.....	108
4.3.2.1. Design of second series of novel naturally inspired FA analogs.....	108-110
4.3.2.2. Cholinesterases inhibition studies.....	110-116
4.3.2.3. Antioxidant activity (DPPH radical scavenging activity).....	117-118

4.3.2.4. Kinetic studies of ChEs inhibition.....	118-119
4.3.2.5. Peripheral anionic site (PAS) binding study.....	119-120
4.3.2.6. Molecular modeling studies.....	120-131
4.3.2.6.1. Molecular docking.....	120-122
4.3.2.6.2. Interaction of ligands 13k and 23b with hAChE.....	122-125
4.3.2.6.3. Interaction of ligands 13k and 23b to hBChE.....	125-127
4.3.2.6.4. Analysis of binding free energy.....	127-129
4.3.2.6.5. Analysis of structural stability, compactness and solvent accessibility surface area.....	129-131
4.3.2.7. Calculation of physicochemical parameters.....	129-130
4.3.2.8. Metal chelation study.....	132-133
4.3.2.9. <i>In-vitro</i> evaluation of cytotoxicity of compound 23b	134
4.3.2.10. Neuroprotective effect of 23b on H ₂ O ₂ induced SH-SY5Y cell injury....	135
4.3.2.11. <i>In-vitro</i> blood-brain barrier permeation assay.....	136
4.3.2.12. 23b ameliorates scopolamine-induced cognitive impairment in the Morris water maze test.....	137-139
5. Experimental Section	140-192
5.1. Materials and methods.....	140-169
5.2. Biological evaluation.....	169-192
6. Summary and Conclusions	193-198
References	199-215
Appendix	216-227
List of Publications	228-229
Curriculum Vitae	230-234

List of Figures

Figure No.	Description	Page No.
1.1	Diagrammatic representation of acetylcholine synthesis, metabolism, and its mode of action.	4
1.2	Mechanism of substrate cleavage through AChE.	5
1.3	Fenton reaction by which H ₂ O ₂ forms hydroxyl radical in iron rich environment.	8
1.4	Schematic representation of role of iron in AD.	9
1.5	Diagrammatic representation indicating the role of ROS and metals in AD progression.	10
1.6	Diagrammatic representation indicating the role of β -secretase in A β aggregation.	11
1.7	Schematic view of the active site of AChE.	13
1.8	NMDA receptor complex as therapeutic target in AD.	14
2.1	Chemical structures of the drugs for the treatment of AD.	17
2.2	Chemical structures of the natural products known for the anti-AD effect.	18
2.3	Structures of novel ferulate ester derivatives with improved water solubility and potency in AD model.	22
2.4	Chemical structures of ferulic acid (FA)-donepezil (DPZ) based hybrid molecules.	28
2.5	Chemical structures of FA-tacrine derived hybrid molecules.	34
2.6	Chemical structures of FA-rivastigmine based hybrid molecules hybrid.	37
2.7	Chemical structures of various developed potent hybrids based on ferulamides derivatives of NBP, DMBA, tetrahydroisoquinoline and memantine.	40
2.8	Chemical structures of potent FA based hybrid analogs derived using fragments from memoquin, rivastigmine, carbazole, and tertiary amine side chain.	44
2.9	Chemical structures of FA based miscellaneous hybrid analogs.	50
3.1	Overview of the designed study.	54
4.1	2D interaction diagram of (A) DPZ and (B) FA with various amino acids of the AChE cavity.	61
4.2	Overlay of docked donepezil and co-crystallized donepezil.	61

4.3	Schematic pipeline toward the development of novel FA derivatives with favorable properties.	62
4.4	2D interaction diagram of (A) 4f with AChE (PDB #4EY7); (B) 4f with BChE (PDB #4BDS); (C) 7a with AChE; and (D) 7a with BChE.	65
4.5	Binding interactions (2D) of 7a (A) Pose I and (B) Pose II with various amino acids of AChE active cavity (two different binding modes).	66
4.6	Binding interactions (2D) of 10b with various amino acids of AChE active cavity.	68
4.7	Structural optimization and a brief summary of SAR with FA analogs.	68
4.8	Percent radical scavenging activity of 4f , 4g , 4i , 4j , 7a , 7b , 10b and FA .	69
4.9	Proposed mechanism for radical scavenging activity of 7a .	70
4.10	Kinetics study on the mechanism of ChE inhibition by compound 7a .	71
4.11	UV absorbance spectrum of compounds 7a and 4f alone and in the presence of FeCl ₃ in water at various pH.	74
4.12	Inhibition of self-induced A β ₁₋₄₂ aggregation in the presence of 7a , FA and DPZ. (AFM images).	76
4.13	(A) Docked pose of 7a and acetylcholine into the active site of AChE. (B) Overlaid docked pose of 7a and DPZ into the active site of AChE.	78
4.14	(A) Protein-ligand RMSD. (B) RMSF (C) 7a interactions with the key amino acid residues. (D) Bar charts of protein interaction with ligand 7a as monitored throughout the simulation.	79
4.15	(A) Protein RMSD and ligand RMSD indicate the stability of the ligand 7a with respect to BChE and its active pocket. (B) RMSF plot based on C α atoms of BChE for the BChE- 7a complex.	80
4.16	The simulation interactions diagram (SID) plot showing the protein-ligand interactions between the amino acid residues of the BChE binding site and 7a .	81
4.17	Binding interactions (2D) of 7a with various amino acids of BChE (PDB ID: 4BDS) active site.	81
4.18	Effect of 7a on cell viability.	84
4.19	Effect of 7a (F24) against H ₂ O ₂ mediated cell death.	85

4.20	Representative images of SH-SY5Y cells morphological alterations induced by H ₂ O ₂ and the effect of 7a (F24) .	87
4.21	Intracellular ROS modulation studies with 7a (F24) .	88
4.22	Detection of TUNEL-positive apoptotic strand breaks.	89
4.23	Protective effect of 7a (F24) against A β induced cell death.	91
4.24	The histogram represents the <i>in-vivo</i> effects of 7a (F24) on wild type and A β ₄₂ expressing flies.	93
4.25	(A) Interaction of 7a (F24) with A β ₁₋₄₂ protofibrils. (B) Surface representation 7a (F24) docked into the pocket of A β ₁₋₄₂ protofibrils. (C) 2D interaction diagram of 7a (F24) with A β ₁₋₄₂ protofibril.	94
4.26	Binding free-energy (kJ/mol) contribution of critical individual residues of different chains in binding 7a (F24) to A β ₁₋₄₂ protofibril.	95
4.27	(A) RMSD, (B) radius of gyration, (C) solvent accessibility surface area, and (D) change in secondary structure content of protofibrils and protofibrils- 7a (F24) complex obtained from molecular dynamics simulation data.	97
4.28	Root mean square fluctuation (RMSF) of each residue for A β ₄₂ and A β ₁₋₄₂ - F24 complex	98
4.29	Projection of the motion of (A) A β ₁₋₄₂ , and (B) A β ₁₋₄₂ - F24 complex in phase space along the first two principal eigenvectors at 300 K.	100
4.30	The eigenvalue rank along with proportion of variance (%) for (A) A β ₁₋₄₂ and (B) A β ₁₋₄₂ - F24 complex.	100
4.31	Histomorphological appearance of liver of mice.	101
4.32	Scopolamine-induced memory deficit in mice.	103
4.33	The <i>ex-vivo</i> AChE, BChE and antioxidant effect of DPZ, compound 7a (F24) , FA and scopolamine.	104
4.34	Scopolamine-induced memory deficit mice model study.	106
4.35	Design of the multifunctional ChEs inhibitors by integrating the structural features of FA derivatives.	113
4.36	ChEs inhibitory activities of selected compounds.	114
4.37	Brief summary of structural activity relationship (SAR).	115
4.38	DPPH scavenging activity by 13k , 23a , 23b , 23c and FA .	117

4.39	Lineweaver-Burk double reciprocal plot showing the mechanism of <i>hAChE</i> and <i>eqBChE</i> inhibition over a range of substrate concentrations	118
4.40	2D interaction diagram of (A) 13k with AChE, (B) 23b with AChE, (C) 13k with BChE, and (D) 23b with BChE.	122
4.41	Interactions fraction histogram of (A) DPZ, (B) 13k , and (C) 23b with <i>hAChE</i> .	123
4.42	Top panel shows the total number of specific contacts the <i>hAChE</i> makes with the (A) DPZ, (B) 13k , and (C) 23b over the course of the trajectory frame.	124
4.43	Interactions fraction histogram of (A) Tacrine, (B) DPZ, (C) 13k , and (D) 23b with <i>hBChE</i> .	125
4.44	Top panel shows the total number of specific contacts the <i>hBChE</i> makes with the (A) Tacrine, (B) DPZ, (C) 13k , and (D) 23b over the course of the trajectory frame.	126
4.45	Binding free energy of (A) AChE with ligands 13k and 23b , and (B) BChE with ligands 13k , and 23b	127
4.46	Backbone RMSD plot for (A) apo-AChE in comparison with AChE-DPZ, AChE- 13k , and AChE- 23b , (B) apo-BChE in comparison with BChE-DPZ, BChE-Tacrine, BChE- 13k , and BChE- 23b .	128
4.47	The radius of gyration plot for (A) apo-AChE in comparison with AChE-DPZ, AChE- 13k , and AChE- 23b , and (B) apo-BChE in comparison with BChE-DPZ, BChE-Tacrine, BChE- 13k , and BChE- 23b .	129
4.48	Percentage decrease in SASA of active site residues (20 Å) away from the bound ligand) of (A) AChE and (B) BChE.	129
4.49	2D interaction diagram of (A) <i>hAChE</i> with DPZ (B) <i>hBChE</i> with DPZ and (C) <i>hBChE</i> with Tacrine.	130
4.50	UV absorbance spectra of 23b alone or in the presence of FeCl ₃ in methanol (pH 4.2 and 7.4).	132
4.51	Effect of 23b on cell viability.	133
4.52	Effect of 23b against H ₂ O ₂ mediated cell death.	134
4.53	Schematic schedule of experimental design to study the anti-amnesic effect of compound 23b .	137

4.54	Escape latency time (in second) from Day 1 to Day 4.	137
4.55	Scopolamine-induced memory deficit mice model study.	138
A1-A3	¹ H-NMR, ¹³ C-NMR and HRMS spectra of (<i>E</i>)-N-(4-hydroxy-3-methoxyphenyl)-N-(2-((3-methoxyphenyl)amino)-2-oxoethyl)acrylamide (4f).	216- 218
A4-A6	¹ H-NMR, ¹³ C-NMR and HRMS spectra of (<i>E</i>)-N-(2-((1H-indol-5-yl) amino)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (7a).	219- 221
A7-A9	¹ H-NMR, ¹³ C-NMR and HRMS spectra of (<i>E</i>)-1-(4-(3-fluorobenzyl) piperazin-1-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (13k.HCl).	222- 224
A10- A12	¹ H-NMR, ¹³ C-NMR and HRMS spectra of (<i>E</i>)-3-(4-hydroxy-3-methoxyphenyl)-N-(2-((2-(5-methoxy-1H-indol-3-yl)ethyl)amino)-2-oxoethyl)acrylamide (23b).	225- 227

List of Tables

Table No.	Description	Page No.
2.1	<i>In-vitro</i> biological data for evaluating the therapeutic potential of hybrid analogs based on novel ferulic acid (FA)-donepezil (DPZ) and their mode of action.	29
2.2	<i>In-vitro</i> biological data for the evaluation of the therapeutic potential of hybrid analogs based on novel FA-tacrine-based hybrid analogs and their mode of action.	35-36
2.3	<i>In-vitro</i> biological data for evaluating the therapeutic potential of hybrid analogs based on FA and their mode of action.	45-46
4.1	Cholinesterase inhibitory activities of the target compounds.	67-68
4.2	Antioxidant activity (DPPH assay) of 4f , 4g , 4i , 4j , 7a , 10b , and FA .	70
4.3	Displacement of propidium iodide from the peripheral anionic site of AChE by 7a , FA and DPZ at the indicated concentrations.	72
4.4	Theoretical prediction of physiochemical parameters of the developed compounds.	82-83
4.5	Permeability in the PAMPA-BBB assay for 7a , testosterone, imipramine, corticosterone, hydrocortisone, and DPZ with their predictive BBB-penetration.	91
4.6	Binding free energy between A β ₁₋₄₂ protofibrils and 7a (F24) in the last 10 ns MD simulation.	96
4.7	Time-average and standard deviation of RMSD, R _g , and SASA value along with the sum of Eigen values for A β ₁₋₄₂ protofibril and A β ₁₋₄₂ protofibril- 7a (F24) Complex.	97
4.8	Average binding free energy along with its individual contributing terms for chain C and D of protofibril in presence and absence of 7a (F24).	99
4.9	Structures, ChEs inhibitory activities, cLogP, and tPSA of compounds 13a-13p , 18a-18c and 23a-23c .	113-114
4.10	Antioxidant activity (DPPH assay) of 13a , 13c , 13f , 13h , 13i , 13k-13l , 18a-18c , 23a-23c and FA .	116

4.11	Displacement of propidium iodide from the peripheral anionic site of AChE by 13k , 23b , 23c , and DPZ at the indicated concentrations.	119
4.12	Docking results (docking score, XP GScore, glide gscore, and glide emodel) and interaction details between the <i>h</i> AChE and different FA analogs.	120
4.13	Docking results (docking score, XP GScore, glide gscore, and glide emodel) and interaction details between the BChE and different FA analogs.	120-121
4.14	The average ΔG_{bind} and its different contributing energy terms for 13k and 23b against hAChE and hBChE calculated from MD trajectories (last 25) ns.	127
4.15	Calculation of physicochemical parameters of the developed molecules.	130-131
4.16	Permeability in the PAMPA-BBB assay for 13k , 23b , DPZ, testosterone, corticosterone, and hydrocortisone with their predictive BBB penetration.	135

List of Schemes

Schemes	Description	Page No.
Scheme 1	Synthesis of FA-acetamide derivatives 4a-4q .	55
Scheme 2	Synthesis of FA-Indole or quinoline derivatives.	55
Scheme 3	Synthesis of FA tethered to <i>N</i> -phenyl-piperazine scaffolds 10a-10g .	56
Scheme 4	Synthesis of benzylpiperazine derivatives 13a-13p .	56
Scheme 5	Synthesis of FA-alanine-amide compounds 18a-18c .	57
Scheme 6	Synthesis of FA-tryptamine-glycine amide 23a-23c .	57