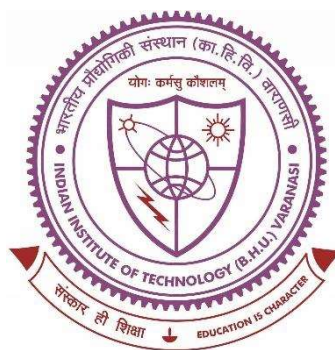


Design, Synthesis and Evaluation of Substituted Acetamides and Carbohydrazones as Dual Monoamine Oxidase and Acetylcholinesterase Inhibitors



**Thesis submitted in partial fulfilment
for the award of degree**

DOCTOR OF PHILOSOPHY

By

Mr. Sandeep Kumar

**PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY (BHU)
VARANASI – 221 005, UP
INDIA**

Senthil Raja A, Professor

25 October 2024

CERTIFICATE

It is certified that the work contained in the thesis titled “**Design, Synthesis and Evaluation of Substituted Acetamidees and Carbohydrazones as Dual Monoamine Oxidase and Acetylcholinesterase Inhibitors**” by **Mr. Sandeep Kumar** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of course work, comprehensive examination, candidacy, state-of-the-art seminar and presubmission seminar.

Supervisor


(Senthil Raja A)

Professor (Pharmaceutical Chemistry),
Pharmaceutical Engineering and Technology
IIT (BHU) Varanasi-221005

DECLARATION BY THE CANDIDATE

I, **Sandeep Kumar** certify that the work embodied in this PhD thesis is my own bona fide work and carried out by me under the supervision of **Prof. Senthil Raja A**, from July 2019 to October 2024, at the Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraphs, text, data, results, *etc.*, reported in journals, books, magazines, dissertations, theses, *etc.*, or available at websites and have not included them in this PhD thesis and have not cited as my own work.

Date: 25 October 2024

Place: Varanasi


(Sandeep Kumar)

CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of my knowledge.


(Dr. Senthil Raja A)
Supervisor


(Dr. S. Hemalatha) 25/10/24

Head of the Department
विभागाध्यक्ष / Head
भेषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग /
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान / INDIAN INSTITUTE OF TECHNOLOGY
(बनारस हिन्दू विश्वविद्यालय) / (BANARAS HINDU UNIVERSITY)
वाराणसी-221005 / Varanasi-221005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: "Design, Synthesis and Evaluation of Substituted Acetamides and Carbohydrazones as Dual Monoamine Oxidase and Acetylcholinesterase Inhibitors"

Name of the Student: Sandeep Kumar

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University) Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the "**Doctor of Philosophy**"

Date: 25 October 2024

Place: Varanasi


(Sandeep Kumar)

ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost respect and obeisance to **Bharat Ratna Pandit Madan Mohan Malaviya Ji** and Late **Prof. M.L. Schroff** for their humanitarian vision in building this temple of learning and values.

I convey my deepest sense of gratitude towards my PhD supervisor, **Prof. (Dr.) Senthil Raja A.** for his constant guidance, support and encouragement throughout my PhD journey and for his unwavering belief in me.

I am grateful to the respected **Prof. (Mrs.) S. Hemalatha**, Head of Department of Pharmaceutical Engineering & Technology, IIT BHU for providing all facilities and allowing me to carry out my research work in the various labs of the department.

My heartfelt gratitude is conveyed to the former Heads of Department, **Prof. S.K. Singh** and **Prof. S.K. Shrivastava** for their kind support and guidance.

I am thankful to all the respected faculty members of my department **Prof. B. Mishra, Prof. Sairam K., Prof. M.S. Muthu, Dr. Ashok Kumar, Dr. A.N. Sahu, Dr. A.K. Agrawal, Dr. G.P. Modi, Dr. Ruchi Chawla, Dr. S.K. Jain, Dr. Vinod Tiwari, Dr. P.K. Naik, Dr. S.K. Mishra, Dr. Rajnish, Dr. Deepak Kumar, Dr. Dinesh Kumar, Dr. Jairam Meena** and **Dr. Arun Khatri** for their kind support, guidance and encouragement.

The valuable suggestions by my RPEC members, **Dr. S.K. Singh, Dr. S.K. Mahto** and **Dr. Jeyakumar K.** and are gratefully acknowledged.

I am also thankful to my colleagues, **Mr. Rangan Mitra, Ms. Shreyasi Majumdar & Mr. Gajendra T.A.** and respected faculty members, **Prof. Sairam K. & Dr. S.K. Mahto** for their invaluable collaboration and for enriching the research work envisaged and carried out.

I happily acknowledge my colleagues and friends, **Dr. K. Bhanukiran, Dr. Vishnu Priya, Dr. Deepa Dehari, Dr. Abhishesh Kr Mehta, Anurag, Himanshu, Sunil, Nilesh, Powsali** and **Ravikiran** for their thought-provoking discussions and uplifting moral support.

The support and the resources provided by ‘PARAM Shivay Facility’, the Central Instrument Facility (CIF), IIT (BHU) and Analytical Laboratory, Department of Chemistry, BHU are gratefully acknowledged.

I am grateful to IIT (BHU) for providing a Teaching Assistantship and the Ministry of Education, GoI, New Delhi for STARS Grant (No: STARS 1/583).

I express my gratitude to every non-teaching staff of the dept., especially Mr. A.N. Upadhyay, Mr. Yashvant Singh and Mr. Anand Kumar for their constant and all-encompassing support.

I would also like to acknowledge all the **animals** used throughout our research work and throughout the history of scientific research, for their ultimate sacrifice in order to advance scientific knowledge, which we, as humans, are eternally grateful for.

Lastly, I would like to acknowledge the unrelenting support and love by my beloved **parents & family** which always encouraged me to carry on...

Date: 25 Oct 2024

Place: Varanasi



Sandeep Kumar

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
TABLE OF CONTENTS	iii
LIST OF FIGURES	x
LIST OF SCHEMES	xii
LIST OF TABLES	xiii
LIST OF ABBREVIATIONS	xiv
LIST OF SYMBOLS	xv
PREFACE	xvi
1. Introduction and literature review	1
1.1. Introduction.....	1
1.1.1. Overview of neurodegenerative diseases (NDDs): Demography & epidemiology ..	1
1.1.2. Overview of NDDs.....	2
1.1.2.1. Alzheimer’s disease	4
1.1.2.2. Parkinson’s disease	7
1.1.3. Neuroprotectants and their role in treating various NDDs	8
1.2. Literature review.....	9
1.2.1. Synthetic hybrids of available drugs as dual MAO-AChE inhibitors	9
1.2.2. Synthetic small molecule dual MAO-AChE inhibitors.....	11
1.2.3. Natural small molecule dual MAO-AChE inhibitors.....	16
1.2.4. Summary	17
1.3. Rationale, objectives and plan of work.....	18
1.3.1. Rationale and design strategy.....	18
1.3.2. Objectives.....	19
1.3.3. Plan of work	20
2. Benzothiazole-derived Thioacetamides	23
2.1. Rationale, objectives and plan of work.....	23
2.1.1. Rationale.....	23
2.1.2. Objectives	24
2.1.3. Plan of work	25
2.2. Experimental work.....	25
2.2.1. Tools and datasets.....	25
2.2.2. Pharmacophore-based virtual screening protocol	26
2.2.3. Validated virtual lead-based library design	30
2.3. Chemistry.....	31

2.3.1.	Synthesis.....	31
2.3.2.	Physicochemical characterization.....	33
2.3.3.	Spectral characterization.....	35
2.4.	Biological studies.....	36
2.4.1.	<i>In vitro</i> studies.....	36
2.4.1.1.	<i>In vitro</i> MAO-A/B enzyme inhibition assay.....	36
2.4.1.2.	<i>In vitro</i> ChEs inhibition assay.....	37
2.4.1.3.	Enzyme kinetics and reversibility assay.....	39
2.4.1.4.	<i>In vitro</i> antioxidant assay.....	40
2.4.1.5.	<i>In vitro</i> metal chelation assay.....	41
2.4.1.6.	<i>In vitro</i> blood-brain-barrier permeation assay.....	41
2.4.1.7.	Cell-based neurotoxicity assay.....	42
2.4.1.8.	Neuroprotection assay using neuroblastoma cell line.....	43
2.4.2.	<i>In vivo</i> studies.....	44
2.4.2.1.	Scopolamine induced amnesia model.....	44
2.4.2.2.	Y-maze test.....	45
2.4.2.3.	<i>Ex vivo</i> biochemical analysis.....	45
2.4.2.4.	<i>In vivo</i> acute oral toxicity evaluation of compound ZBT113	47
2.5.	Computational studies.....	48
2.5.1.	Molecular docking.....	48
2.5.2.	Molecular dynamics simulation.....	50
2.5.3.	Ligand binding efficiency.....	51
2.5.4.	Prediction of ADMET properties.....	51
2.6.	Results and discussion.....	52
2.6.1.	Pharmacophore-based virtual screening (PBVS) studies.....	52
2.6.2.	Validation of virtual lead by molecular docking and MD simulation.....	53
2.7.	Chemistry.....	55
2.7.1.	Synthesis.....	55
2.7.2.	Physicochemical characterization of final compounds.....	55
2.7.3.	Spectral characterization of final compounds.....	56
2.8.	Biological studies.....	65
2.8.1.	<i>In vitro</i> studies.....	65
2.8.1.1.	<i>In vitro</i> MAO inhibition assays.....	66
2.8.2.	<i>In vitro</i> ChEs inhibition assays.....	69

2.8.2.1.	Enzyme kinetics and reversibility assay	70
2.8.2.2.	<i>In vitro</i> antioxidant assay	71
2.8.2.3.	<i>In vitro</i> metal chelation assay.....	72
2.8.2.4.	<i>In vitro</i> blood-brain-barrier permeation assay.....	73
2.8.2.5.	Cell-based toxicity assay.....	74
2.8.2.6.	Neuroprotection assay using neuroblastoma cell line	76
2.8.3.	<i>In vivo</i> studies.....	77
2.8.3.1.	Scopolamine induced amnesia model.....	77
2.8.3.2.	Y-maze test.....	77
2.8.3.3.	<i>Ex vivo</i> biochemical analysis	78
2.8.3.4.	<i>In vivo</i> acute oral toxicity of compound ZBTI13	80
2.9.	Computational studies.....	83
2.9.1.	Molecular docking.....	83
2.9.1.1.	Molecular docking studies against MAO-A	83
2.9.1.2.	Binding analysis of compound ZBTI13 with MAO-A.....	84
2.9.1.3.	Molecular docking studies against MAO-B.....	85
2.9.1.4.	Binding analysis of compound ZBTI13 with MAO-B.....	85
2.9.1.5.	Molecular docking studies against AChE	87
2.9.1.6.	Binding analysis of compound ZBTI13 with AChE	87
2.9.1.7.	Molecular docking studies against BChE	88
2.9.1.8.	Binding analysis of compound ZBTI13 with BChE	89
2.9.2.	Molecular dynamic simulation.....	89
2.9.2.1.	MD simulation of compound ZBTI13 -MAO-B complex	90
2.9.2.2.	MD simulation of compound ZBTI13 -AChE complex.....	91
2.9.3.	Comparative analysis of docking and MDS studies.....	92
2.9.4.	Ligand binding efficiency.....	93
2.9.5.	Predicted ADMETox parameters.....	94
2.10.	Summary	96
3.	Sesamol-derived O-Acetamides & Extended Carbohydrazones	99
3.1.	Rationale, objectives and plan of work.....	99
3.1.1.	Rationale.....	99
3.1.2.	Objectives.....	101
3.1.3.	Plan of work	101
3.2.	Experimental work.....	102
3.2.1.	Chemistry	102

3.2.1.1.	Synthesis.....	102
3.2.1.2.	Physicochemical characterization.....	105
3.2.1.3.	Spectral characterization.....	105
3.2.2.	Biological studies.....	105
3.2.2.1.	<i>In vitro</i> studies	105
3.2.2.1.1.	<i>In vitro</i> MAO inhibition assay	105
3.2.2.1.2.	<i>In vitro</i> ChEs inhibition assays	106
3.2.2.1.3.	Enzyme kinetics and reversibility assay	106
3.2.2.1.4.	<i>In vitro</i> antioxidant assay	106
3.2.2.1.5.	<i>In vitro</i> metal chelation assay.....	107
3.2.2.1.6.	<i>In vitro</i> blood-brain-barrier permeation assay.....	107
3.2.2.1.7.	Cell-based neurotoxicity assay.....	107
3.2.2.1.8.	Neuroprotection assay using neuroblastoma cell line.....	107
3.2.2.2.	<i>In vivo</i> studies	108
3.2.2.2.1.	Scopolamine induced amnesia model	108
3.2.2.2.2.	Y-maze test.....	108
3.2.2.2.3.	<i>Ex vivo</i> biochemical analysis	109
3.2.2.2.4.	<i>In vivo</i> acute oral toxicity evaluation of compound SMA09	109
3.3.	Computational studies.....	109
3.3.1.	Molecular docking.....	109
3.3.2.	Molecular dynamics simulation.....	109
3.3.3.	Ligand binding efficiency.....	110
3.3.4.	Predicted ADMET properties	110
3.4.	Results and discussion.....	111
3.4.1.	Chemistry.....	111
3.4.1.1.	Physicochemical characterization of final compounds (SMA series)	111
3.4.1.2.	Spectral characterization of final compounds (SMA series)	111
3.4.1.3.	Physicochemical characterization of final compounds (SMH series)	118
3.4.1.4.	Spectral characterization of final compounds (SMH series)	119
3.4.2.	Biological studies.....	130
3.4.2.1.	<i>In vitro</i> studies	130
3.4.2.1.1.	<i>In vitro</i> MAO inhibition assay (SMA series)	130
3.4.2.1.2.	<i>In vitro</i> ChEs inhibition assay (SMA series).....	133
3.4.2.1.3.	<i>In vitro</i> MAO inhibition assay (SMH series).....	135

3.4.2.1.4. <i>In vitro</i> ChEs inhibition assay (SMH series)	138
3.4.2.1.5. Enzyme kinetics and reversibility assay	140
3.4.2.1.6. <i>In vitro</i> antioxidant assay	142
3.4.2.1.7. <i>In vitro</i> metal chelation assay	144
3.4.2.1.8. <i>In vitro</i> blood-brain-barrier permeation assay	145
3.4.2.1.9. Cell-based neurotoxicity assay	146
3.4.2.1.10. Neuroprotection assay using neuroblastoma cell line.....	149
3.4.2.2. <i>In vivo</i> studies	150
3.4.2.2.1. Scopolamine induced amnesia model.....	150
3.4.2.2.2. Y-maze test.....	150
3.4.2.2.3. <i>Ex vivo</i> biochemical analysis	152
3.4.2.2.4. <i>In vivo</i> acute oral toxicity of compound SMA09	153
3.4.3. Computational studies	155
3.4.3.1. Molecular docking	155
3.4.3.1.1. Molecular docking studies against MAO-A	156
3.4.3.1.2. Binding mode of compounds SMA09 and SMH06 with MAO-A	157
3.4.3.1.3. Molecular docking studies against MAO-B	160
3.4.3.1.4. Binding mode of compounds SMA09 and SMH06 with MAO-B.....	160
3.4.3.1.5. Molecular docking studies against AChE.....	161
3.4.3.1.6. Binding mode of compounds SMA09 and SMH06 with AChE	161
3.4.3.1.7. Molecular docking studies against BChE.....	164
3.4.3.1.8. Binding mode of compounds SMA09 and SMH06 with BChE	164
3.4.3.2. Molecular dynamics simulation.....	166
3.4.3.2.1. MD simulation of compound SMA09 -MAO-B complex.....	167
3.4.3.2.2. MD simulation of compound SMH06 -MAO-B complex	168
3.4.3.2.3. MD simulation of compound SMA09 -AChE complex	169
3.4.3.2.4. MD simulation of compound SMH06 -AChE complex.....	170
3.4.3.3. Ligand binding efficiency	172
3.4.3.4. Predicted ADMET properties.....	173
3.5. Summary	176
4. Eugenol-derived Carbohydrazones.....	179
4.1. Rationale, objectives and plan of work.....	179
4.1.1. Rationale.....	179
4.1.2. Objectives.....	181

4.1.3.	Plan of work.....	182
4.2.	Experimental work.....	182
4.2.1.	Chemistry.....	182
4.2.1.1.	Synthesis	182
4.2.1.2.	Physicochemical characterization.....	184
4.2.1.3.	Spectral characterization	184
4.2.2.	Biological studies.....	184
4.2.2.1.	<i>In vitro</i> studies	184
4.2.2.1.1.	<i>In vitro</i> MAO inhibition assay	184
4.2.2.1.2.	<i>In vitro</i> ChEs inhibition assays	185
4.2.2.1.3.	Enzyme kinetics and reversibility assay	185
4.2.2.1.4.	<i>In vitro</i> antioxidant assay	186
4.2.2.1.5.	<i>In vitro</i> metal chelation assay.....	186
4.2.2.1.6.	<i>In vitro</i> blood-brain-barrier permeation assay.....	186
4.2.2.1.7.	Cell-based neurotoxicity assay.....	186
4.2.2.1.8.	Neuroprotection assay using neuroblastoma cell line.....	187
4.2.2.2.	<i>In vivo</i> studies	187
4.2.2.2.1.	Scopolamine-induced amnesia model (Y-maze test)	187
4.3.	Computational studies.....	188
4.3.1.	Molecular docking.....	188
4.3.2.	Molecular dynamics simulation.....	188
4.3.3.	Ligand binding efficiency.....	188
4.3.4.	Predicted ADMET properties	188
4.4.	Results and discussion.....	189
4.4.1.	Chemistry.....	189
4.4.1.1.	Physicochemical characterization of final compounds.....	189
4.4.1.2.	Spectral characterization of final compounds.....	190
4.4.2.	Biological studies.....	202
4.4.2.1.	<i>In vitro</i> studies	202
4.4.2.1.1.	<i>In vitro</i> MAO inhibition assay	203
4.4.2.1.2.	<i>In vitro</i> ChEs inhibition assay	206
4.4.2.1.3.	Enzyme kinetics and reversibility assay	208
4.4.2.1.4.	<i>In vitro</i> antioxidant assay	209
4.4.2.1.5.	<i>In vitro</i> metal chelation assay.....	210

4.4.2.1.6. <i>In vitro</i> blood-brain-barrier permeation assay	211
4.4.2.1.7. Cell-based neurotoxicity assay	212
4.4.2.1.8. Neuroprotection assay using neuroblastoma cell line.....	214
4.4.2.2. <i>In vivo</i> studies	214
4.4.2.2.1. Scopolamine-induced amnesia model (Y-maze test).....	214
4.4.3. Computational studies	215
4.4.3.1. Molecular docking	215
4.4.3.1.1. Molecular docking studies with MAO-A	216
4.4.3.1.2. Proposed interactions of compound SIEH10 with MAO-A	217
4.4.3.1.3. Molecular docking studies with MAO-B.....	218
4.4.3.1.4. Observed interactions of compound SIEH10 with MAO-B	219
4.4.3.1.5. Molecular docking studies with AChE	220
4.4.3.1.6. Observed interactions of compound SIEH10 with AChE.....	220
4.4.3.1.7. Molecular docking studies with BChE	222
4.4.3.1.8. Observed interactions of compound SIEH10 with BChE.....	222
4.4.3.2. Molecular dynamics simulation	222
4.4.3.2.1. MD simulation of compound SIEH10 -MAO-B complex.....	223
4.4.3.2.2. MD simulation of compound SIEH10 -AChE complex	224
4.4.3.3. Ligand binding efficiency	225
4.4.3.4. Predicted ADMET properties.....	226
4.5. Summary	228
5. Concluding Remarks and Future Prospective	231
5.1. Conclusion	231
5.2. Future prospective.....	234
References.....	235
Appendix	A1
List of publications	A22

LIST OF FIGURES

Figure 1.1. Global prevalence of NDDs	2
Figure 1.2. Pie chart showing the distribution of neurodegenerative diseases	4
Figure 1.3. Protein structure of target enzymes MAO-B and AChE	7
Figure 1.4. Biological activity of carbohydrazones, sesamol and eugenol derivatives	19
Figure 1.5. Schematic representation of comprehensive plan of work	21
Figure 2.1. Design of compounds ZBTI01-ZBTI14	24
Figure 2.2. Workflow for plan of work	25
Figure 2.3. Workflow of PBVS guided identification of MAO-B inhibitors.....	29
Figure 2.4. 2D chemical structures of designed compounds (ZBTI01-ZBTI14)	31
Figure 2.5 Principle of peroxidase-linked colorimetric assay.....	37
Figure 2.6 Principle involved in the AChE/BChE inhibition assay	38
Figure 2.7 Principle of DPPH-based antioxidant assay	41
Figure 2.8. Principle and reaction of MTT assay.....	43
Figure 2.9. Results of pharmacophore-based virtual screening.....	53
Figure 2.10. 3D molecular docking and MDS interactions of ZINC02181408	54
Figure 2.11. Enzyme kinetics, reversibility and Dixon plot of compound ZBTI13	70
Figure 2.12. Free radical scavenging activity of selected compounds.....	71
Figure 2.13. UV absorption spectra of compounds ZBTI11 , ZBTI13 and ZBTI14	73
Figure 2.14. <i>In vitro</i> cellular assay of compound ZBTI13	75
Figure 2.15. Cell viability graphs of compound ZBTI13	76
Figure 2.16. <i>In vitro</i> neuroprotection assay of compound ZBTI13	77
Figure 2.17. <i>In vivo</i> neuroprotection compound ZBTI13	78
Figure 2.18. <i>Ex vivo</i> biochemical study of compound ZBTI13	80
Figure 2.19. Acute oral toxicity (organ coefficient) of compound ZBTI13	81
Figure 2.20. Acute oral toxicity (histopathology) of compound ZBTI13	82
Figure 2.21. 3D superimposition image of compounds ZBT01-ZBTI14	83
Figure 2.22. 3D binding mode and 2D interaction map of ZBTI13 with MAO	86
Figure 2.23. 3D orientation and 2D interaction of ZBTI13 with ChE.....	88
Figure 2.24. MD simulation of compound ZBTI13 with MAO-B	91
Figure 2.25. MD simulation of compound ZBTI13 with AChE.	92
Figure 2.26. Ligand binding efficiency of ZBTI01-ZBTI14	94
Figure 2.27. Summary of key outcomes from the ZBTI series.....	98

Figure 3.1. Design rationale for compounds SMA01-SMA11 & SMH01-SMH17	99
Figure 3.2. 2D chemical structure of designed compounds SMA01-SMA11	100
Figure 3.3. 2D chemical structure of designed compounds SMH01-SMH17	100
Figure 3.4. Schematic workflow for plan of work	102
Figure 3.5. Possible rotational isomers in compounds SMH01-SMH17	121
Figure 3.6. Representative ¹ H NMR spectrum of SMH02 in CDCl ₃	121
Figure 3.7. Enzyme kinetics, reversibility and Dixon plot of SMA09	141
Figure 3.8. Enzyme kinetics, reversibility and Dixon plot of SMH06	142
Figure 3.9. Free radical scavenging activity of selected of SMA and SMH series	143
Figure 3.10. UV spectra of SMA05 , SMA08 , SMA09 , SMH06 and SMH14	145
Figure 3.11. <i>In vitro</i> toxicity assay of compound SMA09	147
Figure 3.12. <i>In vitro</i> neurotoxicity assay of compound SMH06	148
Figure 3.13. Cell viability graphs of compounds SMA09 and SMH06	149
Figure 3.14. <i>In vitro</i> neuroprotection assay of compound SMA09	150
Figure 3.15. <i>In vitro</i> neuroprotection of compound SMA09 and SMH06	151
Figure 3.16. <i>Ex vivo</i> biochemical study of compound SMA09	153
Figure 3.17. Acute oral toxicity (organ coefficient) of compound SMA09	154
Figure 3.18. Acute oral toxicity (histopathology) of compound SMA09	155
Figure 3.19. 3D superimposition image of SMA01-SMA11 and SMH01-SMH17	156
Figure 3.20. 3D and 2D interaction of SMA09 and SMH06 with MAO	159
Figure 3.21. 3D orientation and 2D interaction of SMA09 and SMH06 with ChE	163
Figure 3.22. MD simulation of compound SMA09 with MAO-B	168
Figure 3.23. MD simulation of compound SMH06 with MAO-B	169
Figure 3.24. MD simulation of compound SMA09 with AChE	170
Figure 3.25. MD simulation of compound SMH06 with AChE	171
Figure 3.26. Ligand binding efficiency of SMA01-SMA11 and SMH01-SMH17	173
Figure 3.27. Summary of key outcomes from the SMA and SMH series	177
Figure 4.1. The design rationale for the design of eugenol-derived carbohydrazones ...	180
Figure 4.2. 2D chemical structure of designed SEH01-SEH11 & SIEH01-SIEH11	181
Figure 4.3. Overview of research workflow	182
Figure 4.4. Enzyme kinetics and reversibility and Dixon plot of compound SIEH10 ...	208
Figure 4.5. Antioxidant activity of selected compounds of SEH and SIEH series	209
Figure 4.6. UV absorption spectra of compounds SEH05 , SIEH06 and SIEH10	211

Figure 4.7. <i>In vitro</i> neurotoxicity assay of compound SIEH10	213
Figure 4.8 Cell viability graph of compound SIEH10	213
Figure 4.9 <i>In vitro</i> neuroprotection assay of compound SIEH10	214
Figure 4.10. <i>In vitro</i> neuroprotection of compound SIEH10	215
Figure 4.11. 3D orientation map of ligands SEH01-SEH11 and SIEH01-SIEH11	217
Figure 4.12. 3D alignment and 2D interaction map of SIEH10 with MAO.	219
Figure 4.13. 3D alignment and 2D interaction map of SIEH10 with ChE.....	221
Figure 4.14. MD simulation of SIEH10 with MAO-B.....	224
Figure 4.15. MD simulation of SIEH10 with AChE	225
Figure 4.16. Ligand binding efficiency of SEH01-SEH11 & SIEH01-SIEH11	226
Figure 4.17. Summary of key findings from SEH and SIEH series	229
Figure 5.1. Conclusive summary and overall outcome.....	233

LIST OF SCHEMES

Scheme 2.1. Synthetic scheme for compounds ZBTI01-ZBTI14	24
Scheme 3.1. Synthetic scheme for compounds SMA01-SMA11 & SMH01-SMH17	99
Scheme 4.1. Synthetic scheme for compounds SEH01-SEH11 & SIEH01-SIEH11	180

LIST OF TABLES

Table 1.1. Structure and activity profile of dual MAO-AChE inhibitory MTDL hybrids	10
Table 1.2. Structure and activity profile of synthetic dual MAO-AChE inhibitors	12
Table 1.3. Structure and activity profile of natural dual MAO-AChE inhibitors.....	16
Table 2.1. Predicted pharmacokinetic properties of ZINC02181408	54
Table 2.2. Physicochemical characterization results of compounds ZBTI01-ZBTI14	56
Table 2.3. <i>In vitro</i> enzyme inhibition results of compounds ZBTI01-ZBTI14	65
Table 2.4. Antioxidant activity % FRS of selected compounds of ZBTI series	72
Table 2.5. PAMPA assay data for compound ZBTI13	74
Table 2.6. Molecular docking results of compounds ZBTI01-ZBTI14	84
Table 2.7. Predicted ADME parameters of compounds ZBTI01-ZBTI14	94
Table 2.8. Predicted toxicity parameters of compounds ZBTI01-ZBTI14	95
Table 3.1. Physicochemical characterization results of compounds SMA01-SMA11 ...	111
Table 3.2. Physicochemical characterization results of compounds SMH01-SMH17 ...	118
Table 3.3. The chemical shift of duplicated signals in ¹ H NMR of SMH01-SMH17	122
Table 3.4. <i>In vitro</i> enzyme inhibition results of compounds SMA01-SMA11	130
Table 3.5. <i>In vitro</i> enzyme inhibition results of compounds SMH01-SMH17	136
Table 3.6. Antioxidant activity % FRS of selected compounds of SMA & SMH series	143
Table 3.7. PAMPA-BBB assay result of compound SMA09	146
Table 3.8 Results of in silico docking studies of compounds SMA01-SMA11	165
Table 3.9. Results of in silico docking studies of compounds SMH01-SMH17	166
Table 3.10. Predicted ADME parameters of compounds SMA01-SMA11	174
Table 3.11. Predicted ADME properties of compounds SMH01-SMH17	174
Table 3.12. Predicted toxicity parameters of compounds SMA01-SMA11	175
Table 3.13. Predicted toxicity properties of compounds SMH01-SMH17	175
Table 4.1. Physicochemical characterization of SEH01-SEH11 & SIEH01-SIEH11 ...	189
Table 4.2. <i>In vitro</i> enzyme inhibition results of SEH01-SEH11 & SIEH01-SIEH11 ...	202
Table 4.3. Percent FRS and EC ₅₀ values of selected compounds	209
Table 4.4. PAMPA-BBB assay result of compound SIEH10	212
Table 4.5. Molecular docking results of SEH01-SEH11 & SIEH01-SIEH11	216
Table 4.6. Predicted ADME properties of SEH01-SEH11 & SIEH01-SIEH11	227
Table 4.7. Predicted toxicity properties of SEH01-SEH11 & SIEH01-SIEH11	228

LIST OF ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
A β	Amyloid beta
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADME	Absorption, distribution, metabolism, and excretion
ALS	Amyotrophic lateral sclerosis
ATCI	Acetylthiocholine iodide
BBB	Blood-brain barrier
BTCI	Butyrylthiocholine iodide
CAS	Catalytic anionic site
CDCl ₃	Deuterated chloroform
DMF	N,N-dimethylformamide
DMSO- <i>d</i> ₆	Deuterated dimethyl sulfoxide
DPZ	Donepezil
DPPH	2,2-Dithphenyl-1-picrylhydrazyl
DTNB	5,5'-Dithiobis-2-nitrobenzoic acid
<i>ee</i> AChE	Electric eel acetylcholinesterase
<i>h</i> AChE	Human acetylcholinesterase
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HRMS	High resolution mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
MTDL	Multitarget directed ligand
PAS	Peripheral anionic site
PDB	Protein data bank
PBL	Porcine brain lipid
SAR	Structure activity relationship
SEM	Standard error of mean
TMS	Tetramethylsilane

LIST OF SYMBOLS

α	Alpha
β	Beta
Δ	Delta
λ	Lambda
$^{\circ}\text{C}$	Degree Celsius
\AA	Angstrom
mg	Milligram
μg	Microgram
μM	Micromolar
mM	Millimolar
mL	Millilitre
μL	Microlitre
h	Hour
nm	Nanometre
ppm	Parts per million
rpm	Revolutions per minute
kcal	Kilocalories
MHz	Megahertz
J	Coupling constant
d	Doublet
t	Triplet
m	Multiplet
dd	Doublet of doublet
m/z	Mass-to-charge ratio
%	Percent
pH	Potential of hydrogen
<	Less than
\leq	Less than, or equal
>	More than
\pm	Plus, or minus
\sim	Approximate

PREFACE

The evolving complex pathophysiology of neurodegenerative diseases (NDDs) poses a big challenge to medicinal chemists to discover potential small molecule therapies. The available anti-neurodegenerative therapies provide only a symptomatic relief to patients and lack the desired efficacy; therefore, effective therapeutics are needed. Multitarget-directed ligands (MTDLs) capable of inhibiting brain monoamine oxidase-B (MAO-B) and acetylcholinesterase (AChE) and chelating brain iron ions have significant advantages in treating NDDs. Several potential MTDLs have been explored, however their further clinical development is limited by their poor neuroprotective properties *in vivo*. Thus, there is a need to develop efficacious MTDLs possessing a balanced multifunctional profile. In the current study, we have employed pharmacophore-based virtual screening and ligand-guided optimization approaches to develop new MTDLs possessing dual MAO-B-AChE inhibition, iron chelation and neuroprotective properties.

The present study is divided into five chapters and are as follows.

Chapter 1 describes the background, pathophysiology, and available drug therapies for NDDs and provides an exhaustive literature review on the reported dual MAO-ChE inhibitory hybrids/scaffolds followed by the research rationale and objectives of the study.

Chapter 2 deals with virtual screening-guided design, synthesis, characterization and biological evaluation of benzothiazole-derived thioacetamides as potential MTDLs (**ZBTI series**).

Chapter 3 deals with the design, synthesis, and biological evaluation of sesamol-derived O-acetamides (**SMA series**) and sesamol-derived extended carbohydrazones (**SMH series**).

Chapter 4 documents the design, synthesis, characterization and biological evaluation of eugenol/isoeugenol-derived carbohydrazones (**SEH & SIEH series**).

Chapter 5 presents the concluding remarks and future scope.

At the end, an appendix containing supporting information, spectral data of representative compounds, and a list of publications is included.