

Acknowledgements

*Prima facie, I am grateful to the God for giving me good health and wellbeing that were necessary to complete this research work. I bow at the lotus feet of Bharat Ratna **Pandit Madan Mohan Malaviyaji**, founder of the Banaras Hindu University.*

*I would like to express my gratitude to my mentor, guide and **guardian Prof. Sushant Kumar Shrivastava**, for his support, patience, guidance, motivation and encouragement throughout my Ph.D. Thank you for believing in me and giving me ample space to grow by myself. Your technical and editorial advice was essential to the completion of this dissertation and has taught me innumerable lessons and insights on the workings of academic research and in general. I am indebted to him for his appreciation for success, backing and support in failures, which ultimately made me keen to tackle any obstructions occurring during the research work. Without his supervision and constant help, this dissertation would not have been possible.*

*I also take this opportunity to express my deepest gratitude **Prof. Sanjay Singh**, Head, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi for allowing me to use the Departmental facilities for carrying out my research work.*

*I am grateful to, **Prof. S.K. Singh** and **Prof. B. Mishra**, Former Heads, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi for their constant inspiration, valuable suggestions and help which have led to the successful completion of this work.*

*I wish to express my heartiest gratitude to **Dr. A.K. Srivastava**, **Prof. Hemalatha S.**, **Prof. K. Sairam**, **Dr. Senthil Raja A.**, **Dr. M.S. Muthu**, **Dr. A.N. Sahu**, **Dr. S.K. Mishra**, **Dr. R. Chawla**, **Dr. P.K. Nayak**, **Dr. V.K. Tiwari**, **Dr. S.K. Jain**, and **Dr. A. Agrawal** for their constant help and support.*

*I owe a warm thanks to **Dr. Gyan Prakash Modi** for helping me in carrying out the homology modeling of GAT-1, which is a very crucial part of my thesis.*

*I owe my gratitude to all my respected **RPC members** for their encouragement and insightful comments leading me to the completion of the research work.*

*I pay my sincere thanks to **Mr. Nandlal, Mr. Madan, Mr. Upadhya, Mr. Sanjeev, Mr. Yashwant, Mr. Jameel, Mr. Pathak, Mr. Arun, Mr. Gopal, Mrs. Saroj Kumari, Mrs. Archana Singh, Ms. Shyamli Ghoshal** and other non-teaching staff of the Department for their timely assistance and co-operation during my Ph.D. tenure.*

*I have been blessed with a friendly and cheerful group of fellow students **Mr. Piyush Sharma, Dr. Anupam Banerjee and Dr. Gireesh kumar Singh**, who were always willing to help and give their best suggestions as well as moral support. I cannot forget the efforts made by them in preparation and submission of the thesis.*

*I would particularly like to acknowledge the contribution of my batch-mates **Dr. Nagendra, Dr. Dileep, Dr. Debapriya, Dr. Sanjay, Anshul, Ranga and Gopal**. It is not sufficient to express my gratitude with only a few words. They have always been there to motivate me during the hour of hardship and helped me unconditionally throughout Ph.D. tenure.*

*I am indeed thankful to have the company of friendly lab mates **Mr. Pavan, Mr. Prabhash, Mr. Avanish, Mr. Tanmaya, Mrs. Priyanka, Dr. Manish, Mr. Digambar, Mrs. Mousami, Ms. Poorvi & Mr. Sombir** who were ever ready to provide me with all possible help. They created a happy working environment and foster camaraderie within the laboratory.*

*Amongst my juniors, I would like to thank **Mukesh, Gopi, Devendra, Sathish and Gunjan** for their alacrity and support during the tenure.*

*I also acknowledge the warm support extended to me by **Dr. Manish Kumar, Dr. Damiki Laloo, Dr. S.K. Prasad, Mr. Ravi B. Singh and Dr. Aditya Singh**. They made my stay at Varanasi a very pleasant and memorable one.*

*I would also like to express my thanks to my ever supportive seniors: **Dr. Nirupam Das, Dr. Meenakshi Dhanawat, Dr. Saurabh K. Sinha, Dr. Chhanda C.***

Danta, Dr. Rahul Tripathi and Dr. Achint Jain who were always there with a helping attitude whenever I was in need.

*A special thanks to my family. Words cannot express how grateful I am to all my family members for all of the sacrifices that they made on my behalf and continuous support they provided to me. Most importantly, none of this would have been possible without the love and patience of my **parents and wife**. They have been a constant source of love, moral support and strength all these years. I owe this achievement to them.*

*Last but not the least, I pray **God** for the **animals** who were mortified for the cause of research and advancement of knowledge. May their souls rest in peace.*

Date:

Place: Varanasi

(Ankit Seth)

INDEX

CONTENTS	Page No.
List of Abbreviations	i-ii
List of Figures	iii-iv
List of Tables	v
Preface	vi-vii
CHAPTER-1 INTRODUCTION	1-13
1.1. Drug Design, Discovery and Development	1
1.2. Epilepsy: An Overview	3
1.3. GABA Hypothesis & Epilepsy	8
1.3.1. GABA Synthesis and Metabolism	9
1.3.2. GABA Receptors	9
1.3.3. GABA Transporters	10
1.4. Piperidine 3-Carboxylic Acid (Nipecotic Acid)	11
CHAPTER-2 REVIEW OF LITERATURE	14-26
2.1. Nipecotic Acid: An <i>In Vitro</i> GABA Reuptake Inhibitor	14
2.2. Piperidine-3-Carboxylic Acid Derivatives as Anticonvulsants	15
2.3. Naphthalene Derivatives as Anticonvulsants	23
CHAPTER-3 RATIONALE, OBJECTIVE AND PLAN OF WORK	27-29
3.1. Rationale and Objective	27
3.2. Plan of Work	28
CHAPTER-4 EXPERIMENTAL	30-43
4.1. Chemistry	30
4.1.1. Chemicals and Reagents	30
4.1.2. Series 1	30
4.1.2.1. Method of Preparation	30
4.1.2.2. Reaction Mechanism for Series 1	31
4.1.3. Series 2	32
4.1.3.1. Method of Preparation	32
4.1.3.2. Reaction Mechanism for Series 2	34
4.1.4. Characterisation of Synthesised Compounds	34
4.1.4.1. Physicochemical Characterisation	35
4.1.4.2. Spectral Characterisation and Elemental Analysis	36
4.2. Biological Activity of Series 1	37
4.2.1. Animals	37
4.2.2. Drug Administration	37
4.2.3. Evaluation of <i>In Vivo</i> Anti-Convulsant Activity	37

CONTENTS	Page No.
4.2.3.1. sc-PTZ Induced Seizures in Mice	37
4.2.3.2. Pilocarpine Induced Seizures in Mice	38
4.2.3.3. DMCM Induced Seizures in Mice	38
4.2.4. Rota-rod Performance Test in Mice	38
4.2.5. MTT Assay on Neuroblastoma Cell Line (SH-SY5Y)	39
4.2.6. In Vitro Parallel Artificial Membrane BBB Permeability Assay (PAMPA)	39
4.3. Biological Activity of Series 2	40
4.4. Statistical analysis	40
4.5. Computational Studies	40
4.5.1. Homology Modelling of GAT-1	40
4.5.2. <i>In Silico</i> Docking	41
4.5.3. Molecular Dynamics (MD)	42
4.4.2.1. MD Simulation Protocol for Compound 4i	42
4.4.2.1. MD Simulation Protocol for Compound 5w	42
4.5.4. Prediction of drug likeliness and <i>in silico</i> ADME	43
CHAPTER-5 RESULTS AND DISCUSSION	44-92
5.A. Synthesis of Acetonaphthones Tethered Piperidine-3-Carboxylic Acid Derivatives. [series 1]	44-67
5.A.1. Chemistry	44
5.A.1.1. Physicochemical Characterisation	44
5.A.1.2. Spectral Characterisation and Elemental Analysis	45
5.A.2. Biological Activity	51
5.A.2.1. Evaluation of Anticonvulsant Activity	51
5.A.2.2. Rota-rod Performance Test in Mice	54
5.A.2.3. Cell viability and neurotoxicity (MTT Assay)	55
5.A.2.4. <i>In Vitro</i> PAMPA-BBB Assay	56
5.A.3. Computational Studies	80
5.A.3.1. Homology modeling of GAT-1	58
5.A.3.2. <i>In Silico</i> Docking	60
5.A.3.3. <i>In Silico</i> Molecular Dynamics	63
5.A.3.4. Estimation of "Drug-Like" Properties	66
5.B. 5,6-diphenyl-1,2,4-triazine-3(2 <i>H</i>)-ones bearing five-member (1,3,4 oxadiazole/thiadiazole, 1,2,4-triazole) heterocyclic moieties [Series 2]	68-92
5.B.1. Chemistry	68
5.B.1.1. Physicochemical Characterisation	68

CONTENTS	Page No.
5.B.1.2. Spectral Characterisation and Elemental Analysis	70
5.B.2. Biological Activity	79
5.B.2.1. <i>In Vitro</i> PAMPA-BBB Assay	79
5.B.2.2. Evaluation of Anticonvulsant Activity	81
5.B.2.3. Rota-rod Performance Test in Mice	84
5.B.2.4. . Cell viability and neurotoxicity (MTT Assay)	85
5.B.3. Computational Studies	85
5.B.3.1. Homology modeling of GAT-1	85
5.B.3.2. <i>In Silico</i> Docking	86
5.B.3.3. <i>In Silico</i> Molecular Dynamics	89
5.B.3.4. Estimation of “Drug-Like” Properties	91
CHAPTER-6 SUMMARY AND CONCLUSION	93-96
CHAPTER-7 REFERENCES	97-113
CHAPTER-8 APPENDIX	114-125
8.1. ¹ H and ¹³ C Spectra of Representative Compounds from Series 1	114
8.2. ¹ H and ¹³ C Spectra of Representative Compounds from Series 2	120
LIST OF RESEARCH PAPERS AND PRESENTATIONS	
REPRINTS OF PUBLISHED RESEARCH PAPERS	

<p>% – Percentage</p> <p>°C – Degree centigrade</p> <p>µl – Microlitre</p> <p>µM – Micromolar</p> <p>ALP – Alkaline phosphatase</p> <p>ALT – Alanine transaminase</p> <p>ANOVA – Analysis of variance</p> <p>AST – Aspartate transaminase</p> <p>ATP – Adenosine triphosphate</p> <p>BUN – Blood urea nitrogen</p> <p>CADD – Computer aided drug design</p> <p>CDCl₃ – Deuterated chloroform</p> <p>cm – Centimeter</p> <p>CNS – Central nervous system</p> <p>CoMFA – Comparative molecular field analysis</p> <p>CoMSIA – Comparative molecular similarity indices analysis</p> <p>DMCM – Methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate</p> <p>ED₅₀ – Median effective dose</p> <p>FDA – Food and drug administration</p> <p>FTIR – Fourier transform infrared spectroscopy</p> <p>g – Gram(s)</p> <p>GABA – γ-aminobutyric acid</p> <p>GAT – GABA transporters</p> <p>h – Hour</p> <p>i.p. – Intraperitoneal</p> <p>IC₅₀ – Half maximal inhibitory concentration</p>	<p>TLC – Thin layer chromatography</p> <p>TMS – Tetramethylsilane</p> <p>UV – Ultra violet</p> <p>XRD – X-ray diffraction</p>
--	---

<p>Kg – Kilogram</p> <p>LBDD – Ligand based drug design</p> <p>m.p. – Melting point</p> <p>MD – Molecular dynamics</p> <p>MES – Maximal electroshock</p> <p>MET – Metrazole</p> <p>MTT – 3-(4,5-Dimethylthiazol-2-yl)- 2,5-Diphenyltetrazolium Bromide</p> <p>mg – Milligram</p> <p>min – Minutes</p> <p>ml – Milliliter</p> <p>mm – Millimeter</p> <p>mmol – Millimole</p> <p>Mol. Eq. – Molar equivalent</p> <p>NCE – New chemical entity</p> <p>NMDA – N-methyl-D-aspartate</p> <p>NMR – Nuclear magnetic resonance</p> <p>ns – Nanosecond</p> <p>OECD – Organization for economic co-operation and development</p> <p>OPLS – Optimised potentials for liquid simulations</p> <p>P – Partition coefficient</p> <p>PAMPA – Parallel artificial membrane permeability assay</p> <p>p.o. – Per oral</p> <p>PDB – Protein data bank</p> <p>ps – Picosecond</p> <p>PTZ</p> <p>QSAR – Quantitative structure-activity relationship</p>	
--	--

<p>R_f – Retention factor</p> <p>RMSD – Root mean square deviations</p> <p>rpm – Revolutions per minute</p> <p>s – Seconds</p> <p>SBDD – Structure-based drug design</p> <p>sc – Subcutaneous</p> <p>scPTZ – Subcutaneous pentylenetetrazole</p> <p>SD – Standard deviation</p> <p>SBDD – Structure based drug design</p>	
--	--

CONTENTS	PAGE NO.
List of Abbreviations	i
List of Figures	ii-iii
List of Tables	iv
Preface	v-vi
1. INTRODUCTION	
1.1. Drug Design, Discovery, and Development	1
1.2. Epilepsy: An Overview	4
1.3. GABA Hypothesis & Epilepsy	10
1.3.1. GABA Synthesis and Metabolism	11
1.3.2. GABA Receptors.....	12
1.3.3. GABA Transporters	13
1.4. Piperidine 3-Carboxylic Acid (Nipecotic Acid)	15
2. REVIEW OF LITERATURE	
2.1. Nipecotic Acid: An <i>In Vitro</i> GABA Reuptake Inhibitor	19
2.2. Piperidine-3-Carboxylic Acid Derivatives as Anticonvulsants	20
2.3. Napthalene Derivatives as Anticonvulsants	30
3. RATIONAL, OBJECTIVE AND PLAN OF WORK	
3.1. Rationale & Objective	35
3.2. Plan of Work	36
3.2.1. Synthesis	36
3.2.2. Characterization of the Synthesized Compounds (Series 1 and 2)	37
3.2.3. Biological Evaluation of Series 1	37
3.2.4. Biological Evaluation of Series 2.....	37
3.2.5. Computational Studies of the Selected Leads from Series 1 & 2	38
4. EXPERIMENTAL	
4.1. Chemistry	39
4.1.1. Chemicals and Reagents	39
4.1.2. Series 1	39
4.1.2.1. <i>Method of Preparation</i>	39
4.1.2.2. <i>Reaction Mechanism for Series 1</i>	41
4.1.3. Series 2	41
4.1.3.1. <i>Method of Preparation</i>	41
4.1.3.2. <i>Reaction Mechanism for Series 2</i>	44

4.1.4. Characterization	44
4.1.4.1. <i>Physicochemical Characterization</i>	45
4.1.4.2. <i>Spectral Characterization and Elemental Analysis</i>	46
4.2. Biological Activity of Series 1	47
4.2.1. Animals	47
4.2.2. Drug Administration	48
4.2.3. <i>In Vivo</i> Anti-convulsant Activity	48
4.2.3.1. <i>sc-PTZ Induced Seizures in Mice</i>	48
4.2.3.2. <i>Pilocarpine Induced Seizures in Mice</i>	48
4.2.3.3. <i>DMCM Induced Seizures in Mice</i>	49
4.2.4. Rota-rod Performance Test in Mice	49
4.2.5. MTT Assay on Neuroblastoma Cell Line (SH-SY5Y)	49
4.2.6. Repeated dose toxicity studies:	50
4.2.7. <i>In Vitro</i> PAMPA-BBB Assay	51
4.3. Biological Activity of Series 2	51
4.4. Statistical Analysis	52
4.5. Computational Studies	52
4.5.1. Homology Modelling of GAT-1	52
4.5.2. Molecular Docking Studies	53
4.5.3. Molecular Dynamics (MD) Simulations	54
4.5.4. Prediction of drug likeliness and <i>in silico</i> ADME properties	54
5. RESULTS AND DISCUSSION	
5A. SERIES 1	56
5.A.1. Chemistry	56
5.A.1.1. Physicochemical Characterization	56
5.A.1.2. Spectral Characterization and Elemental Analysis	57
5.A.2. Biological Activity	65
5.A.2.1. <i>In Vivo</i> Anti-convulsant Activity	65
5.A.2.1.1. <i>sc-PTZ Induced Seizures in Mice</i>	65
5.A.2.1.2. <i>Pilocarpine-induced seizures in mice</i>	67
5.A.2.1.3. <i>DMCM induced seizures in mice</i>	68
5.A.2.2. Rota-rod Performance Test in Mice	69
5.A.2.3. Cell viability and neurotoxicity (MTT Assay)	69
5.A.2.4. Repeated dose toxicity studies	70

5.A.2.5. <i>In Vitro</i> PAMPA-BBB Assay	73
5.A.3. Computational Studies	74
5.A.3.1. Homology modeling of GAT-1	74
5.A.3.3. Molecular Dynamics	81
5.A.3.4. Estimation of “Drug-Like” Properties	84
5.B. SERIES 2	86
5.B.1. Chemistry	86
5.B.1.1. Physicochemical Characterization	86
5.B.1.2. Spectral Characterization and Elemental Analysis	88
5.B.2. Biological Activity	100
5.B.2.1. <i>In vitro</i> PAMPA-BBB assay	100
5.B.2.2. <i>In Vivo</i> Anti-convulsant Activity	102
5.B.2.2.1. <i>sc-PTZ Induced Seizures in Mice</i>	102
5.B.2.2.2. <i>DMCM Induced Seizures in Mice</i>	103
5.B.2.3. Rota-rod Performance Test in Mice	104
5.B.2.2. Cell viability and neurotoxicity (MTT Assay)	105
5.B.2.3. Repeated dose toxicity studies	105
5.B.3. Computational Studies	107
5.B.3.1. Homology modeling of GAT-1	107
5.B.3.2. Molecular Docking Studies	107
5.B.3.3. Molecular Dynamics	112
5.B.3.4. Estimation of “Drug-Like” Properties	114
6. SUMMARY AND CONCLUSION	116
7. REFERENCES	121
8. APPENDIX	140