

CONTENTS

Title	Page No.
Certificate	iii
Declaration by the Candidate	iv
Copyright Transfer Certificate	v
Acknowledgements	vi
Contents	ix-xiv
LIST OF FIGURES	xv-xvii
LIST OF TABLES	xviii
LIST OF ABBREVIATIONS	xix-xx
PREFACE	xxi-xxv
CHAPTER 1: Introduction and Literature Review	1-41
1.1 Introduction	1
1.2 Normal v/s pathophysiological pain	2
1.3 Epidemiology of chronic pain	3
1.4 Pain ontology	4
1.5 Functional anatomy of pain	6
1.5.1 Primary afferents	6
1.5.2 Spinal cord	8
1.5.3 Ascending pain pathway	10
1.5.4 Brain	11
1.5.5 Descending pain pathway	12
1.6 Molecular mechanisms of pain	12
1.6.1 Neurobiology of chronic pain	14
1.6.2 Peripheral sensitization	15
1.6.3 Central sensitization	16
1.7 Neuropathic pain	18
1.8 Inflammatory pain	20
1.9 Pharmacotherapeutics for the treatment of chronic pain and their limitations	22

1.9.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)	23
1.9.2 Anticonvulsants	23
1.9.3 Antidepressants	24
1.9.4 Opioids	25
1.9.5 NMDA antagonists	26
1.9.6 Other pharmacological treatment	26
1.10 Kinesin superfamily proteins and their role in intracellular trafficking	26
1.11 Kinesin nanomotors mediated trafficking of NMDA-loaded cargo as a novel target in chronic pain	28
1.11.1 NMDA receptor system: A key player in the pathophysiology of chronic pain	29
1.11.2 NMDA mediated central sensitization across the ascending and descending pain pathways	30
1.11.3 NR2B assembly sub-unit of NMDA critically mediates chronic pain	33
1.11.4 KIF17 as a major mediator for the trafficking of NR2B subunit	35
1.12 Aurora kinase and its inhibitor tozasertib/VX680	39
CHAPTER 2: Rationale, Objectives and Plan of Work	42-46
2.1 Rationale	42
2.2 Objectives	43
2.3 Plan of work	44
2.3.1 Study I	44
2.3.2 Study II	45
2.2.3 Study III	46
CHAPTER 3: Materials & Methods	47-69
3.1 Drugs, chemicals and antibodies	47
3.2 Equipment and software	50
3.3 In-silico studies	51
3.3.1 Molecular dynamics simulation study	51
3.4 In-vivo studies	52

3.4.1	Experimental animals	52
3.4.2	Ethical committee approval	53
3.4.3	Animal model of neuropathic pain and experimental design	53
3.4.4	Formalin induced acute inflammatory pain model	55
3.4.5	Complete Freund's adjuvant induced chronic inflammatory pain in rats	55
3.4.6	Animal pain behavior tests	56
3.4.6.1	Tail flick test: Analgesic assay	56
3.4.6.2	Tail clip test	56
3.4.6.3	Pinprick test	57
3.4.6.4	Hargreaves test: Thermal hyperalgesia	57
3.4.6.5	von-Frey hair Test: Static allodynia	57
3.4.6.6	Cotton swab test: Dynamic mechanical test	58
3.4.6.7	Ice floor test	58
3.4.6.8	Acetone evaporation test	59
3.4.6.9	Conditioned place preference: Spontaneous ongoing pain assay	59
3.4.7	Behavioral neurotoxicity assays	60
3.4.7.1	Rota-rod test	60
3.4.7.2	Open field test	61
3.4.8	Tissue harvesting and storage	61
3.4.9	Biochemical assays	61
3.4.9.1	Lipid peroxidation	61
3.4.9.2	Nitrite estimation	62
3.4.9.3	Glutathione estimation	62
3.4.10	Molecular biology studies	63
3.4.10.1	Western blot analysis	63
3.4.10.2	Reverse transcription polymerase chain reaction (rtPCR) analysis	65
3.5	Acute toxicity study in rats	66
3.5.1	Animals	66

3.5.2 Animal grouping and experimental design	67
3.5.3 Body weight and food water consumption	67
3.5.4 Gross observations and mortality	67
3.5.5 Hematological index	68
3.5.6 Blood biochemical analysis	68
3.5.7 Histopathological analysis	68
3.6 Statistical analysis	69
CHAPTER 4: Deciphering the Role of KIF17-NR2B Signaling in Nerve Injury Mediated Evoked and Ongoing Pain and its Modulation by Pan-Aurora Kinase Inhibition	70-105
4.1 Introduction	70
4.2 Experimental procedure	72
4.3 Results and discussion	73
4.3.1 In-silico studies	73
4.3.2 Effect of tozasertib on nerve injury-induced pain-like behavior in rats	79
4.3.2.1 Tozasertib treatment attenuates thermal hyperalgesia in nerve injured rats	79
4.3.2.2 Tozasertib treatment inhibits mechanical allodynia (static but not dynamic) and hyperalgesia in nerve-injured rats	82
4.3.2.3 Tozasertib attenuates cold allodynia and cold hyperalgesia in nerve injured rats	85
4.3.3 Tozasertib did not altered the pain threshold of naïve uninjured rats	88
4.3.4 Tozasertib inhibits spontaneous ongoing pain behavior in nerve-injured rats	90
4.3.5 Tozasertib does not affect locomotor or exploratory activity of nerve injured rats	94
4.3.6 Tozasertib treatment attenuates inflammatory signaling in nerve injured rats	96
4.3.7 Tozasertib interferes with KIF17 and NR2B expression in the spinal cord and dorsal root ganglion of nerve-injured rats	99

4.3.7.1	Tozasertib significantly reduced nerve injury induced-NR2B mRNA and protein expression in spinal and DRG tissue	99
4.3.7.2	Tozasertib but not gabapentin suppressed the nerve injury-induced KIF17 expression	103
4.4	Outcomes	104
CHAPTER 5: Modulation of KIF-17/NR2B Crosstalk by Tozasertib in Inflammatory Pain Rat Model		106-127
5.1	Introduction	106
5.2	Experimental design	108
5.3	Results and discussion	109
5.3.1	Tozasertib attenuates only the second phase of formalin-induced inflammatory pain	109
5.3.2	Effect of tozasertib on thermal, mechanical and cold pain hypersensitivities in CFA injected rats	111
5.3.2.1	Pan aurora kinase inhibition decreases heat hyperalgesia in CFA injected rats	111
5.3.2.2	Tozasertib attenuates cold-hyperalgesia in CFA injected rats	113
5.3.2.3	Pan aurora kinase inhibition reduced CFA induced mechanical hyperalgesia in rats	114
5.3.2.4	Tozasertib attenuates mechanical allodynia in CFA injected rats	116
5.3.2.5	Tozasertib attenuates cold allodynia in chronic inflammatory pain rat model	117
5.3.3	Effect of tozasertib on CFA induced biochemical and molecular alterations	118
5.3.3.1	Tozasertib attenuates oxidative-nitrosative stress in the sciatic nerve of CFA-injected rats	118
5.3.3.2	Tozasertib inhibits glial cell activation in dorsal root ganglion and spinal cord of CFA injected rats	121
5.3.3.3	Tozasertib suppressed the KIF17/NR2B/mlin10 expression in dorsal root ganglion and spinal cord of CFA injected rats	123
5.4	Outcomes	127

CHAPTER 6: Acute Toxicity Study for Tozasertib in Rats	128-137
6.1 Introduction	128
6.2 Experimental design	129
6.3 Results and discussion	130
6.3.1 Tozasertib administration did not affect the on gross behavior in rats	130
6.3.2 Tozasertib did not altered body weight and food-water consumptions in rats	130
6.3.3 Hematology index	131
6.3.4 Tozasertib did not affected the blood biochemical profile of rats	132
6.3.5 Gross necroscopy	134
6.3.6 Tozasertib single dose administration has no effect on organ body weight of rats	134
6.3.7 Histopathology	135
6.4 Outcomes	137
CHAPTER 7: Summary & Conclusions	138-143
7.1 Summary	138
7.2 Conclusion	142
7.3 Limitations and outlook for future work	142
References	144-161
List of Publications	162-168

LIST OF FIGURES

		Page No.
Chapter I	Introduction and Literature review	
Fig. 1.1	Stimulus response function depicting normal v/s pathophysiological pain	3
Fig.1.2	Illustration representing the different categories of pain	5
Fig.1.3	Spinal organization of nociceptive central terminals	10
Fig.1.4	Neurobiology of pain processing	11
Fig.1.5	Peripheral sensitization under chronic pain	16
Fig.1.6	Molecular mechanism of central sensitization in chronic pain	17
Fig.1.7	Mechanism of kinesin mediated cargo transport	28
Fig.1.8	Role of NMDA receptor system in the neurobiology of chronic pain	31
Fig.1.9	Schematic representation of KIF17 and cargo (NR2B) binding	36
Fig.1.10	Kinesin mediated transport of NMDA receptor	37
Fig.1.11	2D chemical structure of tozasertib	40
Chapter III	Materials & Methods	
Fig. 3.1	CCI model being performed in our lab at IIT (BHU)	53
Fig. 3.2	Experimental timeline	54
Fig. 3.3	Redness and swelling in rat paw before and after CFA injection	56
Chapter IV	Deciphering the Role of KIF17-NR2B Signaling in Nerve Injury Mediated Evoked and Ongoing Pain and its Modulation by Pan-Aurora Kinase Inhibition in Rats	
Fig.4.1	A) The molecule of tozasertib bound in the binding site of human aurora kinase A according to X-ray data (structure code 3E5A) B) Comparative analysis of primary sequences of human and rat aurora kinase A	74
Fig.4.2	Protein-ligand RMSD for tozasertib and aurora kinase A	75
Fig.4.3	Protein-ligand contacts for tozasertib and aurora kinase	76

Fig.4.4	Root Mean Square Fluctuation for tozasertib and aurora kinase	77
Fig.4.5	Timeline representation of the interactions and contacts for tozasertib and aurora kinase A	78
Fig.4.6	A schematic of detailed ligand atom interactions with the protein residues	79
Fig.4.7	Effect of pan-aurora kinase inhibition on pain-like behavior in nerve-injured rats.	81
Fig.4.8	Effect of pan-aurora kinase inhibition on (A) Cotton swab test (D) Pinprick test (E) Acetone test (F) Cold hyperalgesia test	84
Fig.4.9	Effect of tozasertib on pain-like behavior in the contralateral paw of nerve injured rats	87
Fig.4.10	Effect of tozasertib on normal pain threshold of healthy naïve rats (A and B) tail flick test	89
Fig.4.11	Effect of tozasertib, a pan-Aurora kinase inhibitor, on spontaneous ongoing pain in nerve injured rats	91
Fig.4.12	Effect of tozasertib on spontaneous ongoing pain behavior in nerve injured rats	92
Fig.4.13	Effect of morphine on spontaneous ongoing pain behavior in nerve injured rats	93
Fig.4.14	Effect of gabapentin on spontaneous ongoing pain behavior in nerve injured rats	93
Fig.4.15	Effect of tozasertib on locomotor activity of nerve injured rats (A, B and C) using open field test	95
Fig.4.16	Effect of tozasertib on NFκβ mRNA and protein expressions in dorsal root ganglion and spinal cord of nerve injured rats	97
Fig.4.17	Effect of pan-Aurora kinase inhibition on nerve injury-induced inflammatory signaling in dorsal root ganglion and spinal cord of rats	98
Fig.4.18	Effect of tozasertib on KIF17 and NR2B mRNA expressions in dorsal root ganglion (DRG) and spinal cord of nerve injured rats	100
Fig.4.19	Effect of tozasertib on KIF17 and NR2B mRNA expressions in contralateral dorsal root ganglion and spinal cord of nerve injured rats	102

Fig.4.20	Effect of pan-aurora kinase inhibition on protein expressions of KIF17 and NR2B in dorsal root ganglion and spinal cord of nerve injured rats	103
Chapter V	Modulation of KIF-17/NR2B Crosstalk by Tozasertib in Inflammatory Pain Rat Model	
Fig.5.1	Effect of tozasertib on formalin induced acute inflammatory pain	110
Fig.5.2	Effect of pan aurora kinase inhibition on CFA-induced thermal hyperalgesia in rats using Hargreaves apparatus	112
Fig.5.3	Effect of pan aurora kinase inhibition on CFA-induced cold hyperalgesia in rats	113
Fig.5.4	Effect of pan aurora kinase inhibition on CFA-induced mechanical hyperalgesia in rats	115
Fig.5.5	Effect of tozasertib on CFA induced mechanical allodynia in rats	116
Fig.5.6	Effect of tozasertib on CFA-induced oxido-nitrosative stress in sciatic nerve of rats	120
Fig.5.7	Effect of aurora kinase inhibitor on IBA1 and ICAM1 protein expressions in dorsal root ganglion and spinal cord of CFA injected rats	122
Fig.5.8	Effect of tozasertib treatment on KIF17 and NR2B expressions in DRG and spinal cord of CFA injected rats	125
Fig.5.9	Effect of pan-aurora kinase inhibition on mLIN10 mRNA expressions in dorsal root ganglion and spinal cord of CFA injected rats	126
Chapter VI	Acute Toxicity Study for Tozasertib in Rats	
Fig.6.1	Effect of tozasertib single dose administration on body weight and food consumption of rats	131
Fig.6.2	Effect of tozasertib on histopathological architecture of liver and kidney of rats	135
Chapter VII	CHAPTER 7: Summary & Conclusions	
Fig.7.1	Summary of the thesis work	141

LIST OF TABLES

		Page No.
Chapter II	Rationale, Objective & Work Plan	
Table 2.1	Animal grouping to investigate the effect of pan aurora kinase inhibition on evoked and ongoing pain behavior in nerve injured rats	44
Table 2.2	Animal grouping to investigate the effect of pan aurora kinase inhibition on normal pain threshold	45
Table 2.3	Animal grouping to investigate the effect of tozasertib on acute inflammatory pain rat model	45
Table 2.4	Animal grouping to study the effect of pan aurora kinase inhibition on chronic inflammatory pain model in rats	46
Table 2.5	Animal grouping to study the acute toxicity study of tozasertib in rats	46
Chapter III	Materials & Methods	
Table 3.1	List of drugs, chemicals and antibodies	47
Table 3.2	List of equipment & software	50
Table 3.3	Composition of RIPA buffer	63
Table 3.4	Loading buffer recipe	64
Table 3.5	Running buffer recipe	64
Table 3.6	Primers used in rtPCR analysis	66
Chapter VI	Acute Toxicity Study for Tozasertib in Rats	
Table 6.1	Effect of tozasertib on hematological profile of rats	132
Table 6.2	Effect of single dose administration of tozasertib on biochemical parameters of rats	133
Table 6.3	Effect of tozasertib single dose administration on body organ weight of rats	134