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It is certified that the work contained in the thesis titled **"Investigating Aurora Kinase Mediated Regulation of Kinesin Nanomotors as A Novel Therapeutic Target for The Treatment of Chronic Pain"** by **ANKIT UNIYAL** has been carried out under my supervision and that this work has been not submitted elsewhere for a degree.

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Dr. Vinod Tiwari
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DECLARATION BY THE CANDIDATE

I, ***Ankit Uniyal***, certify that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of ***Dr. Vinod Tiwari*** from ***January, 2019*** *to* ***February, 2022*** at the ***Department of Pharmaceutical Engineering & Technology***, Indian Institute of Technology (B.H.U.), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

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Date:

Place: Varanasi **(Ankit Uniyal)**

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ASIC	Acid sensing ion channels
ATP	Adenosine tri-phosphate
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
COX	Cyclooxygenase
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CPP	Conditioned place preference
CREB	Camp response element-binding protein
DMSO	Dimethyl sulfoxide
DRG	Dorsal root ganglion
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid
ERK	Extracellular signal-related kinase
GABA	Gamma-aminobutyric acid

GPCR	G protein coupled receptors
GSH	Reduced glutathione
IASP	International Association for the Study of Pain
ICAM1	Intercellular adhesion molecule 1
ICD	International Classification of Disease
KIFs	Kinesin superfamily proteins
LTP	Long-term potentiation
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
NFKβ	Nuclear factor kappa β
NMDA	N-methyl-D-aspartate
NPT	Normal pressure and temperature
NS	Non-significant

PREFACE

International Association for the Study of Pain defines pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". Pain is a protective mechanism of our body that acts as an alarm against various tissue-damaging stimuli, thus it is regarded as the 6th sense which is essential for the survival and wellbeing of organisms. When pain becomes chronic it develops into a devastating medical condition imposing a huge burden on society and healthcare costs. Nociception is the neural process that encodes the noxious stimuli and manipulation in the nociceptive pathways can degrade the usefulness of pain as a protective phenomenon. Despite the progress made in unraveling the pathophysiology behind chronic pain the current therapeutics shows limited efficacy and elicits several side effects, ultimately leading to treatment withdrawal and poor quality of life. Opioids are the most frequently prescribed medication for chronic pain but carry several side effects including sedation, drug addiction, motor incoordination, respiratory depression, hypotension, sleep apnea, constipation, etc. Other treatment options including non-steroidal anti-inflammatory drugs, ion channel blockers, gamma-aminobutyric acid analogs have raising contraindications with high reports of adverse effects along with drug-drug interaction. Thus, there is an unmet need for effective pharmacotherapeutics for the treatment of chronic pain without causing severe side effects.

Intracellular transport is essential for the cellular homeostasis and survival. Kinesins (KIFs) are the ATP dependent motor proteins that transport variety of receptors from cytosol to the synaptic membrane in an anterograde direction. Kinesin

generates mechanical force by utilizing ATP and cause displacement over the microtubule via hand-over-hand movement. On reaching the synaptic membrane the whole kinesin and cargo complex gets disassembled and the receptors are delivered to the cell surface making them functional. Any impairment in the expression and functioning of KIFs results in maladaptive neuronal circuits that cause improper propagation of neuronal signals. Recent literature has suggested the role of kinesin nanomotors in trafficking of various ion channels and thereby regulating the nociceptive response. KIF17 is a homodimeric kinesin motor protein that belongs to the osmotic avoidance abnormal protein-3 (OSM3)/KIF17 family, involved in trafficking of N-methyl D-aspartate receptor subtype 2B (NR2B) subunit of NMDA receptor system from cytosol to periphery. The NR2B subunit is essential for the localization of NMDARs into the synaptic membrane and plays crucial role in regulating synaptic plasticity and chronic pain pathophysiology. Central sensitization is a primary feature of chronic pain which develops due to the over-activity of NMDARs at the spinal and supra-spinal regions. Whereas in dorsal root ganglion (DRG) the NMDARs play critical role in development of peripheral hyperalgesia. Targeting NMDARs through direct pharmacological blockade affects the basal physiological role of this receptors system leading to severe side effects. Therefore, an indirect approach of targeting the NMDA receptor function by interfering with receptor maturation, synthesis, and transport to the synaptic membrane could be an attractive strategy for the treatment of neuropathic pain.

Many regulatory proteins govern the transit of receptors by activating kinesin, and aurora kinases are one of them. Aurora kinase is a serine-threonine class of enzymes belonging to the phosphotransferase group that maintains cellular processes including

proliferation, mitosis, and several intracellular signaling. Tozasertib is a pan aurora kinase inhibitor with demonstrated efficacy against various type of cancers and promising potential against neurodegenerative, somatosensory, immune system and metabolism related disorders. In the present work, we have performed the *in-silico* molecular dynamics simulation to delineate the dynamic interaction of aurora kinase with its pharmacological inhibitor, tozasertib. Further, we investigated the effect of tozasertib, on nerve injury- and complete Freund's adjuvant-induced evoked and chronic ongoing pain and involvement of KIF17-NR2B crosstalk in the same.

The present thesis is divided into seven chapters and a brief description is given below:

Chapter 1 introduces pain as a protective mechanism and chronic pain as a devastating disorder with a wide literature survey. This chapter illustrates the motivation of work and the background of the study. Further, it includes definitions, terminologies, mechanisms, and limitations of currently available therapeutics for chronic pain. Moreover, the section presents the comprehensive literature on the interplay of kinesins in multifarious signaling involved in the neurobiology of chronic pain.

Chapter 2 of this thesis is dedicated to the rationale and objectives of the work. This chapter consists of the hypothesis of the study along with experimental design. It also includes the details of different objectives that were framed using a multidisciplinary state-of-the-art approach including *in-silico* and *in-vivo* tools.

Chapter 3 illustrates a detailed description of the material and methods used to carry out the present work. A complete overview of the different experimental techniques including the working principles and modifications performed is presented in this section. *In-silico* techniques, sample size, chronic pain models, surgical procedures,

tissues harvesting method, sample processing procedures, reagent preparation and composition, biochemical assays nd molecular biology techniques are discussed in a detailed manner along with the source of materials and reagents used in the experimental work.

Chapter 4 presents the experimental work and findings of the first study conducted to evaluate various aspects of the hypothesis. The sections started with the computational modeling of aurora kinase and tozasertib architectural interplay. Here, we investigated the effect of pan aurora kinase inhibition on nerve injury-induced neuropathic pain and KIF17-NR2B crosstalk in DRG and spinal cord in the same. This section consists of a behavioral battery for the assessment of tozasertib action on different stimulus-evoked pain hypersensitivities and CNS toxicity. The section also presents the comparison of tozasertib with gabapentin and morphine activity on spontaneous ongoing pain inhibition and the addictive potential profiling of these compounds. Next, the findings from molecular studies including rtPCR and western blotting, are discussed in detail to elucidate the mechanism of action of tozasertib.

Chapter 5 represents another part of the experimental work that has been carried out to study the effect of pan aurora kinase inhibitor on acute and chronic inflammatory pain models. The study revealed the effect of tozasertib in inflammatory pain and suggest the role of KIF17-NR2B interplay, microglial activity, and oxido-nitrosative signaling in modulation of the same.

Chapter 6 shows the experimental data for the acute toxicity study of tozasertib in rats using behavioral, necropsy, hematological, and histopathological approaches.

Chapter 7 summaries and the key findings of the experimental work of the thesis and includes the discussion on the results observed in the present work and describes the

advantages of aurora kinase inhibition mediated kinesin regulation for the treatment of chronic pain. The section gives detailed insight on the novel mechanisms of tozasertib antinociceptive action against chronic pain. Finally, this chapter conclude the thesis work and illustrate the future scope of the research.