Chapter-2

Rationale, Objectives and Plan of Work

2.1 Rationale

Chronic pain is among the major health burden concerning 30% of the population worldwide. The current pharmacotherapeutic treatment is inadequate further raising the concern of researchers toward this unmet medical need. The pathophysiology of chronic pain involves impaired nociceptive signaling and the generation of uncontrolled and inappropriate responses. Treatment with the best available drugs such as opioids leads to inadequate relief and significant adverse effects (sedation, respiratory depression, psychotropic effects, cognitive dysfunction, addiction, abuse, and hyperalgesia) that present substantial barriers to their clinical use. Thus, a better understanding of the underlying mechanism responsible for chronic pain is needed for the development of better and safer analgesics. Under chronic pain, there is increased production and delivery of receptors leading to increased sensitivity and neuroplasticity. Kinesins are the motor-proteins responsible for the anterograde transport of receptors. When new receptors are synthesized and packed into vesicles, kinesins attach to their respective cargos and the motor-protein complex formation occurs which begins its transit toward synapse. On reaching synapse the motor-protein complex dissociates and releases receptors, and it is expressed in membrane thus contributing to the receptor up-regulation. Aurora kinase is a serine-threonine kinase, and it has three isoforms aurora kinase A, aurora kinase B, and aurora kinase C. We hypothesized that inhibition of aurora kinases could lead to the regulation of kinesin mediated receptor trafficking and attenuation of pain hypersensitivity.

2.2 Objectives

The objective of the present study was to investigate the involvement of kinesins (KIF17) in the pathophysiology of chronic pain and to target its downstream signaling using pan aurora kinase inhibitor. Below are the key objectives of this study:

Aim 1. Investigating the effect of pan-aurora kinase inhibition on nerve injury induced chronic pain

Aim1A. To investigate the architectural interplay between aurora kinase and tozasertib using computational analysis

Aim1B. To investigate the effect of pan aurora kinase inhibition on evoked and ongoing pain behavior in nerve-injured rats.

Aim1C. To study the role of nerve injury on KIF17-NR2B crosstalk in DRG and spinal cord of rats and its modulation by tozasertib.

Aim 2. Investigating the effect of tozasertib on acute and chronic inflammatory pain models and dissecting the role of KIF17/mLin10/NR2B signaling.

Aim2A. To investigate the effect of pan aurora kinase inhibition on acute and chronic inflammatory pain hypersensitivity in rats.

Aim 2B. To dissect the role of KIF17-mLin10-NR2B assembly in tozasertib mediated pain relief.

Aim 3. To perform acute toxicity study of tozasertib in rats using single-dose administration.

Aim. 3A. To identify the gross behavioral toxicities associated with tozasertib administration

Aim 3B. To study the effect of tozasertib on hematological and histopathological profiling in rats

2.3 Plan of work

2.3.1 Study I

In the first set of experiments, we studied the architectural interplay between tozasertib and aurora kinase using molecular dynamics simulation. Next, we investigated the effect of pan aurora kinase inhibition on evoked (mechanical and thermal) and spontaneous ongoing pain behavior in nerve-injured rats. Then we examined the role of KIF17-NR2B and inflammatory axis in nerve injury-induced neuropathic pain and its regulation using pharmacological inhibitor of pan aurora kinase enzymes, tozasertib. CNS associated side effects are the major limitation of currently available analgesics in clinic therefore we examine the effect of tozasertib on motor coordination and locomotion activity. The animal grouping was designed as below:

Table 2.1 Animal grouping to investigate the effect of pan aurora kinase inhibition on evoked and ongoing pain behavior in nerve-injured rats.

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	8
2.	Nerve injury + Vehicle	8
3.	Nerve injury + Tozasertib 10mg/kg *i.p.*	8
4.	Nerve injury + Tozasertib 20mg/kg *i.p.*	8
5.	Nerve injury + Tozasertib 40mg/kg *i.p.*	8
6.	Nerve injury + Gabapentin 30mg/kg *i.p.*	8

We also examined the effect of tozasertib on the pain threshold of naïve rats (healthy animals) to check if this compound interferes with normal nociception or not

and for comparison purposes, we choose the clinically used analgesic morphine. The grouping for this set of experiment was:

Table 2.2 Animal grouping to investigate the effect of pan aurora kinase inhibition on normal pain threshold:

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Naïve	8
2.	Naïve + Tozasertib 10mg/kg *i.p.*	8
3.	Naïve + Tozasertib 20mg/kg *i.p.*	8
4.	Naïve + Tozasertib 40mg/kg *i.p.*	8
5.	Naïve + Gabapentin (30mg/kg i.p.)	8
6.	Naïve + Morphine 10mg/kg *i.p.*	8

2.3.2 Study II

In this study, we have used the acute and chronic inflammatory pain model in rats and investigated the effect of tozasertib against the same. Firstly, we have used an acute model of formalin-induced inflammatory pain in rats and evaluated the efficacy of tozasertib against the same. The grouping for the study was as follows:

Table 2.3 Animal grouping to investigate the effect of tozasertib on acute inflammatory model of pain in rats

S. No	Group	Number of animals (Male Sprague Dawley rats)
1.	Formalin + Vehicle	8
2.	Formalin + Tozasertib 10 mg/kg *i.p*	8
3.	Formalin + Tozasertib 20 mg/kg *i.p*	8
4.	Formalin + Tozasertib 40 mg/kg *i.p*	8
5.	Formalin + Diclofenac 10 mg/kg *i.p*	8

In the next set of experiment, we have used complete Freund's adjuvant (CFA) to induce chronic inflammatory pain in rat and study the effect pan-aurora kinase

inhibition on regulation of KIF17-NR2B-mLIN10 assembly and glial cell activation in DRG and spinal cord of rats. The grouping for this set of the study was as follows:

Table 2.4 Animal grouping to study the effect of pan aurora kinase inhibition on chronic inflammatory pain model in rats

S. No	Equipment/software	Number of animals (Male Sprague Dawley rats)
1.	Naive	8
2.	CFA + Vehicle	8
3.	CFA + Tozasertib 10 mg/kg *i.p*	8
4.	CFA + Tozasertib 20 mg/kg *i.p*	8
5.	CFA + Tozasertib 40 mg/kg *i.p*	8
6.	CFA + Diclofenac 10 mg/kg *i.p*	8

2.2.3 Study III

Finally, we have examined the acute toxicity profiling of highest dose of tozasertib used against neuropathic and inflammatory pain model in rats. The study was performed in rats using behavioral, hematological, biochemical, and histopathological analysis. The grouping for this set of study was as follows:

Table 2.5 Animal grouping to study the acute toxicity study of tozasertib in rats

S. No	Equipment/software	Number of animals (Male Sprague Dawley rats)
1.	Naïve + saline	6
2.	Naïve + Tozasertib 40mg/kg *i.p.*	6