TARGETING PERIPHERAL G-PROTEIN COUPLED RECEPTORS FOR THE TREATMENT OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

Thesis submitted in partial fulfilment for the Award of

Degree

DOCTOR OF PHILOSOPHY

By

GADEPALLI ANAGHA

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI – 221005
INDIA

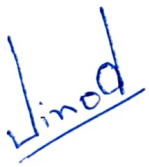
Roll No: 19161014 **Year: 2024**

Dedicated to those enduring the immense burden of cancer and chemotherapy-induced neuropathic pain

CERTIFICATE

It is certified that the work contained in the thesis titled **"Targeting Peripheral G-Protein Coupled Receptors for the Treatment of Chemotherapy-induced Neuropathic Pain"** by **GADEPALLI ANAGHA** has been carried out under my supervision and that this work has been not submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Course work, Comprehensive, Candidacy, SOTA and Pre-submission seminar.



Dr. Vinod Tiwari

Department of Pharmaceutical Engineering & Technology

Indian Institute of Technology (BHU), Varanasi

डा० विनोद तिवारी/Dr. Vinod Tiwari
सह-आचार्य/Associate Professor
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग/
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान/Indian Institute of Technology
(काशी हिन्दू विश्वविद्यालय)/(Banaras Hindu University)
वाराणसी-२२१००५/Varanasi-221005

DECLARATION BY THE CANDIDATE

I, ***Gadepalli Anagha***, 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 ***July, 2019*** *to* ***February, 2024*** 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.

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 willfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports, dissertations, thesis, etc., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

Date: 26/02/24
Place: Varanasi

Anagha

(Gadepalli Anagha)

CERTIFICATE FROM THE SUPERVISOR

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

Vinod

(Dr. Vinod Tiwari)

Supervisor

डा० विनोद तिवारी/Dr. Vinod Tiwari
सह-आचार्य/Associate Professor
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग/
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान/Indian Institute of Technology
(काशी हिन्दू विश्वविद्यालय)/(Banaras Hindu University)
वाराणसी-२२१००५/Varanasi-221005

S. Hemalatha 26/2/24

(Prof. S. Hemalatha)

Head of the Department

विभागाध्यक्ष / Head
भैषजकीय अभियांत्रिकी एवं प्रौद्योगिकी विभाग/
Department of Pharmaceutical Engineering & Technology
भारतीय प्रौद्योगिकी संस्थान/INDIAN INSTITUTE OF TECHNOLOGY
(बनारस हिन्दू विश्वविद्यालय)/(BANARAS HINDU UNIVERSITY)
वाराणसी-२२१००५/Varanasi-221005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis : **"Targeting Peripheral G-Protein Coupled Receptors for the Treatment of Chemotherapy-induced Neuropathic Pain"**

Name of the Student : Ms. Gadepalli Anagha

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (B.H.U.), Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the "**Doctor of Philosophy**"

Anagha

(Gadepalli Anagha)

Date: 26/02/24

Place: Varanasi

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the Institute's copyright notice are indicated.

ACKNOWLEDGEMENT

My first and foremost heartfelt gratitude and indebtedness would be towards the founder of Banaras Hindu University, Bharat Ratna Pandit Madan Mohan Malaviya Ji, who established this artistic temple of knowledge and wisdom.

I would like to express my sincere gratitude to my supervisor Dr. Vinod Tiwari, for his unwavering support, invaluable guidance, and scholarly mentorship throughout the journey of completing my Ph.D. research work. His profound expertise, insightful feedback, and commitment to academic excellence have been instrumental in shaping the trajectory of my research. I am truly fortunate to have had the privilege of working under his supervision. His encouragement, boundless patience, and dedication have not only enriched my academic pursuits but have also been instrumental in my personal and professional development. I am deeply appreciative of the encouragement and trust he placed in me, making this academic endeavor both challenging and rewarding. Thank you for being an inspiring mentor and for contributing significantly to the successful completion of my Ph.D. journey.

I owe my sincere thanks to Prof. S. Hemalatha, Head, Department of Pharmaceutical Engineering & Technology, I.I.T. (B.H.U.), Varanasi, for providing the necessary infrastructure facilities during the course of the work.

I would also like to express my sincere thanks to RPEC members Prof. M.S. Muthu, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University) and Dr. Vishal Mishra, School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), for their valuable inputs and criticism which motivated me to widen my research from various perspectives.

I would like to thank all the faculty members of the department Prof. B. Mishra, Prof. S. K. Singh, Prof. Sanjay Singh, Prof. S. K. Shrivastava, Prof. K.

Sairam, Prof. A. Senthil Raja, Dr. Ashok Kumar, Dr. G. P. Modi, Dr. Ruchi Chawla, Dr. A. N. Sahu, Dr. Shreyans K. Jain, Dr. Ashish K. Agarwal, Dr. S. K. Mishra, Dr. P. K. Nayak, Dr. Rajnish, Dr. Deepak, Dr. Dinesh, Dr. Jairam Meena & Dr. Arun Khatri for their kind support during the progress of my research.

I greatly acknowledge the Central Instrumentation Lab, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (B.H.U), for providing the instrumental facility for the research work.

I am very thankful to Prof. S. P. Singh, Department of Biochemistry, BHU, for providing the research support and facilities that I needed to successfully complete my studies.

The efforts of the non-teaching staff Mr. Yashwant Singh, Mr. Atul, Mr. Anand, Mr. A. N. Upadhyay, Mr. Arun, Mr. Virendra, Mr. Jameel, and other non-teaching staff members of the department could never go unnoticed, who continuously lent their support and technical assistance during the tenure of the research.

I extend my sincere gratitude to my lab seniors, colleagues, and juniors: Dr. Anurag Kumar Singh, Dr. Ankit Uniyal, Mr. Akhilesh Kotiyal, Mr. Obulapathi Ummadisetty, Mr. Deepak Chouhan, Ms. Allani Meghana, Mr. Somesh Agarwal, Ms. Priyanka Pandey, Mr. Vaibhav Thakur, and Mr. Utkarsh Patil. Engaging in stimulating discussions with them and sharing moments of joy provided a welcomed respite from research, making the journey more enjoyable. I would like to thank all my M. Pharm. and B. Tech IDD juniors Mr. Tapas, Ms. Sneha, Mr. Narendra, Mr. Nagendra, Mr. Lavan, Mr. Akash, Mr. Ajay Modi, Ms. Mousmi Rani, Ms. Shreya Khanna, Ms. Shreya Solanki, Mr. Abhipshit Kalita, Ms. Dipa, Mr. Sangam, Mr. Nipun, Mr. Krushna Eknath Yadav, Ms. Anmol, Mr. Anuj Mehta, Ms. Nivedita Verma, Ms. Madhuri Dhande, Mr. Rohan Londhe, and Ms. Nisha Sahu and the interns Ms. Khushi Shukla, Mr. Arjun Menon, and Ms. Hasitha Reddy for their pleasant company, co-operation, and maintaining a positive environment in the lab. I would be amiss if I did not mention the students from the Department of

Biochemistry, BHU, Dr. Aaina Singh Rathore, Dr. Richa Singh, Dr. Hagera Dilnashin, Ms. Priyanka Kumari Keshri, and Mr. Shekhar for their support and cooperation whenever needed.

I would like to thank all my batchmates and my friends Ms. Bhagwati Bharadwaj, and Mrs. Mohini Mishra for their unwavering support and affection throughout this journey.

The moral support and constant motivation which I have always received from my friends cannot be expressed in words and I feel blessed with such wonderful friends and seniors. Some of them who deserve special thanks are Ms. Ajitha Reddy, Ms. Leela Mounica, Mr. Goutham Reddy, Ms. Ruksana, Mr. Nishant Sharma, Mr. Surya Narayana, Mr. Pathik Parekh.

I would like to express my heartfelt gratitude to my late grandparents (Mr. G. Kameswara Rao, Mrs. G. Bhanumathi, Dr. V. V. S. S. Tilak, Mrs. V. Lakshmi), whose love, wisdom, and encouragement continue to inspire me every day. I am profoundly thankful to my mother Mrs. G. Surya Ramani, my father Mr. G.S.K. Chaitanya, my uncle Mr. G. Sree Ramamurthy, and my brother Mr. G. Prasanna Chaitanya for their unwavering support and endless love. Their encouragement and faith in my abilities have been a constant source of strength. I could not have achieved this without them by my side.

I express my sincere thanks to the funding organizations without whose support this work would not have been possible: Ministry of Human Resource Development (MHRD), Science & Engineering Research Board (SERB), and Indian Institute of Technology (B.H.U).

Finally, I extend my deepest reverence and gratitude to Lord Shiva, Mata Saraswati, and Sri Ganapathi Sachchidananda Swamiji. Their divine blessings have bestowed upon me the opportunity and the inner strength necessary to undertake and complete this work.

Date: 26/02/24

Place: Varanasi

Anagha

(Gadepalli Anagha)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine tri-phosphate
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CBR	Cannabinoid Receptor
CCI	Chronic constriction injury
CGRP	Calcitonin gene-related peptide
CINP	Chemotherapy Induced Neuropathic Pain
CIPN	Chemotherapy-induced Peripheral Neuropathy
CNS	Central nervous system
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CPP	Conditioned place preference
DALDA	Dermorphin [d-Arg2, Lys4] (1–4) amide
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
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
LOP	Loperamide
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
MOR	Mu-opioid Receptor
NFKβ	Nuclear factor kappa β
NMDA	N-methyl-D-aspartate

NS	Non-significant
PINP	Paclitaxel Induced Neuropathic Pain
PNS	Peripheral Nervous System
RIPA	Radioimmunoprecipitation Assay
SNRIs	Serotonin and Norepinephrine Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TRP	Transient Receptor Potential channels
VGCCs	Voltage Gated Calcium Channels
VGSCs	Voltage Gated Sodium Channels

PREFACE

PREFACE

Cancer is a leading cause of mortality worldwide, significantly impacting global life expectancy. In addition to the physical challenges posed by malignancy, individuals undergoing cancer treatment frequently confront the adverse impact of chemotherapy-induced neuropathic pain (CINP). This distressing consequence of cancer treatment is characterized by a myriad of symptoms, ranging from tingling and numbness to excruciating pain, significantly diminishing the quality of life for those already grappling with the immense challenges of cancer. The profound nature of CINP is highlighted by its enduring presence, persisting even after the completion of chemotherapy. The burden imposed on patients is not solely physical; psychological and emotional well-being are intricately intertwined, exacerbating the challenges faced by individuals already navigating the complexities of cancer diagnosis and treatment.

Although CINP presents significant challenges, the available therapeutic options are unfortunately quite restricted. Current treatments for neuropathic pain often come with debilitating side effects, including hepatic impairment, renal insufficiency, fatigue and central nervous system toxicities like anxiety, dizziness, sedation, respiratory depression, cognitive dysfunction, addiction, and abuse potential, compounding the challenges faced by patients. The lack of precision in targeting neuropathic pain at its source contributes to the suboptimal outcomes observed in many cancer patients.

Amidst this therapeutic void, targeting of peripheral G Protein- Coupled Receptors (GPCRs) emerges as a promising avenue for innovative interventions. Notably, the mu-opioid receptors (MORs) and cannabinoid receptors (CBRs) within

the peripheral nervous system hold significant potential for therapeutic exploitation. By focusing on these receptors at the periphery, we aim to mitigate the unwanted central side effects associated with conventional therapies. Targeting the peripheral MORs, which are key players in pain modulation, has garnered attention for providing pain relief without the unwanted cognitive and sedative side effects often observed with centrally acting opioids. Additionally, CBRs, present an intriguing prospect, as they are predominantly expressed in the peripheral nervous system and immune cells, offering a more localized and targeted approach to pain management.

Recognizing the critical need for effective therapeutics that address the specificities of CINP, this research aims to explore and elucidate the intricacies of peripheral GPCR modulation. By unraveling the complexities of MORs and CBRs within the peripheral nervous system, we aspire to develop interventions that not only alleviate the burden of pain but also do so with a precision to avoid unwanted adverse effects.

In the present work, we have performed pre-clinical investigations to evaluate the analgesic potential of peripherally restricted MOR and CBR agonists in rat model of CINP. Further, we have investigated the downstream molecular signalling resulting in mitigation of CINP. The present thesis is divided into seven chapters, the brief description of which is given below:

Chapter 1 introduces neuropathic pain as an adverse consequence experienced by cancer patients receiving chemotherapy. It discusses in detail the unique symptomology and pathophysiology of CINP. The chapter further illustrates the motivation of work and the background of the study by providing the limitations of currently available

therapeutics for the management of CINP. Additionally, a comprehensive exploration of the literature is presented, shedding light on the pivotal role played by various GPCRs in pain modulation. The mechanisms orchestrating analgesia through MORs and CBRs are intricately detailed. The literature review extends to encompass an in-depth discussion on the drugs utilized in this thesis, providing a thorough foundation for the subsequent research exploration.

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 using *in-vivo* behavioural and molecular 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. Sample size, rodent model of CINP, method of tissue harvesting, sample processing procedures, reagent preparation and composition, biochemical assays and 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 section starts with the development of rodent model of paclitaxel induced neuropathic pain. Here, we investigated the effect of peripheral MOR agonist on chemotherapy-induced evoked and spontaneous ongoing pain. We further evaluated if loperamide administration showed any Central Nervous System (CNS) toxicities. The section also presents the comparison of loperamide 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 biochemical, RT-PCR and western blotting, are discussed in detail to elucidate the mechanism of action of loperamide.

Chapter 5 represents another part of the experimental work that has been carried out to further confirm our findings from the previous study. In this we have evaluated the effect of a preferential peripheral MOR agonist, Dermorphin [d-Arg2, Lys4] (1–4) amide (DALDA) on rat model of paclitaxel induced neuropathic pain. The study revealed the effect of DALDA on chemotherapy-induced evoked and spontaneous ongoing pain while further demonstrating its non-addictive potential. The multifaceted mechanisms underlying DALDA's efficacy in mitigating CINP are elucidated in detail.

Chapter 6 presents the experimental work and findings conducted to investigate the effect of peripheral CBR agonist, CB13 on chemotherapy-induced neuropathic pain. We further evaluated if CB13 administration showed any CNS toxicities. Next, the findings from molecular studies including biochemical, RT-PCR and western blotting, are discussed in detail to elucidate the mechanism of action of CB13.

Chapter 7 summarizes 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 peripherally restricted therapeutics targeting specific peripheral GPCRs, notably MORs and CBRs, for the management of CINP while steering clear of CNS toxicities. Finally, this chapter concludes the thesis work and illustrates the future scope of the research