

**NOVEL MEMBRANE ACTIVE PEPTIDES DERIVED  
FROM MARINE ORGANISMS AND THEIR  
MECHANISMS OF ACTION**

**ANJALI PRIYA**



**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES  
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**JUNE 2023**

© Indian Institute of Technology Delhi (IITD), New Delhi, 2023

**NOVEL MEMBRANE ACTIVE PEPTIDES DERIVED  
FROM MARINE ORGANISMS AND THEIR  
MECHANISMS OF ACTION**

*by*

**ANJALI PRIYA**

**Kusuma School of Biological Sciences**

Submitted

*in fulfillment of the requirements of the degree of Doctor of Philosophy*

*to the*



Indian Institute of Technology Delhi

**JUNE 2023**

## **CERTIFICATE**

This is to certify that the thesis titled “**Novel membrane active peptides derived from marine organisms and their mechanisms of action**”, being submitted by **Ms. Anjali Priya** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi**, for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the ‘Indian Institute of Technology Delhi’. The research report and the results described in it have not been submitted in part or full to any other University for the award of any other degree or diploma.

**Dr. Archana Chugh**

Professor

Kusuma School of Biological Sciences

Indian Institute of Technology Delhi

New Delhi 110016, India

## **ACKNOWLEDGEMENTS**

***“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”***

***-Marie Curie***

Joining a PhD program at IIT Delhi was the start of my journey towards expanding my understanding and doing so required a lot of courage and conviction. There are many individuals who helped me in this amazing journey because of whom this process became pleasant as well as exciting.

First and foremost, I would like to express my sincere gratitude to my **PhD supervisor, Prof. Archana Chugh** for her constant guidance and support throughout my work. She provided critical feedback from time to time and pushed me to achieve my potential as a PhD student. She not only provided the freedom to shape my work and carry out experiments but also helped with any requirements even at the last moments. Her kind words of motivation always fueled me up in the moments of failure and frustrations. Her faith in me resulted in my overall growth as a PhD student. Outside the lab, I also learned work life balance from her that helped shape my mindset towards better time management in my personal life as well.

I am extremely thankful to my student research committee members, **Prof. Bishwajit Kundu, Prof. Manidipa Banerjee and Prof. Pramit Kumar Chowdhury** (Department of Chemistry, IIT Delhi) for their regular suggestions and guidance during semester progress presentations. It really helped with providing direction to my work and refining it. I am also thankful to all the Faculty members of Kusuma School of Biological Sciences (KSBS) for allowing me to use the instruments as and when required.

I would also like to extend my gratitude towards **Prof. Kasturi Mukhopadhyay** (Jawaharlal Nehru University, New Delhi) for her help in starting biofilm work. I am also thankful to **Prof. Aditya Mittal** for providing confocal microscope facility and **Prof. Tapan K Chaudhari** for CD spectroscopy instrument. I would also like to thank KSBS staff members, **Ms. Mini Sharma, Mr. Pradeep and Garg Sir** for facilitating the administrative and accounts related work. I am also thankful to **Mr. Praveen, Pushpalta Ma'am and Mr. Vijaypal** for their assistance during TA duty.

I would like to acknowledge **IIT Delhi** for providing financial assistance in the form of GATE fellowship during my PhD program along with a safe and vibrant campus environment. I am also thankful to **IIT Delhi** for providing me with the research scholar travel assistance (RSTA) to present my work at University of Poitiers, France in 2018 and travel contingency for presenting my work and attending workshop at IIT Roorkee in 2019. I would also like to thank **Department of Biotechnology (DBT, Government of India)** for providing me travel grant for presenting my work at Gordon Research Conference on Antimicrobial Peptides at Renaissance Tuscany II, Italy in 2019.

I would like to thank **Kusuma Trust, UK** for providing funds for procurement of FACS instrument which has been extensively used for my work. I am also thankful to **Central Research Facility (CRF), IITD** for providing facilities of scanning electron microscopy (SEM), high resolution-transmission electron microscopy (HR-TEM) and FE-SEM that were crucial for my study. I would like to extend my gratitude to **Dr. Sahil** and **Akshay** from Centre for Biomedical Engineering (CBME), IITD for letting me use their spectrofluorometer for my experiments. I would also like to thank **Dr. Devanshu** and **Pragati** for helping with CD experiments and data analysis. I am thankful to my friend and batchmate, **Dr. Anshu** for helping with immunological experiments.

A friendly and happy lab environment was provided by all my labmates (current and past) to whom I am heartily grateful for sharing their uniqueness in the best way possible. I would especially like to thank **Dr. Deepthi Budagavi** with whom I started my research training and got to learn a lot about how to plan and execute experiments properly. **Dr. Anusha Aditya** was extremely helpful in carrying out experiments diligently for my first objective with whom I enjoyed time outside the lab as well. I am very much thankful to **Dr. Nisha, Dr. Vivek, Dr. Harsha, Dr. Pankhuri, Dr. Nirupama, Dr. Aparupa, Dr. Chayanika, Dr. Sujithra, Saurabh, Saiguru, Prasanjeet, Aditi, Gagan, Mayank and Malay** for our tea breaks and discussions (“chai pe charcha”). All these people made my experience really memorable. I would especially like to thank **Mayank** for actively helping me out with my last remaining experiments. He showed great enthusiasm and willingness to learn that accelerated the pace of experiments as well as lifted some work-load off my shoulders so that I could focus on writing my thesis.

I would like to thank **Dr. Juhi** (Department of Maths, IITD), **Aman** and **Dipshikha** with whom I met on day one of my IITD journey. We shared our interests in badminton, dance, painting, cooking, swimming, yoga and many other activities which were great stress busters. They became friends for life and have been a constant source of joy and happiness in my life away from home. I would also like to thank some

friendly faces in KSBS and Kailash hostel like ***Dibyakanti, Uzma, Late Ms. Devanshi, Dr. Shikha, Dr. Sapna*** and ***Dr. Supriya*** for the lovely interactions that we used to have.

One of the strongest support system in my journey was my ***Family*** and words cannot express my gratitude towards them. My father was my pillar of strength during this whole time and always used to motivate me by sharing his own PhD days and the ups and downs of it. My mother always cared and worried about my health despite telling her that I am quite well here. Her love and concern kept me going despite challenges. My younger sister, ***Shalu***, was my biggest cheerleader and my radio channel that talked non-stop for hours, sometimes annoying but most of the times entertaining. My elder brother, ***Chiranjeev Sagar*** (PhD student, IIT BHU), is kind of my twin in a way. We both started our PhD journey at the same time but at different institutions and area of research. That way, we shared our experiences with each other and he always guided me about anything and everything. I am also thankful to my sister-in-law and friend, ***Dr. Sumedha Mukherjee***, for helping me with my scientific problems as well as life in general. Finally, I would like to thank my husband, ***Satyajeet Singh***, for bringing calmness to my overthinking mind and always motivating me during moments of self-doubt. These last two years have been actually made easy by his efforts making me utilize maximum time for finishing my PhD work. I cannot thank him enough for all his love and support.

Last but not the least, I would like to thank Almighty God for giving me this life and all the lovely people that I got to share this life with. His blessings and grace will pave the way forward.

ANJALI PRIYA

## **ABSTRACT**

In the current era of multi-drug resistance, the conventional antibiotics are proving inefficient in the treatment of serious life-threatening infections along with their debilitating side-effects and development of resistance. To tackle this major challenge, alternative therapeutic compounds are required and membrane active peptides (MAPs) are one such group of therapeutics that have gained attention due to their ability to interact with various biological membranes in both disruptive and non-disruptive manner. Concomitantly, the inability of pathogens to mutate their membrane components as frequently in response to MAPs, keeps the spread of antimicrobial resistance (AMR) under check. Two major classes of MAPs, namely cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs) have been extensively studied and employed in therapeutics as drug delivery vectors and antimicrobial agents respectively. MAPs from marine organisms were chosen for this study because of their uniqueness and biochemical diversity that can be utilized for development of better peptide therapeutics with multi-functional properties.

The present thesis addresses the antimicrobial, anti-biofilm, cell penetrating and other functions of marine and toxin derived CPPs and AMPs along with elucidation of their mechanisms of action. It is hypothesized that the peptides used in this study will prove to be effective and multi-functional in their bioactivity with distinct mechanisms of action that can be utilized for the development of better peptide therapeutics.

The research questions targeted in the first objective of the study were: How the marine derived peptide Tachyplestin and snake-toxin derived peptide CyLoP-1 will fare as an anti-mycobacterial peptide? What will be the role of cysteine and arginine residues in imparting antimicrobial and cell penetrating activity to these peptides? What will be their mechanisms to inhibit or kill mycobacterium cells? Will they be able to enter macrophage cells infected with mycobacterium and kill the intracellular pathogen without disturbing the host cell? Will these peptides would also be able to inhibit or eradicate mycobacterium biofilms? To answer these questions, antimicrobial and cell culture based studies and assays were performed. For determination of anti-mycobacterial and anti-biofilm activity of peptides, minimum inhibitory

concentration (MIC), minimum bactericidal concentration (MBC), time-kill kinetics, co-culture and crystal violet assays were employed. Further, the mechanistic insights were gained by utilizing various microscopy and membrane-based assays such as transmission electron microscopy (TEM), membrane integrity and membrane depolarization assays. Intracellular reactive oxygen species (ROS) production was determined using fluorescent dye. The major outcomes of this objective were potent anti-mycobacterial and anti-biofilm activity of both the peptides with the ability to kill intracellular mycobacteria as well. Cysteine and arginine residues proved to be crucial for cell penetrating and anti-mycobacterial activity of both the peptides. Tachyplesin mainly followed membranolytic pathway whereas, CyLoP-1 employed intracellular ROS production as the mechanism to kill mycobacterium cells.

The research questions to be addressed in the second objective were: How to design a novel CPP from marine antimicrobial peptide and improve its functionality? What will be the overall bioactivity and toxicity profile of the novel peptides and will they be suitable candidates for further drug development pipeline? These research questions were answered using various *in silico* and *in vitro* techniques. In this direction, a novel peptide from marine AMP clavanin was designed *in silico* and an immunomodulatory sequence was added to its C-terminal. The peptides were named clavanin derived peptides (CDPs) with 3 variants - CDP-1 (with immunomodulatory sequence), CDP-2 (without immunomodulatory sequence) and CDP-3 (mutated version of CDP-2). The most significant results were excellent cell penetrating, antimicrobial and anti-biofilm activity without considerable cytotoxicity demonstrated by CDP-1 as compared to the other two variants. However, cargo delivery abilities and cytotoxicity profile of all the three peptides were similar.

In conclusion, novel AMPs and CPPs from marine sources displayed multi-functionality that can be translated into viable therapeutics. This study highlights the importance of understanding structure-activity relationship and mechanistic pathways of peptides against a host of cells and microorganisms so as to develop antimicrobial peptide therapeutics for better management of diseases.

## सार

बहु-दवा प्रतिरोध के वर्तमान युग में, पारंपरिक एंटीबायोटिक्स अपने दुर्बल करने वाले दुष्प्रभावों और प्रतिरोध के विकास के साथ-साथ गंभीर जीवन-धमकाने वाले संक्रमणों के उपचार में अक्षम साबित हो रहे हैं। इस बड़ी चुनौती से निपटने के लिए, वैकल्पिक चिकित्सीय यौगिकों की आवश्यकता होती है और मेम्ब्रेन एक्टिव पेप्टाइड्स (एमएपी) चिकित्सीय का एक ऐसा समूह है, जिसने विघटनकारी और गैर-विघटनकारी तरीके से विभिन्न जैविक झिल्लियों के साथ बातचीत करने की अपनी क्षमता के कारण ध्यान आकर्षित किया है। सहवर्ती रूप से, एमएपी के जवाब में अक्सर अपने झिल्ली घटकों को बदलने के लिए रोगजनकों की अक्षमता, रोगाणुरोधी प्रतिरोध (एएमआर) के प्रसार को जांच के दायरे में रखती है। एमएपी के दो प्रमुख वर्ग, अर्थात् सेल पेनेट्रेटिंग पेप्टाइड्स (सीपीपी) और एंटीमाइक्रोबियल पेप्टाइड्स (एएमपी) का बड़े पैमाने पर अध्ययन किया गया है और क्रमशः दवा वितरण वैक्टर और रोगाणुरोधी एजेंटों के रूप में चिकित्सीय में नियोजित किया गया है। इस अध्ययन के लिए समुद्री जीवों के एमएपी को उनकी विशिष्टता और जैव रासायनिक विविधता के कारण चुना गया था जिसका उपयोग बहु-कार्यात्मक गुणों के साथ बेहतर पेप्टाइड चिकित्सीय के विकास के लिए किया जा सकता है।

वर्तमान थीसिस एंटीमाइक्रोबियल, एंटी-बायोफिल्म, सेल पेनेट्रेटिंग और समुद्री और टॉक्सिन व्युत्पन्न सीपीपी और एएमपी के अन्य कार्यों के साथ-साथ उनकी क्रिया के तंत्र की व्याख्या के साथ संबोधित करती है। यह परिकल्पना की गई है कि इस अध्ययन में उपयोग किए गए पेप्टाइड्स अपनी जैव-सक्रियता में प्रभावी और बहु-कार्यात्मक साबित होंगे, जिसमें क्रिया के विशिष्ट तंत्र होंगे जिनका उपयोग बेहतर पेप्टाइड चिकित्सीय के विकास के लिए किया जा सकता है।

अध्ययन के पहले उद्देश्य में लक्षित शोध प्रश्न थे: समुद्री व्युत्पन्न पेप्टाइड टैचीप्लेसिन और साँप-विष व्युत्पन्न पेप्टाइड CyLoP-1 एक एंटी-माइक्रोबैक्टीरियल पेप्टाइड के रूप में कैसे काम करेगा? इन पेप्टाइड्स को रोगाणुरोधी और सेल पेनेट्रेटिंग गतिविधि प्रदान करने में सिस्टीन और आर्जिनिन अवशेषों की क्या भूमिका होगी? माइक्रोबैक्टीरियम कोशिकाओं को बाधित करने या मारने के लिए उनका तंत्र क्या होगा? क्या वे माइक्रोबैक्टीरियम से संक्रमित मैक्रोफेज कोशिकाओं में प्रवेश करने और मेजबान सेल को परेशान किए बिना इंट्रासेल्युलर रोगजनक को मारने में सक्षम होंगे? क्या ये पेप्टाइड माइक्रोबैक्टीरियम बायोफिल्म्स को बाधित या मिटाने में भी सक्षम होंगे? इन सवालों का जवाब देने के लिए, रोगाणुरोधी और सेल कल्चर आधारित अध्ययन और परीक्षण किए गए।

पेप्टाइड्स की एंटी-माइक्रोबैक्टीरियल और एंटी-बायोफिल्म गतिविधि के निर्धारण के लिए, न्यूनतम निरोधात्मक एकाग्रता (एमआईसी), न्यूनतम जीवाणुनाशक एकाग्रता (एमबीसी), टाइम-किल कैनेटीक्स, सह-संस्कृति और

क्रिस्टल वायलेट परख कार्यरत थे। इसके अलावा, विभिन्न माइक्रोस्कोपी और झिल्ली-आधारित जांच जैसे ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (टीईएम), झिल्ली अखंडता और झिल्ली विधुवण जांच का उपयोग करके यंत्रवत अंतर्दृष्टि प्राप्त की गई। फ्लोरोसेंट डाई का उपयोग करके इंद्रासेल्युलर प्रतिक्रियाशील ऑक्सीजन प्रजातियों (आरओएस) का उत्पादन निर्धारित किया गया था। इस उद्देश्य के प्रमुख परिणाम इंद्रासेल्युलर माइकोबैक्टीरिया को मारने की क्षमता के साथ दोनों पेप्टाइड्स की शक्तिशाली एंटी-माइकोबैक्टीरियल और एंटी-बायोफिल्म गतिविधि थे। दोनों पेप्टाइड्स की कोशिका भेदन और एंटी-माइकोबैक्टीरियल गतिविधि के लिए सिस्टीन और आर्जिनिन अवशेष महत्वपूर्ण साबित हुए। टैचीप्लेसिन ने मुख्य रूप से मेम्ब्रेनोलिटिक मार्ग का अनुसरण किया, जबकि CyLoP-1 ने माइकोबैक्टीरियम कोशिकाओं को मारने के तंत्र के रूप में इंद्रासेल्युलर आरओएस उत्पादन को नियोजित किया। दूसरे उद्देश्य में संबोधित किए जाने वाले शोध प्रश्न थे: समुद्री रोगाणुरोधी पेप्टाइड से एक उपन्यास सीपीपी कैसे डिजाइन करें और इसकी कार्यक्षमता में सुधार करें? उपन्यास पेप्टाइड्स की समग्र जैव-सक्रियता और विषाक्तता प्रोफाइल क्या होगी और क्या वे आगे की दवा विकास पाइपलाइन के लिए उपयुक्त उम्मीदवार होंगे? सिलिको और इन विट्रो तकनीकों में विभिन्न का उपयोग करके इन शोध प्रश्नों का उत्तर दिया गया था। इस दिशा में, समुद्री एएमपी क्लैविनिन से एक उपन्यास पेप्टाइड को सिलिको में डिजाइन किया गया था और इसके सी-टर्मिनल में एक इम्यूनोमॉड्यूलेटरी सीक्वेंस जोड़ा गया था। पेप्टाइड्स को क्लैविनिन व्युत्पन्न पेप्टाइड्स (सीडीपी) नाम दिया गया था जिसमें 3 प्रकार थे - सीडीपी-1 (इम्यूनोमॉड्यूलेटरी अनुक्रम के साथ), सीडीपी-2 (इम्यूनोमॉड्यूलेटरी अनुक्रम के बिना) और सीडीपी-3 (सीडीपी-2 का उत्परिवर्तित संस्करण)। अन्य दो रूपों की तुलना में सीडीपी-1 द्वारा प्रदर्शित काफी साइटोटोक्सिसिटी के बिना सबसे महत्वपूर्ण परिणाम उत्कृष्ट सेल मर्मज, रोगाणुरोधी और एंटी-बायोफिल्म गतिविधि थे। हालांकि, तीनों पेप्टाइड्स की कार्गो डिलीवरी क्षमता और साइटोटोक्सिसिटी प्रोफाइल समान थे। अंत में, समुद्री स्रोतों से उपन्यास एएमपी और सीपीपी ने बहु-कार्यक्षमता प्रदर्शित की जिसे व्यवहार्य चिकित्सीय में अनुवादित किया जा सकता है। यह अध्ययन कोशिकाओं और सूक्ष्मजीवों के एक मेजबान के खिलाफ पेप्टाइड्स के संरचना-गतिविधि संबंध और यंत्रवत मार्गों को समझने के महत्व पर प्रकाश डालता है ताकि रोगों के बेहतर प्रबंधन के लिए रोगाणुरोधी पेप्टाइड चिकित्सीय विकसित किया जा सके।

# TABLE OF CONTENTS

CERTIFICATE .....	i
ACKNOWLEDGEMENTS .....	ii
ABSTRACT .....	v
TABLE OF CONTENTS .....	vii
LIST OF FIGURES .....	x
LIST OF TABLES .....	xii
ABBREVIATIONS AND SYMBOLS .....	xiii
<b>Chapter 1: Introduction and objectives</b> .....	<b>1</b>
1.1. Membrane active peptides (MAPs) .....	2
1.1.1. Cell penetrating peptides (CPPs): classes and their mechanisms of uptake/transport .....	2
1.1.2. Antimicrobial peptides (AMPs): classes and their mechanisms of action.....	7
1.2. Therapeutic significance and clinical potential of MAPs .....	10
1.2.1. Delivery vector .....	10
1.2.2. Anti-infective agents.....	11
1.3. Marine ecosystem as treasure trove for novel therapeutics.....	11
1.3.1. MAPs derived from marine organisms .....	12
1.3.2. Classification and mechanisms of action .....	13
1.4. Objectives .....	13
1.5. Hypotheses .....	14
<b>Chapter 2: Review of literature</b> .....	<b>15</b>
2.1. Marine biodiversity .....	16
2.2. Bioactive compounds from marine organisms (MNPs) .....	16
2.3. MAPs from marine organisms and their pharmacological properties .....	18

2.3.1. Linear peptides with $\alpha$ -helical structure .....	21
2.3.2. Helical/linear peptides with abundance of particular amino acids .....	22
2.3.3. Peptides with $\beta$ -hairpin or mixture of $\alpha$ -helical and $\beta$ -sheet structures .....	23
2.3.4. Cyclic peptides .....	25
2.4. Post-translational modifications present in marine MAPs and their role in synthetic peptide design .....	26
2.5. Challenges and way forward .....	27
2.6. Marine MAPs currently in clinical trials .....	29
<b>Chapter 3: Materials and methods</b> .....	30
3.1. Materials .....	31
3.1.1. Peptides .....	31
3.1.2. Reagents and media for mammalian cell culture .....	33
3.1.3. Microbial culture media, reagents and strains .....	33
3.2. Objective 1: Investigating the anti-mycobacterial activity of marine CPP Tachyplestin (Tpl) and snake toxin derived CPP CyLoP-1.....	33
3.2.1. Anti-mycobacterial and antibiofilm activity of peptides .....	33
3.2.2. Cellular viability and uptake of peptides .....	36
3.2.3. Mechanistic studies of the anti-mycobacterial activity of peptides .....	36
3.3. Objective 2: Design of novel CPPs from marine tunicate <i>Styela clava</i> and assessment of their <i>in vitro</i> activities .....	39
3.3.1. Peptide design and secondary structure determination .....	39
3.3.2. Cellular uptake studies .....	40
3.3.3. Mechanism of cellular uptake of peptides .....	40
3.3.4. Cargo-delivery capability of peptides .....	41
3.3.5. Cytotoxicity of peptides towards mammalian cells .....	41
3.3.6. Antimicrobial and anti-biofilm activity of peptides .....	41
3.3.7. Immune-modulatory function of peptides in macrophage cells .....	42
3.4 Statistical analysis .....	42

<b>Chapter 4: Investigating the anti-mycobacterial activity of marine CPP Tachyplestin (Tpl) and snake toxin derived CPP CyLoP-1 .....</b>	<b>44</b>
4.1. Introduction .....	45
4.2. Results .....	48
4.3. Discussion .....	63
<b>Chapter 5: Design of novel CPPs from marine tunicate <i>Styela clava</i> and assessment of their bioactivities <i>in vitro</i> .....</b>	<b>66</b>
5.1. Introduction .....	67
5.2. Results .....	68
5.3. Discussion .....	89
<b>SUMMARY .....</b>	<b>93</b>
<b>REFERENCES .....</b>	<b>96</b>
<b>APPENDICES .....</b>	<b>118</b>
Appendix 1: List of reagents and equipment .....	119
Appendix 2: Preparation of peptide stocks and media .....	121
Appendix 3: Preparation of buffers and stock solutions .....	124
Appendix 4: Characterization data of peptides .....	126
<b>AUTHOR'S RESUME .....</b>	<b>146</b>

## LIST OF FIGURES

Figure 1.1: Mechanisms of translocation of cell penetrating peptides across lipid bilayer membranes employing various endocytic pathways .....	4
Figure 1.2: Mechanisms or models of peptide internalization in cells via direct translocation through interaction with lipid bilayer membranes .....	5
Figure 2.1: Model of AMP's non-membrane mechanisms of action .....	19
Figure 2.2: Classification of marine bioactive peptides based on various functions along with suitable examples .....	21
Figure 4.1: Time-kill kinetics study of Tpl and CyLoP-1 against <i>M. smegmatis</i> .....	49
Figure 4.2: Inhibition of <i>M. smegmatis</i> biofilm formation quantified by crystal violet assay.....	50
Figure 4.3: Percentage eradication of pre-formed <i>M. smegmatis</i> biofilm by Tpl and CyLoP-1...	51
Figure 4.4: Reduction in intracellular bacterial load by Tpl and CyLoP-1 .....	52
Figure 4.5: Percentage viability of RAW 264.7 cells with Tpl and CyLoP-1 at various concentrations evaluated by MTT assay .....	53
Figure 4.6: Quantitative uptake of peptides in RAW 264.7 cells as analyzed by flow cytometry .....	54
Figure 4.7: Qualitative analysis of peptide uptake in macrophage cells observed by confocal laser scanning microscopy .....	57
Figure 4.8: Transmission electron micrographs of <i>Mycobacterium smegmatis</i> after peptide treatment .....	58
Figure 4.9: Uptake of FITC-tagged peptides and PI in <i>M. smegmatis</i> cells as analyzed by CLSM .....	59
Figure 4.10: Membrane depolarization assay of Tpl and CyLoP-1 in <i>M. smegmatis</i> cells as assessed by DiSC <sub>3</sub> (5) dye .....	60
Figure 4.11: Assessment of ROS production in <i>M. smegmatis</i> cells after treatment with various concentrations of Tpl and CyLoP-1 for 4 hours .....	61
Figure 4.12: Scatter plots of FITC-Annexin V and PI uptake in <i>M. smegmatis</i> .....	62

Figure 5.1: CD spectra of CDPs depicting secondary structures in PBS, 300nM SDS micelles and 90% TFE as solvents .....	69
Figure 5.2: Uptake of FITC-tagged CDPs in HeLa cells .....	71
Figure 5.3: Confocal laser scanning microscopy images of HeLa cells depicting uptake of peptides .....	73
Figure 5.4: Confocal images of HeLa cells to visualize sub-cellular localization of CDP-1 .....	74
Figure 5.5: (A) Uptake of CDPs in HeLa cells assessed by various endocytic and non-endocytic inhibitors. (B) Combination of inhibitors employed to confirm the uptake mechanism of CDP-1 and 2 .....	75
Figure 5.6: Assessment of cargo-delivery ability of CDPs in HeLa cells .....	76
Figure 5.7: Percentage viability of cells treated with various concentrations of CDPs for 24 hours as determined by MTT assay .....	77
Figure 5.8: SEM images of (A) <i>S. aureus</i> and (B) <i>S. epidermidis</i> (C) MRSA and (D) <i>A. baumannii</i> treated with CDPs for 6 hours and imaged at 30000X magnification .....	80
Figure 5.9: Graph depicting biofilm formation capacity of <i>Staphylococcus species</i> in BHI growth medium .....	82
Figure 5.10: (A) Inhibition of <i>Staphylococcus</i> biofilm formation by CDPs (B) Eradication of pre-formed biofilms by CDPs .....	83
Figure 5.11: Concentration dependent inhibition of <i>S. epidermidis</i> biofilm formation by CDP-1 .....	84
Figure 5.12: Uptake of FITC-tagged CDPs in RAW 264.7 cells .....	85
Figure 5.13: Confocal microscopy images of RAW 264.7 cells depicting uptake of FITC-tagged CDPs .....	87
Figure 5.14: Percentage viability of RAW 264.7 cells treated with various concentrations of CDPs for 24 hours .....	88
Figure 5.15: Comparative analysis of IL-1 $\beta$ production by CDPs in LPS-stimulated and non-stimulated murine macrophage cells .....	89

## LIST OF TABLES

Table 1.1: Various classes of CPPs with their examples, source and mechanism of internalization in cells .....	6
Table 1.2: Various classes of AMPs and their examples along with their sources .....	8
Table 2.1: List of marine drugs recently approved by FDA, EU (European Union) or Australian Regulatory Agency .....	17
Table 2.2: List of bioactive peptides from major phyla of marine organisms .....	20
Table 3.1: Peptides used in this study with their sequences and other parameters .....	32
Table 4.1: Minimum inhibitory concentration and minimum bactericidal concentration of peptides and antibiotics against mycobacterium species .....	48
Table 5.1: CDPs with their peptide sequences and other physico-chemical parameters .....	68
Table 5.2: MIC of CDPs against various Gram-positive, negative and acid-fast bacteria along with their respective antibiotic controls .....	78
Table 5.3: MBC of CDPs against various Gram-positive, negative and acid-fast bacteria along with their respective antibiotic controls .....	79
Table 5.4: MIC of CDPs against various filamentous and non-filamentous fungi along with their respective antibiotic controls .....	79
Table 5.5: Categories of biofilm formation with their corresponding biofilm formation scores .....	81
Table 5.6: Categories of biofilm formation of <i>Staphylococcus species</i> based on their BF score .....	81

## **ABBREVIATIONS AND SYMBOLS**

$\alpha$	Alpha
$\beta$	Beta
Da	Dalton
$\mu$	Micro
$\mu\text{g}$	Microgram
$\mu\text{L}$	Microlitre
$\mu\text{M}$	Micromolar
%	Percent
$^{\circ}\text{C}$	Degree Celcius
$\text{\textcircled{R}}$	Registered
w/w	weight/weight
w/v	weight/volume
rpm	rotations per minute
ACE	Angiotensin Converting Enzyme
AMP	Antimicrobial Peptide
AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
APD	Antimicrobial Peptide Database
BEC	Biofilm Eradication Concentration
BHI	Brain Heart Infusion
BIC	Biofilm Inhibitory Concentration
CD	Circular Dichroism
CDP	Clavanin Derived Peptide
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CLSM	Confocal Laser Scanning Microscope

CPP	Cell Penetrating Peptide
CyLoP-1	Cytoplasmic Localizing Peptide-1
DAPI	4', 6-diamidino-2-phenylindole
DCF	2',7'-dichlorofluorescein
DiSC <sub>3</sub> (5)	3,3'-Dipropylthiadicarbocyanine Iodide
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
ECP	Eosinophilic Cationic Protein
ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> and <i>Enterobacter</i> species
EU	European Union
FACS	Fluorescence Activated Cell Sorting
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FITC	Fluorescein Isothiocyanate
Fmoc	Fluorenylmethyloxycarbonyl
H <sub>2</sub> DCFDA	2',7'-dichlorodihydrofluorescein diacetate
HDPs	Host Defence Peptides
HEK	Human Embryonic Kidney
HEPES	N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid
HR-TEM	High Resolution-Transmission Electron Microscope
IL-1 $\beta$	Interleukin-1 $\beta$
INH	Isoniazid
LC	Lethal Concentration
LPS	Lipopolysaccharides
MAP	Model Amphipathic Peptide
MAPs	Membrane Active Peptides
MBC	Minimum Bactericidal Concentration

MBF	Moderate Biofilm Former
MDR	Multi-drug Resistance
MIC	Minimum Inhibitory Concentration
miRNA	micro Ribonucleic Acid
MNPs	Marine Natural Products
MOI	Multiplicity of Infection
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MTCC	Microbial Type Culture Collection and Gene Bank
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NAC	N-Acetyl Cysteine
NBF	Non-biofilm Former
NO	Nitric oxide
OADC	Oleic acid, Albumin, Dextrose, Catalase
PBS	Phosphate Buffered Saline
PI	Propidium Iodide
PrP	Prion Protein
PS	Phosphatidylserine
PTD	Protein Transduction Domain
PTMs	Post-translational Modifications
qPCR	Quantitative Polymerase Chain Reaction
RFU	Relative Fluorescence Unit
RIF	Rifampicin
ROS	Reactive Oxygen Species
SAP	Sweet Arrow Peptide
SBF	Strong Biofilm Former
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscope
siRNA	small-interfering Ribonucleic Acid

SVM	Support Vector Machine
SV40	Simian Virus 40
TAT	Trans Activator of Transcription
TB	Tuberculosis
TFE	Trifluoroethanol
Tpl	Tachyplesin
XDR	Extremely Drug Resistant
WBF	Weak Biofilm Former
WHO	World Health Organization