

# Contents

## Summary

## List of Abbreviations

<b>Chapter 1 Introduction</b> .....	1
1.1 Pulmonary Arterial Hypertension .....	2
1.2 Apelin Receptor Discovery, Signalling and Distribution .....	17
1.3 Endogenous Apelin Receptor Ligand Apelin .....	22
1.4 Novel Apelin Receptor Ligand Elabela/Toddler .....	26
1.5 Physiological Functions of Apelin Receptor Signalling .....	29
1.6 Synthetic Agonists, Biased Agonists and Antagonists.....	40
1.7 Hypotheses and Aims.....	50
<b>Chapter 2 Materials and Methods</b> .....	51
2.1 Materials .....	52
2.2 Human Tissue Collection.....	53
2.3 Cell-based Assays of Receptor Activation and Signalling .....	54

2.4	Immunohistochemistry.....	60
2.5	Enzyme-Linked Immunosorbent Assay.....	64
2.6	Reverse Transcription and Real-Time Polymerase Chain Reaction.....	68
2.7	Detection of Peptide Hydrolysis <b>in vitro</b> .....	71
2.8	Cardiovascular Hemodynamic Measurements.....	73
2.9	Monocrotaline-Induced Pulmonary Arterial Hypertension.....	77
2.10	Statistical Analyses.....	79
<b>Chapter 3 [Pyr<sup>1</sup>]apelin-13<sub>(1-12)</sub> is a Biologically Active ACE2 Metabolite of [Pyr<sup>1</sup>]apelin-13.....</b>		
		81
3.1	Introduction.....	82
3.2	Methods - Cell-based Assays and Signalling Bias Analysis.....	87
3.3	Results.....	89
3.4	Discussion.....	102
<b>Chapter 4 Characterisation of Elabela/Toddler in the Human Cardiovascular System.....</b>		
		113
4.1	Introduction.....	114
4.2	Methods – ELA in the MCT Rat Model.....	125
4.3	Results.....	127
4.4	Discussion.....	152
<b>Chapter 5 Prevention of Pulmonary Arterial Hypertension by the Biased Apelin Receptor Agonist MM07.....</b>		
		163
5.1	Introduction.....	164
5.2	Methods – MM07 in the MCT Rat Model.....	169
5.3	Results.....	170
5.4	Discussion.....	178