

Table of Contents

CONTENTS	PAGE NO
CHAPTER 1	1
LEISHMANIASIS INFECTION, CURRENT TREATMENT METHODS, AND EMERGING INTERVENTIONS	1
ABSTRACT*	1
1. INTRODUCTION TO LEISHMANIASIS	2
1.2 COENZYME A	5
1.3 COA-ASSOCIATED METABOLIC PATHWAYS	8
1.4 EFFECT OF INHIBITION OF COENZYME A ON CELL SURVIVAL AND PROLIFERATION	9
1.5 LOCALISATION OF COENZYME A	10
1.6 RATIONALE BEHIND CHOOSING DPCK AS A DRUG TARGET	11
1.7 DPCK ENZYME STRUCTURE AND MECHANISM	12
1.8 INHIBITION OF DPCK	14
CHAPTER 2	16
INTEGRATED COMPUTATIONAL AND EXPERIMENTAL APPROACH FOR NOVEL ANTI-LEISHMANIAL MOLECULES BY TARGETING DEPHOSPHO-COENZYME A KINASE	16
ABSTRACT*	16
2.1 INTRODUCTION	17
2.2 MATERIALS AND METHODS	21

2.2.1 PREPARATION OF TARGET PROTEIN AND LIGANDS	21
2.2.2 VIRTUAL SCREENING AND MOLECULAR DOCKING STUDY	22
2.2.3 ADME ANALYSIS	24
2.2.4 MD SIMULATIONS STUDY	24
2.2.5 MM-PBSA FREE ENERGY ANALYSIS	25
2.2.6 CULTURES AND CHEMICALS	25
2.2.7 EFFECT OF SELECTED LDDPCK INHIBITORS ON <i>L. DONOVANI</i> PROLIFERATION	25
2.2.8 CYTOTOXIC EFFECT OF LDDPCK INHIBITORS ON J774A.1 CELL LINE	26
2.2.9 STATISTICAL ANALYSIS	27
2.3 RESULTS	27
2.3.1 PROTEIN MODEL	27
2.3.2 MOLECULAR DOCKING ANALYSIS	28
2.3.3 MOLECULAR DYNAMIC SIMULATION (MD SIMULATIONS) ANALYSIS	32
2.3.4 MM-PBSA ANALYSIS	37
2.3.5 HYDROGEN BOND ANALYSIS	37
2.3.6 CYTOTOXICITY ANALYSIS OF LDDPCK INHIBITORS ON <i>L. DONOVANI</i> PROMASTIGOTES AND J774A.1 CELL LINE	39
2.4 DISCUSSION	40
2.5 CONCLUSION	43
CHAPTER 3	45
MOLECULAR MECHANISMS OF CELL DEATH IN <i>LEISHMANIA DONOVANI</i>	45
INDUCED BY SELECTED STEROIDAL ALKALOIDS	45

ABSTRACT	45
3.1 INTRODUCTION	46
3.2 MATERIALS AND METHODS	48
3.2.1 BIOLOGICAL STUDIES	48
3.2.2 CULTURES AND REAGENTS	48
3.2.3 ESTIMATION OF REACTIVE OXYGEN SPECIES	48
3.2.4 ESTIMATION OF CELL CYCLE	49
3.2.5 DETECTION OF CHANGE IN MORPHOLOGY	49
3.2.6 DETECTION OF PHOSPHATIDYLSERINE EXTERNALISATION	50
3.2.7 ESTIMATION OF MITOCHONDRIAL-MEMBRANE POTENTIAL	50
3.2.8 ESTIMATION OF ACIDIC ORGANELLES	50
3.2.9 DETERMINATION OF GENE EXPRESSION	51
3.2.10 DETECTION OF AUTOPHAGIC VACUOLES	51
3.2.11 STATISTICAL ANALYSIS	52
3.3 RESULTS	52
3.3.1 ANALYSIS OF REACTIVE OXYGEN SPECIES	52
3.3.2 CELL-CYCLE ANALYSIS	54
3.3.3 CELLULAR MORPHOLOGY ANALYSIS	54
3.3.4 EXAMINATION OF PHOSPHATIDYLSERINE EXTERNALISATION	57
3.3.5 ANALYSIS OF MITOCHONDRIAL-MEMBRANE POTENTIAL	57
3.3.6 ANALYSIS OF ACIDIC ORGANELLES	60
3.3.7 ESTIMATION OF CHANGE IN GENE EXPRESSION	63

3.3.8 DETECTION OF AUTOPHAGIC VACUOLES	63
3.4 DISCUSSION	67
CHAPTER 4	70
COMBATING MILTEFOSINE UNRESPONSIVENESS IN <i>LEISHMANIA DONOVANI</i>: SYNERGISTIC EFFICACY OF VERATRAMINE CO-TREATMENT	70
ABSTRACT*	70
4.1 INTRODUCTION	71
4.2 MATERIALS AND METHODS	73
4.2.1 BIOLOGICAL STUDIES	73
4.2.2 CULTURES AND CHEMICALS	73
4.2.3 ESTIMATION OF THE INHIBITORY EFFECT OF TEST COMPOUNDS ON MILTEFOSINE-UNRESPONSIVE <i>LEISHMANIA DONOVANI</i>	74
4.2.4 DETERMINATION OF ULTRASTRUCTURAL CHANGES AFTER DRUG TREATMENT	74
4.2.5 EVALUATION OF PHOSPHATIDYLSERINE EXTERNALISATION	75
4.2.6 DETECTION OF ACIDIC ORGANELLE PRODUCTION	75
4.2.7 DETECTION OF AUTOPHAGIC VACUOLE PRODUCTION	76
4.2.8 STATISTICAL ANALYSIS	76
4.3 RESULTS	77
4.3.1 INHIBITORY ANALYSIS OF SELECTED DRUG COMPOUNDS ON MILTEFOSINE-UNRESPONSIVE <i>LEISHMANIA DONOVANI</i>	77
4.3.2 ANALYSIS OF THE MICROSTRUCTURAL ALTERATIONS	80
4.3.3 ASSESSMENT OF PHOSPHATIDYLSERINE EXTERNALISATION	80
4.3.4 ANALYSIS OF ACIDIC ORGANELLES PRODUCTION	82

4.3.5 DETECTION OF AUTOPHAGIC VACUOLE PRODUCTION	82
4.4 DISCUSSION	83
APPENDIX A	92
PATENTS AND PUBLICATIONS	92
APPENDIX B	93
CONFERENCES ATTENDED	93
REFERENCES	94