

Contents

Chapter 1. Introduction	1
1.1 Cancer	1
1.2 Pancreatic ductal adenocarcinoma (PDAC)	1
1.3 Current therapies for PDAC	1
1.4 Reprogrammed tumour metabolism and introduction to malic enzymes	5
1.5 Collateral lethality and role of malic enzyme 3 in PDAC	6
1.6 Objective of the research project	8
1.7 Strategy for identification of hits for ME3 as target	9
1.8 Selection of ME3 hit for further optimization in to lead molecule	10
Chapter 2. Identification of pharmacophore for ME3 inhibition and study of structure activity relationship (SAR).	11
2.1 Docking study of compound A in malate binding pocket of ME3	11
2.2 Design strategy – Ring opening and optimization of linker in compound A	11
2.3 Investigating the role of phenolic hydroxyl group on ME3 inhibition	18
2.4 Investigating the role of piperazine nitrogens on ME3 inhibition	20
2.5 Chemistry	21
2.5.1 General information	21
2.5.2 Experimental procedures and spectral data for compounds	21
2.6 Biological evaluation of compounds	51
2.7 Conclusion	51
2.8 Spectral data	53
Chapter 3. Selectivity enhancement for ME3 and in vivo preclinical evaluation	85
3.1 Heterocyclic modifications to enhance selectivity for ME3	85
3.2 Lipophilic modification of ring-B in compound 31 to improve ME3 potency	86
3.3 Investigating role of logP to improve cellular permeation in BxPC-3 cells	90
3.4 Safety evaluation of selected compounds by screening on non-oncogenic cells.	92
3.5 <i>in vitro</i> mechanism of action study and target engagement study for compound 31	93
3.6 <i>in vivo</i> pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of compound 31	94
3.6.1 <i>in vivo</i> pharmacokinetic (PK) profile	94
3.6.2 <i>in vivo</i> anti-tumour activity evaluation	95
3.7 Chemistry	96
3.7.1 General information	96
3.7.2 Experimental procedures and spectral data for compounds	97
3.8 Biological evaluation of compounds	121

3.8.1 ME2 and ME1 inhibition data for selected compounds	121
3.9 Conclusion.....	122
3.10 Spectral data.....	124
Chapter 4. Design and synthesis of indole-piperazine carboxamide series and <i>in vitro</i> evaluation of tool compound in combination with trametinib	156
4.1 Structural diversification by incorporating rigidity.....	156
4.2 Lead optimization of indole-piperazine carboxamides	158
4.3 <i>in vitro</i> screening of selected compounds in Hs766T PDAC cells	164
4.4 Combination study of compound 62 and trametinib on Hs766T cells	164
4.5 Chemistry.....	165
4.5.1 General information	165
4.5.2 Experimental procedures and spectral data for compounds (55-74).....	166
4.6 Biological evaluation of the prepared compounds.....	181
4.6.1 ME2 and ME1 inhibition data for selected compounds	181
4.7 Conclusion.....	182
4.8 Spectral data	183
Chapter 5. Design and synthesis of dual ME3-tubulin inhibitors for the treatment of PDAC	204
5.1 Designing dual ME3-tubulin inhibitors for enhanced cell growth inhibition of PDAC cell lines....	204
5.2 Docking studies of compounds 75 and 76 with ME3 and tubulin.	205
5.3 Synthesis and biological evaluation of the designed compounds and related SAR.	206
5.4 Chemistry.....	210
5.4.1 General information	210
5.4.2 Experimental procedures and spectral data for compounds	210
5.5 Conclusion.....	220
5.6 Spectral data	221
Chapter 6. Comprehensive biological evaluation protocols of selected compounds for their mechanism of action, efficacy and safety.	233
6.1 Molecular docking methodology for docking studies of compounds with ME3	233
6.1.1 Ligand Structure Preparation	233
6.1.2 Protein structure preparation.....	233
6.1.3 Grid Generation and GLIDE Docking.....	233
6.2 <i>in vitro</i> screening protocols for screening of compounds in ME isoforms and PDAC cell lines	234
6.2.1 <i>in vitro</i> enzymatic assay	234
6.2.2 <i>in vitro</i> BxPC-3 cell growth inhibition assay:.....	235
6.2.3 <i>in vitro</i> Hs766T cell growth inhibition assay:.....	236

6.3 Mechanism of ME3 inhibition studies for compounds 31 and 64.	236
6.3.1 <i>in vitro</i> enzymatic assay to investigate the mode of ME3 inhibition	236
6.3.2 Mode of inhibition study for compound 31	237
6.3.3 Mode of inhibition study for compound 62	239
6.4 Target (ME3) engagement studies for compound 31 and 62 in BxPC-3 cells	240
6.4.1 CETSA (Cellular Thermal Shift Assay) for compound 31 with BxPC-3 live cells	240
6.4.2 CETSA (Cellular Thermal Shift Assay) for compound 62 with BxPC-3 cells	241
6.5 <i>in vitro</i> off target screening: Screening data for compound 31 to evaluate its safety in terms of off target side effects.	242
6.6 Antitumor activity of compound 31 in BxPC-3 subcutaneous (s.c.) xenograft in athymic nude mice	251
6.6.1 Brief protocol for <i>in vivo</i> study	251
6.6.2 Results	252
6.7 Synergy study of compound 62 in combination with trametinib to assess synergistic effect on Hs766T cell growth inhibition.	254
6.7.1 Brief study protocol for combination study	254
Overall summary and conclusion	256
References	257
Publication and conference presentation	262