

Table of Contents

Abstract	(i)
Declaration	(vi)
Certificate of the Principal Supervisor	(vii)
Certificate of the External Examiner and ODEC	(viii)
Acknowledgements	(ix)
Table of Contents	(xi)
List of Figures	(xxi)
List of Tables	(xxx)
List of Abbreviations	(xxxiv)
List of Publications	(xxxvi)
Conference Proceedings	(xxxviii)

CHAPTER 1 :	Motivation and Outline of the Thesis	1- 10
1.1	Motivation of the present work	2
1.2	Outline of the thesis	9
CHAPTER 2 :	Introduction and Review of Literature	11-61
2.1	Cancer	12
2.2	Causes of Cancer	13
2.3	Cancer Cells	14
2.4	Cancer Treatment Options	15
2.5	Protein-Protein Interactions	18
2.5.1	The p53-MDM2 Interacting Partners	18
2.6	p53 – An Introduction	19
2.6.1	The <i>TP53</i> Family	21
2.6.2	Transcriptional role of p53	22
2.6.3	Structure of p53	22
2.6.4	The Quaternary Structure of p53	23
2.6.5	The cell cycle and the role of p53	24
2.6.6	Cellular functions of p53	27
2.6.6.1	Cell-cycle arrest	28
2.6.6.2	DNA repair	28
2.6.6.3	Apoptosis	30
2.6.6.4	Senescence	30
2.6.7	Post-translational modifications (PTMs) on p53	30
2.7	MDM2 – An Introduction	32
2.7.1	Structure of MDM2	32
2.7.2	Transcriptional regulation of MDM2	34
2.7.3	Post-translational regulation of MDM2	34
2.8	The p53-MDM2 Sites of Interaction	35
2.8.1	The Primary Site of Interaction – Between the NTD of MDM2 and TAD1 of p53	36

Table of Contents

2.8.2	Between the central AD of MDM2 and core DBD of p53	37
2.8.3	Between the NTD of MDM2 and CTD of p53	37
2.8.4	Between the central AD of MDM2 and CTD of p53	38
2.8.5	Between the NTD of MDM2 and Oligomerization Domain (OD) of p53	38
2.8.6	Between the Really Interesting New Gene (RING) Domain of MDM2 and DBD of p53	38
2.9	p53-MDM2 Interaction Inhibitors	38
2.9.1	Potent Small Molecule Inhibitors of the p53/MDM2 Interaction and their Development	38
2.9.2	Discovery of Nutlins: an Imidazoline-Based Library	39
2.9.3	Optimisation of Nutlins - RG7112	41
2.9.4	The Pyrrolidines	42
2.9.5	Key Features of the optimum p53-MDM2 Small Molecule Inhibitors and their Further Development	43
2.9.6	Peptide-Based Inhibitors	46
2.9.7	Circular RNA (circRNA)	48
2.9.8	MicroRNA (miRNA)	48
2.9.9	Small-molecule MDM2 inhibitors in clinical trials	49
2.9.10	Synthesis of some of the MDM2 inhibitors	52
2.10	Inhibitors of Interest	57
2.11	Scope of the Thesis	60
2.12	Main Objectives of the Thesis	61
CHAPTER 3 :		Materials and Methods
3.1	Tools and Techniques	62-117
3.1.1	Molecular dynamics (MD) simulation	63
3.1.1.1	History of Simulation	64
3.1.1.2	Theory of molecular dynamics simulation	64
3.1.1.3	Force field (FF)	66
3.1.1.4	Long range interactions: Ewald sum	69
3.1.1.5	Dealing with molecules: SHAKE algorithm	70
3.1.1.6	Boundary conditions	72
3.1.1.7	Temperature and Pressure computation and control	73
3.1.1.8	Water molecule models	75
3.1.1.9	Molecular Dynamics steps	79
3.1.2	Potential of mean force (PMF)	87
3.1.2.1	Umbrella Sampling (US)	88
3.1.2.2	Bias potentials	89
3.1.2.3	Harmonic bias potentials	90

Table of Contents

3.1.2.4	Adaptive bias umbrella sampling	91
3.1.2.5	Specialized umbrella potentials	91
3.1.2.6	Running the umbrella sampling calculations	91
3.1.2.7	The weighted histogram analysis method (WHAM) for free-energy calculations	92
3.1.2.8	Umbrella Integration	94
3.1.3	Molecular Docking	95
3.1.3.1	PatchDock	95
3.1.3.2	ClusPro web server	101
3.1.4	Binding free energy calculation using Molecular Mechanics energies combined with the Poisson-Boltzmann or Generalized Born and Surface Area continuum solvation method (MM- PBSA/GBSA)	102
3.1.4.1	Free energy calculation using Perl Script (mm_pbsa.pl)	102
3.1.4.2	Free energy decomposition using Python Script MMPBSA.py	104
3.1.5	<i>In silico</i> prediction of protein-protein interaction	106
3.1.5.1	PDBsum web server	107
3.1.5.1.1	Wiring diagram	107
3.1.5.1.2	Topology diagram	109
3.1.5.1.3	Protein-protein interfaces	109
3.1.5.2	Hot spot residue prediction	112
3.1.6	Analysis of trajectories	113
3.1.7	3-D structure visualization tools	116
3.1.8	3-D structure modelling tools	117
3.1.8.1	I-TASSER web server	117
CHAPTER 4 :	Computational Investigation on the p53(TAD1)-MDM2(NTD) Interaction Using the Potential of Mean Force Study	118-147
4.1	Abstract	119
4.2	Introduction	120
4.3	Materials & Methods	124
4.3.1	Preparation of the p53(TAD1)-MDM2(NTD) system	124
4.3.2	MD simulation of p53(TAD1)-MDM2(NTD) complex	124
4.3.3	PMF Calculation	125
4.3.4	MD simulation of the lowest energy structure of the p53-MDM2 complex	126
4.3.5	Determination of the interface residues	126
4.3.6	BFE analyses for the p53(TAD1)-MDM2(NTD) complex	127

Table of Contents

4.4	Results & Discussions	128	
4.4.1	PMF profile of p53(TAD1)-MDM2(NTD) complex	128	
4.4.2	Analysis of conformational dynamics of p53 as a function of its CoM distance from MDM2	129	
4.4.3	RMSD analysis for p53 as a function of its CoM distance from MDM2	130	
4.4.4	DSSP analysis of p53 as a function of its CoM distance from MDM2	131	
4.4.5	Analysis of probable secondary structure per residue of p53 as a function of its CoM distance from MDM2	132	
4.4.6	Intramolecular hydrogen bond analyses for p53 as a function of CoM distance	133	
4.4.7	Density, temperature, pressure, potential energy, kinetic energy and total energy of the p53-MDM2 complex	134	
4.4.8	RMSD Analysis of the p53(TAD1)-MDM2(NTD) Complex	135	
4.4.9	RMSF Analysis of the p53(TAD1)-MDM2(NTD) Complex	135	
4.4.10	Rg Analysis of the p53(TAD1)-MDM2(NTD) Complex	136	
4.4.11	SASA Analysis of the p53(TAD1)-MDM2(NTD) Complex	136	
4.4.12	Hydrogen Bond Analysis of the p53(TAD1)-MDM2(NTD) Complex	137	
4.4.13	Determination of the interface interactions of the p53(TAD1)-MDM2(NTD) Complex	138	
4.4.14	BFE and PRED analyses of the p53(TAD1)-MDM2(NTD) Complex	142	
4.5	Conclusion	147	
CHAPTER 5 :		Computational Investigation on Molecular Interactions between the DNA Binding Domain of p53 and Acidic Domain of MDM2	148-162
5.1	Abstract	149	
5.2	Introduction	149	
5.3	Materials & Methods	152	
5.3.1	Preparation of the p53(DBD)-MDM2(AD) system	152	
5.3.2	MD simulation of p53(DBD)-MDM2(AD) complex	152	
5.3.3	Analysis of the MD Trajectories	152	
5.3.4	Determination of the interface residues	153	

Table of Contents

5.3.5	BFE analysis for the p53(DBD)-MDM2(AD) complex	153	
5.4	Results & Discussions	153	
5.4.1	Analysis of the conformational changes of the p53(DBD)-MDM2(AD) complex throughout the course of MD Simulation	153	
5.4.2	DSSP analysis of the p53(DBD)-MDM2(AD) complex	154	
5.4.3	Analysis of probable secondary structure per residue of p53(DBD) and (MDM2(AD)) in the p53(DBD)-MDM2(AD) complex	155	
5.4.4	RMSD Analysis of the p53(DBD)-MDM2(AD) complex	156	
5.4.5	RMSF Analysis of the p53(DBD)-MDM2(AD) complex	156	
5.4.6	Rg Analysis of the p53(DBD)-MDM2(AD) complex:	157	
5.4.7	SASA Analysis of the p53(DBD)-MDM2(AD) complex	157	
5.4.8	Hydrogen Bond Analysis of the p53(DBD)-MDM2(AD) complex	158	
5.4.9	Determination of the interface interactions of the p53(DBD)-MDM2(AD) complex	159	
5.4.10	BFE and PRED analyses of the p53(DBD)-MDM2(AD) complex	160	
5.5	Conclusion	162	
CHAPTER 6 :		In silico Investigation on the Conformational Dynamics of N-Terminal Lid of MDM2 in the presence and absence of p53 C-Terminal Domain	163-180
6.1	Abstract	164	
6.2	Introduction	164	
6.3	Materials & Methods	166	
6.3.1	Molecular docking and the preparation of initial structures	166	
6.3.2	Setup for MD simulations	167	
6.3.3	Analysis of the MD Trajectories	167	
6.3.4	BFE and PRED analysis	167	
6.4	Results & Discussions	167	
6.4.1	Analysis of the conformational dynamics of MDM2 in apo and in p53-MDM2 complex states	167	
6.4.2	RMSD Analysis	169	
6.4.3	RMSF Analysis	170	
6.4.4	Rg Analysis	171	
6.4.5	SASA Analysis	172	

Table of Contents

6.4.6	Analysis of the Average Distance between the Centers of Mass of the N-Terminal Lid and the N-Terminal Binding Cavity of MDM2	173
6.4.7	DSSP Analysis	173
6.4.8	Secondary structure analysis of the initial and final structures of p53(CTD)-MDM2(NTD) complex	174
6.4.9	Intramolecular Hydrogen Bond Analysis	174
6.4.10	Determination of Interface Residues	175
6.4.11	BFE and PRED Analyses	177
6.5	Conclusion	179
CHAPTER 7 :		Computational investigation on the molecular interactions between MDM2 and its photoactivatable inhibitor
7.1	Abstract	182
7.2	Introduction	182
7.3	Materials & Methods	184
7.3.1	Preparation of the MDM2 (Apo), MDM2-idasanutlin, and MDM2-idasanutlin+PPG systems	184
7.3.2	MD simulation of the MDM2 (Apo), MDM2-idasanutlin, and MDM2-idasanutlin-PPG systems	185
7.3.3	Analysis of the MD Trajectories of the three systems	186
7.4	Results & Discussions	186
7.4.1	Density, temperature, pressure, potential energy, kinetic energy and total energy of the three systems	186
7.4.2	Analysis of the conformational changes of the three systems	188
7.4.3	RMSD, RMSF, Rg, and SASA analyses of the three systems	189
7.4.4	Hydrogen Bond Analyses of the three systems	191
7.4.5	Analysis of probable secondary structure per residue and DSSP analysis of the three systems	192
7.4.6	Determination of the interface interactions of the two complexes	194
7.4.7	Visualization of the two complexes and the p53(TAD1)-MDM2(NTD) complex (PDB ID: 1YCR)	199
7.5	Conclusion	200
CHAPTER 8 :		Computational Investigation on the MDM2-Idasanutlin Interaction Using the Potential of Mean Force Method
		201-214

Table of Contents

8.1	Abstract	202
8.2	Introduction	202
8.3	Materials & Methods	204
8.3.1	PMF Calculation	204
8.3.2	RMSD and Intramolecular hydrogen bond analyses for MDM2 as a function of CoM distance from Idasanutlin	204
8.3.3	BFE and PRED of the MDM2-idasanutlin Complex	204
8.4	Results & Discussions	205
8.4.1	PMF profile of MDM2-idasanutlin complex	205
8.4.2	Determination of the interface interactions of the p53(TAD1)-MDM2(NTD) Complex	206
8.4.3	Secondary Structure comparison of the MDM2-idasanutlin Complex with the p53(TAD1)-MDM2(NTD) complex (PDB ID:1YCR)	206
8.4.4	Analysis of conformational dynamics of MDM2(NTD) as a function of its CoM distance from idasanutlin	209
8.4.5	RMSD analysis for MDM2(NTD) as a function of its CoM distance from idasanutlin	210
8.4.6	Intramolecular hydrogen bond analysis for MDM2(NTD) as a function of its CoM distance from idasanutlin:	211
8.4.7	BFE and PRED analyses of the MDM2-idasanutlin complex	213
8.5	Conclusion	214
CHAPTER 9 :		Computational investigation on the molecular interactions between MDM2 and its inhibitor XR-2
9.1	Abstract	215-227
9.2	Introduction	216
9.3	Materials & Methods	216
9.3.1	Preparation of the MDM2(NTD)-XR-2 system	217
9.3.2	MD simulation of the MDM2(NTD)-XR-2 system	218
9.3.3	Analysis of the MD Trajectories	218
9.3.4	Secondary Structure comparison of the MDM2(NTD)-XR-2 Complex	218
9.3.5	BFE and PRED analyses of the MDM2(NTD)-XR-2 Complex	218
9.4	Results & Discussions	219
9.4.1	Analysis of the conformational changes of the MDM2(NTD)-XR-2 Complex	219

Table of Contents

9.4.2	LigPlot analysis of the various conformers of the MDM2(NTD)-XR-2 Complex obtained during the MD simulation	219
9.4.3	RMSD analysis of the MDM2(NTD)-XR-2 Complex	220
9.4.4	RMSF analysis of the MDM2(NTD)-XR-2 Complex	221
9.4.5	Rg analysis of the MDM2(NTD)-XR-2 Complex	221
9.4.6	SASA analysis of the MDM2(NTD)-XR-2 Complex	222
9.4.7	Hydrogen bond analysis of the MDM2(NTD)-XR-2 Complex	222
9.4.8	Analysis of probable secondary structure per residue of MDM2(NTD) in the MDM2(NTD)-XR-2 Complex	223
9.4.9	DSSP analysis of MDM2(NTD) in the MDM2(NTD)-XR-2 Complex	224
9.4.10	Secondary Structure comparison of the MDM2(NTD)-XR-2 Complex with the p53(TAD1)-MDM2(NTD) complex (PDB ID:1YCR)	225
9.4.11	BFE and PRED analyses of the MDM2(NTD)-XR-2 Complex	225
9.5	Conclusion	227
CHAPTER 10 :	<i>In silico investigation on the effect of Epigallocatechin gallate (EGCG) on the interaction between p53 (NTD) and MDM2 (NTD)</i>	228-242
10.1	Abstract	229
10.2	Introduction	229
10.3	Materials & Methods	230
10.3.1	Preparation of the p53(NTD)-EGCG system	230
10.3.2	MD simulation of the p53(NTD)-EGCG complex	231
10.3.3	Analysis of the MD Trajectories	231
10.3.4	LigPlot Analysis of the p53(NTD)-EGCG complex	231
10.3.5	BFE and PRED Analyses of the p53(NTD)-EGCG complex	231
10.3.6	Determination of the interface residues	232
10.4	Results & Discussions	232
10.4.1	Analysis of the conformational changes of the p53(NTD)-EGCG Complex	232
10.4.2	RMSD analysis of the p53(NTD)-EGCG Complex	233

Table of Contents

10.4.3	RMSF analysis of the p53(NTD)-EGCG Complex	234
10.4.4	Rg analysis of the p53(NTD)-EGCG Complex	234
10.4.5	SASA analysis of the p53(NTD)-EGCG Complex	235
10.4.6	Hydrogen bond analysis of the p53(NTD)-EGCG Complex	235
10.4.7	Analysis of probable secondary structure per residue of p53(NTD) in the p53(NTD)-EGCG Complex	236
10.4.8	DSSP analysis of p53(NTD) in the p53(NTD)-EGCG Complex	237
10.4.9	Secondary Structure analysis of the conformers of the p53(NTD)-EGCG Complex	238
10.4.10	BFE and PRED analyses of the p53(NTD)-EGCG Complex	238
10.4.11	LigPlot analysis of the conformers of the p53(NTD)-EGCG Complex	240
10.4.12	Determination of the interface residues of the conformers of the p53(NTD)-EGCG Complex	241
10.5	Conclusion	242
<hr/>		
CHAPTER 11 :	Computational investigation on the molecular interactions of the p53(NTD)-RITA complex	243-258
<hr/>		
11.1	Abstract	244
11.2	Introduction	244
11.3	Materials & Methods	246
11.3.1	Preparation of the p53(NTD)-RITA system	246
11.3.2	MD simulation of the p53(NTD)-RITA complex	246
11.3.3	Analysis of the MD Trajectories	246
11.3.4	LigPlot Analysis of the p53(NTD)-RITA complex	247
11.3.5	BFE and PRED Analyses of the p53(NTD)-RITA complex	247
11.3.6	Determination of the interface residues	247
11.4	Results & Discussions	247
11.4.1	Analysis of the conformational changes of the p53(NTD)-RITA Complex	247
11.4.2	LigPlot Analysis of the various conformations of the p53(NTD)-RITA Complex	248
11.4.3	RMSD analysis of the p53(NTD)-RITA Complex	249
11.4.4	RMSF analysis of the p53(NTD)-RITA Complex	250
11.4.5	Rg analysis of the p53(NTD)-RITA Complex	251

Table of Contents

11.4.6	SASA analysis of the p53(NTD)-RITA Complex	251
11.4.7	Hydrogen bond analysis of the p53(NTD)-RITA Complex	252
11.4.8	Analysis of probable secondary structure per residue of p53(NTD) in the p53(NTD)-RITA Complex	253
11.4.9	DSSP analysis of p53(NTD) in the p53(NTD)-RITA Complex	254
11.4.10	Secondary Structure analysis of the conformers of the p53(NTD)-RITA Complex	254
11.4.11	BFE and PRED analyses of the p53(NTD)-RITA Complex	255
11.4.12	Determination of the interface residues of the conformers of the p53(NTD)-RITA Complex	256
11.5	Conclusion	258
CHAPTER 12 :		Summary and Future Prospects
11.1	Overview of Results	260
11.2	Future Prospects	261
	Bibliography	
	Publications (First Page)	