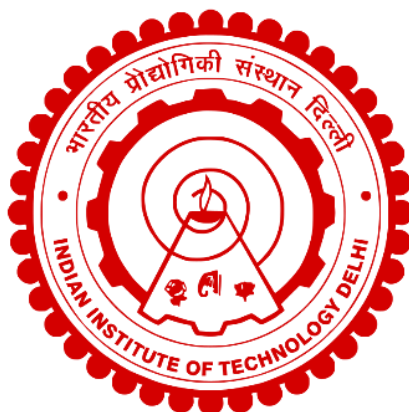


MECHANISTIC INSIGHTS INTO BIOACTIVITY AND PERMEABILITY OF NATURAL COMPOUNDS FOR SELECTIVE TARGETING OF CANCER

NAVANEETHAN RADHAKRISHNAN



**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

MAY 2023

MECHANISTIC INSIGHTS INTO BIOACTIVITY AND PERMEABILITY OF NATURAL COMPOUNDS FOR SELECTIVE TARGETING OF CANCER

by

NAVANEETHAN RADHAKRISHNAN
Department of Biochemical Engineering and Biotechnology

Submitted in
fulfillment of the requirements of the degree of Doctor of Philosophy
to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

MAY 2023

Certificate

This is to certify that the thesis entitled '**Mechanistic Insights into Bioactivity and Permeability of Natural Compounds for Selective Targeting of Cancer**' being submitted by **Mr. Navaneethan Radhakrishnan** to the Indian Institute of Technology Delhi for the award of the degree of '**Doctor of Philosophy**', is a record of the bonafide research work carried out by him, which has been prepared under my supervision in conformity with the rules and regulations of the Indian Institute of Technology Delhi. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Prof. D. Sundar

Professor

Department of Biochemical Engineering and Biotechnology

Indian Institute of Technology Delhi

Acknowledgements

I express my sincere gratitude to my supervisor Prof. D. Sundar for the constant support and motivation during the Ph.D. His guidance has helped me a lot in shaping the way I do, document, and present my research. Interactions with him has always motivated me.

I thank Department of Biotechnology (DBT), Govt. of India for the BINC Fellowship. I am grateful to Prof. Lee Wei Yang, NTHU, Taiwan for the guidance and support during my time at NTHU. I thank Prof. Renu Wadhwa and Prof. Sunil Kaul from AIST, Japan for their guidance and motivation. My sincere thankfulness to the rest of the members of my thesis committee: Prof. Ritu Kulshreshtha, Prof. Ravikrishnan Elangovan and Prof. Ashok Kumar Patel (Kusuma School of Biological Sciences, IITD) for their insightful comments and encouragement.

I appreciate and thank all the lab mates who were with me at some point of time between 2018 and 2023.

Lastly, but most importantly, I cannot thank enough my father Mr. M. Radhakrishnan, my mother Ms. R. Chitra and my sister Ms. R. Swathi Krishna, for their persistent support and for believing in me.

Navaneethan Radhakrishnan

Abstract

Cancer chemotherapy is often associated with serious adverse side effects. This is due to the lack of selectivity of drugs towards cancer cells. The need for selectivity led to the development of the targeted therapy approaches. Targeted therapy aims to selectively target cancer cells, however, drug resistance arising from heterogeneity of the tumor is an issue. To counter heterogeneity, molecules that could target multiple cancer pathways are considered promising. Several small molecules are actively being explored in cancer research for their therapeutic potential. Natural compounds are of great interest in cancer drug development due to their molecular rigidity and drug-like properties.

Cancer drug development is associated with high failure rates during clinical trials due to the lack of efficacy of the drug candidates. This could be overcome by proper elucidation of molecular mechanism of action of drug candidates during preclinical trials. Drug activity also depends on permeability of the active compounds through biological barriers like cell membranes. This study presents mechanistic insights into different modes of selectivity by focusing on bioactivity and permeability of few select natural compounds that are already reported for their anticancer properties.

In silico molecular modeling methods have been used to study the bioactivity and permeability of the chosen compounds. Molecular mechanisms underlying the anticancer activities of natural compounds fucoxanthin, withaferin A and caffeic acid phenethyl ester have been interpreted from protein-ligand docking and molecular dynamics simulation studies. The results suggested that fucoxanthin, withaferin A and caffeic acid phenethyl ester can cause p53 activation in cancer cells leading to apoptosis. Molecular dynamics simulations with umbrella sampling protocol were performed using lipid bilayer models to

explore the effect of phosphatidylserine exposure in cancer cells on the membrane permeation of natural compounds withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C. The results indicated that the exposure of phosphatidylserines in cancer cell membranes facilitated the permeation of withaferin A, withanone, caffeic acid phenethyl ester through a cancer cell membrane compared to a normal cell membrane. The presented data demonstrated the potential of drug-membrane interactions in rendering selectivity to drugs. An atomistic model of stratum corneum lipid matrix of skin for transdermal permeation studies of small molecules has been developed and validated with experimental data from literature. In summary, this thesis sheds light into specific molecular-level mechanisms underlying bioactivity and permeability using *in silico* modeling techniques that will aid in advancing the studied natural compounds as cancer drugs and develop new compounds for selective targeting of cancer.

सारांश

कैंसर की रसोचिकित्सा अक्सर गंभीर प्रतिकूल दुष्प्रभावों से जुड़ी होती है। यह कैंसर कोशिकाओं के प्रति दवाओं की चयनात्मकता की कमी के कारण है। चयनात्मकता की आवश्यकता ने लक्षित चिकित्सा पद्धतियों के विकास को प्रेरित किया। लक्षित चिकित्सा का उद्देश्य कैंसर कोशिकाओं को चुनिंदा रूप से लक्षित करना है, हालांकि, ट्यूमर की विषमता से उत्पन्न होने वाली दवा प्रतिरोध एक मुद्दा है। विषमता का मुकाबला करने के लिए, कई कैंसर मार्गों को लक्षित करने वाले अणुओं को आशाजनक माना जाता है। कैंसर अनुसंधान में उनकी चिकित्सीय क्षमता के लिए कई छोटे अणुओं का सक्रिय रूप से पता लगाया जा रहा है। प्राकृतिक यौगिकों उनके आणविक कठोरता और दवा-जैसा गुणों के कारण कैंसर दवा के विकास में बहुत रुचि रखते हैं।

कैंसर दवा विकास दवा उम्मीदवारों की प्रभावकारिता की कमी के कारण नैदानिक परीक्षणों के दौरान उच्च विफलता दर के साथ जुड़ा हुआ है। प्रीक्लिनिकल परीक्षणों के दौरान दवा उम्मीदवारों की कार्रवाई के आणविक तंत्र के उचित स्पष्टीकरण से इसे दूर किया जा सकता है। दवा गतिविधि कोशिका झिल्ली जैसे जैविक बाधाओं के माध्यम से सक्रिय यौगिकों की पारगम्यता पर भी निर्भर करती है। यह अध्ययन कुछ चुनिंदा प्राकृतिक यौगिकों, जो पहले से ही उनके एंटीकैंसर गुणों के लिए रिपोर्ट किए गए हैं, उनकी जैवसक्रियता और पारगम्यता पर ध्यान केंद्रित करके चयनात्मकता के विभिन्न तरीकों में यांत्रिक अंतर्दृष्टि प्रस्तुत करता है।

कम्प्यूटेशनल आणविक मॉडलिंग विधियों का उपयोग चयनित यौगिकों की जैवसक्रियता और पारगम्यता का अध्ययन करने के लिए किया गया है। प्राकृतिक यौगिकों फ्यूकोक्सेथिन, विथफेरिन ए और कैफिक एसिड फिनिथिल एस्टर के एंटीकैंसर गतिविधियों को अंतर्निहित करने वाले आणविक तंत्र को प्रोटीन-लिगेंड डॉकिंग और आणविक

गतिशीलता सिमुलेशन अध्ययनों से व्याख्या की गई है। परिणामों ने सुझाव दिया कि फ्यूकोक्सैथिन, विथफेरिन ए और कैफिक एसिड फिनिथिल एस्टर कैंसर कोशिकाओं में p53 सक्रियण का कारण बन सकता है, और जिससे एपोटोसिस हो सकता है। विथफेरिन ए, विथानोन, कैफिक एसिड फिनिथिल एस्टर और आर्टेपिलिन सी की झिल्ली पारगम्यता पर, कैंसर कोशिकाओं में पाए जाते फॉस्फेटिडाइल्सेरिन एक्सपोज़र के प्रभाव का पता लगाने के लिए, लिपिड द्विपरत मॉडलों को उपयोग करके आणविक गतिशीलता सिमुलेशन, अम्ब्रेला सैंपलिंग तकनीक के साथ किया गया था। परिणामों ने संकेत दिया कि फॉस्फेटिडाइल्सेरिन एक्सपोज़र ने, सामान्य कोशिका झिल्ली की तुलना में, कैंसर कोशिका झिल्ली के माध्यम से विथफेरिन ए, विथानोन, कैफिक एसिड फिनिथिल एस्टर के पारगमन की सुविधा प्रदान की। प्रस्तुत आंकड़ों ने दवाओं को चयनात्मकता प्रदान करने में दवा-झिल्ली अन्योन्यक्रिया की क्षमता का प्रदर्शन किया। छोटे अणुओं के ट्रांसडर्मल पारगम्यता अध्ययन के लिए त्वचा के स्ट्रेटम कॉर्नियम लिपिड मैट्रिक्स का एक परमाणु मॉडल, साहित्य से प्रयोगात्मक डेटा के साथ विकसित और मान्य किया गया है। संक्षेप में, यह थीसिस कम्प्यूटेशनल आणविक मॉडलिंग तकनीकों का उपयोग करके जैवसक्रियता और पारगम्यता को अंतर्निहित विशिष्ट आणविक स्तर के तंत्र में प्रकाश डालता है, जो अध्ययन किए गए प्राकृतिक यौगिकों को कैंसर दवाओं के रूप में आगे बढ़ाने में, और कैंसर के चयनात्मक लक्ष्यीकरण के लिए नए यौगिकों का विकास में मदद करेगा।

Contents

Certificate	i
Acknowledgements	iii
Abstract.....	v
सारांश	vii
Contents	ix
List of figures.....	xiii
List of tables.....	xvii
List of equations	xix
List of abbreviations and acronyms	xxi
1. Introduction.....	1
1.1 Cancer chemotherapy: strategies and challenges	3
1.2 Landscape of cancer drug development	5
1.3 Natural compounds as small molecule therapeutics for cancer.....	7
1.4 Role of molecular modeling in cancer drug development.....	9
1.5 Problem statement	11
1.6 Research objectives	13
2. Investigating the molecular mechanism of action of natural compounds	15
2.1 Rationale and study design	17
2.1.1 Natural compounds of interest.....	17
2.1.2 Shortlisted molecular targets.....	19
2.1.3 Molecular modeling methods used for studying ligand-target interactions.....	22
2.2 Inhibition of mortalin-p53 complex formation by fucoxanthin.....	25
2.2.1 Background.....	25
2.2.2 Methodology	26
2.2.3 Results and Discussion	26

2.3 Inhibition of p53-mortalin complex formation by combination of withaferin A and caffeic acid phenethyl ester.....	29
2.3.1 Background	29
2.3.2 Methodology	29
2.3.3 Results and Discussion.....	30
2.4 Activation of p53 ^{Y220C} mutant by caffeic acid phenethyl ester	33
2.4.1 Background	33
2.4.2 Methodology	33
2.4.3 Results and Discussion.....	35
2.5 Implications of the study	41
3. Exploring drug-membrane interactions for cancer cell selectivity	43
3.1 Drug-membrane interaction studies in pharmacology	45
3.2 Molecular modeling of membrane permeability	46
3.2.1 Theory	46
3.2.2 Protocol for membrane permeability studies using umbrella sampling	48
3.3 Altered lipid distribution in cancer cell membranes.....	50
3.4 Rationale and study design	51
3.5 Effect of phosphatidylserine exposure on the permeation of natural compounds.....	52
3.5.1 Background	52
3.5.2 Methodology	54
3.5.3 Results and discussion.....	58
3.6 Implications of the study	69
4. Predicting skin permeability of natural compounds using stratum corneum lipid matrix models	73
4.1 Transdermal drug delivery for cancer	75
4.2 Structure of stratum corneum lipid matrix: literature review	78
4.3 Molecular modeling of skin permeability	79
4.4 Rationale and study design	80

4.5 Predicting the skin permeability of natural compounds	81
4.5.1 Background.....	81
4.5.2 Methodology.....	83
4.5.3 Results and discussion	86
4.6 Atomistic model of stratum corneum lipid matrix based on experimental data for skin permeation studies	94
4.6.1 Background.....	94
4.6.2 Methodology.....	95
4.6.3 Results and discussion	100
4.7 Implications of the study	110
5. Conclusions and future prospects.....	113
References.....	121
List of publications from the thesis	145
Curriculum vitae of the author.....	149

List of figures

Figure 1.1: Drug development pipeline for cancer	7
Figure 1.2: Depiction of molecular docking.....	10
Figure 2.1: Natural compounds of interest	18
Figure 2.2: Schematic representation of p53 signaling pathway	20
Figure 2.3: Schematic representation showing the role of mortalin in cancer.	22
Figure 2.4: Interfacial residues of mortalin-p53 interaction.	26
Figure 2.5: Interaction of fucoxanthin at mortalin binding site (323-337) of p53.	27
Figure 2.6: Stability of the p53-fucoxanthin interaction.	28
Figure 2.7: Experimental inputs from DAILAB@AIST: p53 activation by fucoxanthin.....	28
Figure 2.8: Experimental inputs from DAILAB@AIST: Combination of withaferin A and caffeic acid phenethyl ester possesses stronger cytotoxicity and selectivity.	30
Figure 2.9: Interaction of withaferin A at mortalin binding site (323-337) of p53	31
Figure 2.10: Interaction of caffeic acid phenethyl ester at p53 binding site (323-337) of mortalin.....	32
Figure 2.11: Mutation crevice of p53 ^{Y220C} mutant.	34
Figure 2.12: Redocking of PK7242 to p53 ^{Y220C}	35
Figure 2.13: Interaction of caffeic acid phenethyl ester at the mutation crevice of p53 ^{Y220C} mutant.....	36
Figure 2.14: Stability of interaction of caffeic acid phenethyl ester at the mutation crevice of p53 ^{Y220C} molecule	37
Figure 2.15: Residues of p53 ^{Y220C} interacting with caffeic acid phenethyl ester and PK7242.....	39
Figure 2.16: Structural integrity of p53 ^{Y220C} upon caffeic acid phenethyl ester binding.....	40

Figure 2.17: Experimental inputs from DAILAB@AIST: Activation of wild-type like p53 activity in cell line possessing p53 ^{Y220C}	40
Figure 3.1: Illustration of altered lipid distribution in cancer cell membranes.	50
Figure 3.2: Natural compounds taken for membrane permeation studies	54
Figure 3.3: Built normal and cancer cell membrane models based on phosphatidylserine exposure after equilibration.	59
Figure 3.4: Densities of components of the equilibrated membrane systems..	60
Figure 3.5: Area per lipid values of the normal and cancer membrane models	60
Figure 3.6: Order parameters of hydrophobic tails of phosphatidylcholine and phosphatidylserine molecules in membrane models.....	61
Figure 3.7: Potential of mean force curves of withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C calculated after 10, 20, 30 and 40 ns.....	62
Figure 3.8: Potential of mean force profiles of withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C in normal and cancer membrane models	63
Figure 3.9: Snapshots of last frame from umbrella sampling windows showing the preferred orientations of the compounds withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C in the leaflets of normal and cancer membrane models	65
Figure 3.10: Diffusion coefficients of withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C in normal and cancer membrane models	66
Figure 3.11: Resistivity profiles of withaferin A, withanone, caffeic acid phenethyl ester and artemisinin C in normal and cancer membrane models	67
Figure 4.1: Natural compounds taken for skin permeation studies	82
Figure 4.2: Equilibrated lipid bilayer system made of stratum corneum lipids.....	87
Figure 4.3: Densities of the system components along the direction of the bilayer normal	87

Figure 4.4: Potential of mean force profiles of the natural compounds withaferin A, withanone, caffeic acid phenethyl ester, cucurbitacin B, curcumin, and resveratrol through the lipid bilayer model made up of stratum corneum lipids.....	88
Figure 4.5: Snapshots of last frame from umbrella sampling windows showing the orientations of the compounds withaferin A, withanone and caffeic acid phenethyl ester at different positions along reaction coordinate	90
Figure 4.6: Snapshots of last frame from umbrella sampling windows showing the orientations of the compounds cucurbitacin B, curcumin and resveratrol at different positions along reaction coordinate	91
Figure 4.7: Diffusion coefficients of the natural compounds withaferin A, withanone, caffeic acid phenethyl ester, cucurbitacin B, curcumin, and resveratrol through stratum corneum bilayer model	92
Figure 4.8: Schematic representation of the built model of stratum corneum lipid matrix.	95
Figure 4.9: Properties of the built model of stratum corneum lipid matrix during production run	100
Figure 4.10: Angle distributions between the two tails of ceramide NP and ceramide AP molecules	101
Figure 4.11: Built model of stratum corneum lipid matrix. Snapshot of the built model during production simulation and densities of the all the lipid molecules and water molecules in the system along the direction of the bilayer normal.	101
Figure 4.12: Comparison of neutron scattering length density profiles with experimental data	103
Figure 4.13: Density of the components of the model systems along 'z' upon hydration.	105
Figure 4.14: Potential of mean force profiles and diffusion coefficients of water, benzene and estradiol through the built model..	106

Figure 4.15: Snapshots from simulation trajectories showing the localization of permeation enhancers.....	108
Figure 4.16: Densities of system components after equilibrating with permeation enhancers.....	109
Figure 4.17: Properties of the stratum corneum lipid matrix systems equilibrated with permeation enhancers.....	110

List of tables

Table 2.1: Docking scores and binding free energies of binding of caffeic acid phenethyl ester and PK7242 at p53 ^{Y220C} mutation crevice.....	37
Table 3.1: The distribution of phosphatidylcholine and phosphatidylserine molecules in the leaflets of cancer and normal membrane models	54
Table 3.2: Calculated permeability coefficients of the drug molecules	68
Table 4.1: Permeability coefficients of the studied anticancer compounds calculated using bilayer model of stratum corneum lipids	93
Table 4.2: Permeability coefficients of water, benzene and estradiol calculated using the built model of stratum corneum lipid matrix compared with experimental values	107

List of equations

Equation 2.1: Binding free energy	25
Equation 3.1: Flux or rate of permeation of a solute across a membrane.....	45
Equation 3.2: Homogeneous solubility-diffusion model	46
Equation 3.3: Inhomogeneous solubility-diffusion model.....	47
Equation 3.4: Lipid tail order parameter	48
Equation 3.5: Diffusion coefficients	49
Equation 4.1: Free energy of transfer of permeants from water to lipid.....	98

List of abbreviations and acronyms

ARC	Artepillin C
CAPE	Caffeic Acid Phenethyl Ester
CucB	Cucurbitacin B
DAILAB@AIST	DBT-AIST International Laboratory for Advanced Biomedicine, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan
MD simulation	Molecular Dynamics simulation
PC	Phosphatidylcholine
PDB	Protein Data Bank
PE	Phosphatidylethanolamine
PMF	Potential of Mean Force
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
POPS	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine
PS	Phosphatidylserine
RMSD	Root-mean-square deviations
SM	Sphingomyelin
SMD simulation	Steered Molecular Dynamics simulation
WHAM	Weighted Histogram Analysis Method
Wi-A	Withaferin A
Wi-N	Withanone