Computational and Medicinal Chemistry Approach to Explore the Pharmacological Potential of Naturally-Occurring Lactones

Thesis submitted in partial fulfilment for the
Award of Degree

Doctor of Philosophy

By

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List of Abbreviations

Abbreviation	Full Form
2D	Two-dimensional
3D	Three-dimensional
A549	Human lung adenocarcinoma cell line
ACN	Acetonitrile
ADMET	Absorption, Distribution, Metabolism, Excretion & Toxicity
Akt	Protein kinase B
ALOX5	Arachidonate-5 lipoxygenase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AP-1	Activator protein-1
ASC	Apoptosis-associated speck-like protein
AST	Aspartate aminotransferase
BBB	Blood-brain barrier
BIOS	Biology-oriented synthesis
BRD4	Bromodomain-containing protein 4
Calc.	Calculated
CASP1	Caspase-1
CDK9	Cyclin-dependent kinase 9
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
CDP	Consensus diversity plot
CFA	Complete Freund’s adjuvant
$CHCl_3$	Chloroform
CMAUP	Collective molecular activities of useful plants
CNS	Central nervous system
COCONUT	Collection of open natural products
COSY	Correlation spectroscopy
COX	Cyclooxygenase
COX 1	Cyclooxygenase 1
COX-2	Cyclooxygenase 2
CTRC	Chymotrypsin C
CTSG	Cathepsin G
CYP2D6	Cytochrome P2D6
DCM	Dichloromethane
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNP	Dictionary of natural products
DOGS	De novo design of generic structures
DOS	Diversity-oriented synthesis

Abbreviation	Full Form
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DTNB	5,5′-dithiobis (2-nitrobenzoic acid)
EGFR	Epidermal growth factor receptor
EIC	Extracted ion chromatogram
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated kinase
ERBB2	Erythroblastic oncogene B homolog 2
ESI-MS	Electronspray ionization-Mass spectrometry
EtOAc	Ethyl acetate
F2	Coagulation factor II
FAME	Fast metabolizer
FASTA	Fast adaptive shrinkage threshold algorithm
FAX	Fatty acid export
FBS	Foetal bovine serum
FLT1	Fms related receptor tyrosine kinase 1
GMQE	Global model quality estimate
GO	Gene ontology
GSH	Glutathione
H-Bond	Hydrogen bond
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HeLa	Human cervical cancer cell line
hERG	human ether-a-go-go-related gene
HIA	Human Intestinal Absorption
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence spectroscopy
HTS	High-throughput screening
HTVS	High-throughput virtual screening
iNOS	Inducible nitric oxide synthase
IC_{50}	Half-maximal inhibitory concentration
ID_{50}	Half-maximal infectious dose
IL-1	Interleukin-1
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL1R1	Interleukin-1 receptor
IKBKB	Inhibitor of nuclear factor kappa B kinase subunit beta
JAK1	Janus kinase 1
JNK	c-Jun N-terminal kinase
JNK3	c-Jun N-terminal kinase 3
KDR	Kinase insert domain

Abbreviation	Full Form
KEGG	Kyoto encyclopedia of genes and genomes
KNIME	Konstanz Information Miner
LC-MS	Liquid chromatography-mass spectrometry
LC-QTOF	Liquid chromatography quadrupole time-of-flight
LGA	Lamarckian genetic algorithm
LOX	Lipoxygenase
LPO	Lipid peroxidation
LPS	Lipopolysaccharide
LTA4H	Leukotriene A4 hydrolase
MAPK/ MEK	Mitogen-activated protein kinase
MAPK8	Mitogen-activated protein kinase 8
MD	Molecular dynamic
MDA	Malondialdehyde
MDA-MB-231	Human metastatic breast adenocarcinoma cell line
MEM	Minimum essential medium
MolSA	Molecular surface area
MMP2	Matrix metallopeptidase 2
MM-GBSA	Molecular mechanics-Generalized born surface area
MM-PBSA	Molecular mechanics-Poisson boltzmann surface area
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NCE	New chemical entity
NC-MFP	Natural compound molecular fingerprint
NF-κB	Nuclear factor-kappa B
NLRP3	NLR family pyrin domain containing 3
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOESY	Nuclear overhauser effect spectroscopy
NOS2	Nitric oxide synthase-2
NP	Natural product
Nrf2	Nuclear factor erythroid 2-related factor 2
NSAID	Non-steroidal anti-inflammatory drugs
NSCLC	Non-small cell lung cancer
PAINS	Pan-assay interference compounds
PBS	Phosphate buffer saline
PDB	Protein data bank
PDBQT	Protein data bank, partial charge (Q), & atom type (T)
PCA	Principal component analysis
PGE2	Prostaglandin E_2
PGH2	Prostaglandin H2

Abbreviation	Full Form
PI3K	Phosphoinositide-3-kinase
PKC	Protein kinase C
PLA2R1	Phospholipase A2 receptor 1
PLCγ	Phosphoinositide phospholipase C
PMA	Phorbol myristate acetate
PME	Particle mesh ewald
PPB	Plasma protein binding
PPI	Protein-protein interaction
PRKCA	Protein kinase C alpha
PRKCBP1	Protein kinase C binding protein 1
PRKCG	Protein kinase C gamma
PRSS1	Protease serine 1
PSA	Polar surface area
PTGES	Prostaglandin E synthase
PTGES1	Prostaglandin E synthase 1
PTGES2	Prostaglandin E synthase 2
PWF	Paw withdrawal frequency
PWL	Paw withdrawal latency
PWT	Paw withdrawal threshold
Rg/RoG/rGyr	Radius of gyration
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
ROCS	Rapid overlay of chemical structures
Ro5	Rule of five
ROS	Reactive oxygen species
SAR	Structure-activity relationship
SASA	Solvent-accessible surface area
SB-DFP	Statistical-based database fingerprint
SCLC	Small cell lung cancer
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Standard error mean
SHB	SH2 domain containing adaptor protein B
SL	Sesquiterpene lactone
SRC	Proto-oncogene tyrosine-protein kinase
SP	Standard precision
SPiDER	Self-organizing map-based prediction of drug equivalence relationships
STarFish	Stacked ensemble target fishing
STAT3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor-β (TGF-β)-activated kinase 1
TBA	Thio barbituric acid

Abbreviation	Full Form
TBARS	Thiobarbituric acid reactive substances
TBX2	T-Box transcription factor 2
TCM	Traditional Chinese Medicine
TIP3P	Transferable intermolecular potential with 3 points
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
TNB	Thionitro benzoic acid
TNF-α	Tumor necrosis factor-α
TNBC	Triple-negative breast cancer
t-SNE	T-distributed stochastic neighbor embedding
TIGER	Target inference generator
TSAd	T cell-specific adapter protein
UMAP	Uniform manifold approximation and projection
US FDA	United States Food & Drug administration
UV	Ultraviolet
VEGFs	Vascular endothelial growth factors
VEGFRs	Vascular endothelial growth factor receptors
VEGFR-2	Vascular endothelial growth factor receptor- 2
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRE	Vancomycin-resistant *Enterococci*
XP-	Extra precision
XRD	X-ray diffraction
YES1	YES proto-oncogene 1

List of Symbol

Symbols	Meaning
α	Alpha
β	Beta
γ	Gamma
δ	Delta
ω	Omega
~	Approximately
Å	Angstrom
κ	Kappa
±	Plus or Minus
<	Less than
>	Greater than
≥	Greater than equal to
≤	Less than or equal to
%	Percent
µM	Micromolar
nM	Nanomolar
mM	Millimolar
µL	Microliter
mL	Milliliter
L	Liter
µg/mL	Micrograms per milliliter
pH	Potential of hydrogen
K	Kelvin
ns	Nanosecond
ps	Picosecond
kcal/mol	Kilocalories per mole
nm	Nanometer
sec	Second
min	Minute
hr	Hour
Hz	Hertz
MHz	Megahertz
J	Coupling constant
ppm/δ	Parts per million
s	Singlet
d	Doublet
t	Triplet
dd	Doublet of doublet
dt	Doublet of triplet
m	Multiplet

Symbols	Meaning
m/z	Mass-to-charge ratio
mg	Milligrams
mg/dl	Milligrams per deciliter
g	Grams
kg	Kilograms
mg/kg	Milligrams per kilogram
rpm	Revolutions per minute
i.p.	Intraperitoneal

Preface

Natural products (NPs) are and have remained a rich source of inspiration in drug discovery and development. They exhibit a broad spectrum of structural diversity and complexity with vast pharmacological potential. A significant portion of approved anticancer and anti-infective drugs have natural origins. Till now, nearly one-third of the approved drugs and two-thirds of small-molecule drugs had some connection to NPs, whether in their original form, as derivatives, or mimetics. While successful drugs have historically emerged from NPs, there has recently been a noticeable decline in the number of new drugs derived from them, but despite the challenges, the significant impact of NPs on drug discovery remains evident.

Plant-derived cyclic esters, known as lactones, are abundant in nature's chemical reservoir and offer a wide range of structural diversity, thus serving as unique frameworks for lead compounds. Among naturally occurring lactones, γ- and δ-lactones are the most abundant. The present thesis work was aimed at the discovery of new lead molecules with cytotoxic and anti-inflammatory potential derived from natural lactones. The thesis consists of ten chapters in which the natural lactones were explored for their biological potential using a multitude of approaches that included computational screenings, phytochemical investigations, semi-synthetic derivatization, and pharmacological screenings.

Chapter 1 initiates a comprehensive exploration of NPs, encompassing their role in drug discovery, their precedence over synthetic molecules, their limitations and challenges, and their contribution toward approved drugs. It also discusses about natural lactones in drug discovery and their current therapeutic landscape. Additionally, the role of computational approaches in NP-based drug discovery and the advantages of amalgamating them are discussed.

In **Chapter 2**, the objectives of the study and plan of work are enumerated.

Chapter 3 describes the identification of natural coumarin compounds from the COCONUT database as potential VEGFR-2 inhibitors (anti-angiogenic). By developing a pharmacophore of lenvatinib and applying pharmacophore-based virtual screening and molecular modeling, the chapter identifies promising candidates associated with VEGFR-2 inhibition, contributing to the development of inhibitors of cancer-related angiogenesis.

Chapter 4 mentions the computational exploration of coumestans (type of lactone-containing phytoconstituents) of *Psoralea corylifolia* (L.) through molecular modeling studies for their EGFR (an important target for anticancer therapies) inhibitory potential and subsequent lead identification. The chapter then proceeds to extraction, isolation, and *in vitro* cytotoxicity screening of identified coumestan hit from *P. corylifolia*.

Chapter 5 describes the phytochemical investigations of leaves and seed of *P. corylifolia* (L.) for the presence of potential coumarin (benzenoid δ-lactones) phytoconstituents. The study proceeds through extraction of *P. corylifolia* leaves and seeds, followed by isolation and purification of various fractions for pure compounds. Later, the chapter mentions the evaluation of cytotoxic potential of isolated phytoconstituents and molecular modeling studies of the compounds that possessed potent cytotoxicity.

Chapter 6 explores a network pharmacology-based approach to identify anti-inflammatory lactones from a NP database and their possible mechanism of action. The study then proceeds to docking studies of selected lactone candidates with the inflammatory targets and identification of potential lactone hit and its potential targets. Later, the section describes molecular dynamic studies, extraction, isolation and purification of lactone hit and *in vitro* validation of its target inhibitory potential.

Chapter 7 mentions the semi-synthetic modification of lactone hit identified in Chapter 6. The chapter describes the development of naproxen-like analogs from santonin, a

sesquiterpene lactone identified through network pharmacology-based approach. The section then describes the evaluation of the anti-inflammatory potential of synthesized analogs through enzyme inhibition assay and later evaluation of the *in vivo* anti-inflammatory potential of the best analog.

Chapter 8 describes the phytochemical exploration of *Vitex negundo* (L.) leaves for potential phytoconstituents. The chapter encompasses the extraction of *V. negundo* leaves, resin-based fractionation, and repeated purification of fractions to obtain pure phytoconstituents. The section mentions the isolation of 2,3-Dehydrosilychristin, a silymarin flavonolignan, from the leaf extract of *V. negundo* along with 12 other phytoconstituents. It also describes the pharmacological exploration of 2,3-Dehydrosilychristin through *in vitro* and *in vivo* studies.

Chapter 9 outlines the summary and conclusions of the research work undertaken.

In **Chapter 10**, the references used to carry out the research work are enumerated.

An appendix containing additional supporting information, spectral data of representative compounds and a bibliography of publications during the course of the Ph.D. is included.